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EDITORIAL OFFICE

Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/csp/helpdesk.aspx>
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One center in Brussels has consistently had the lowest HbA1c values in the 4 studies (1994-2009) by the Hvidoere International Study Group on Childhood Diabetes: What are the "recipes"?

Harry Dorchy

Harry Dorchy, Diabetology Clinic, University Children's Hospital Queen Fabiola, Université Libre de Bruxelles, 1020 Brussels, Belgium

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Correspondence to: Harry Dorchy, MD, PhD, DHC, Professor, Diabetology Clinic, University Children's Hospital Queen Fabiola, Avenue JJ Crocq 15, 1020 Brussels, Belgium. hdorchy@ulb.ac.be
 Telephone: +32-2-4773185

Fax: +32-2-4773156

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Abstract

The principal aims of therapeutic management of the child, adolescent and adult with type 1 diabetes are to allow good quality of life and to avoid long-term complications (retinopathy, neuropathy, nephropathy, cardiovascular disease, *etc.*) by maintaining blood glucose concentrations close to normal level. Glycated hemoglobin levels (HbA1c) provide a good criterion

of overall glycemic control. The Hvidoere Study Group (HSG) on Childhood Diabetes, founded in 1994, is an international group representing about twenty highly experienced pediatric centers from Europe, North America, Japan and Australia. Four international comparisons of metabolic control (1995, 1998, 2005, 2009) have been performed. The one center that has consistently had the lowest HbA1c values (approximate 7.3% or 56.3 mmol/mol) is my center in Brussels. This is more often obtained with a twice-daily free-mixed regimen with additional supplemental fast insulins ad hoc. The so-called "Dorchy's recipes" are summarized. The conclusion is that the number of daily insulin injections, 2 or ≥ 4 , or the use of pumps, by itself does not necessarily give better results. Intensified therapy should not depend upon the number of insulin doses per day, by syringe, pen or pump but rather should be redefined as to intent-to-treat ascertainment (*i.e.*, goals). When there are no mutually agreed upon goals for BG and/or HbA1c, when there is insufficient education and psychosocial support by the medical team or at home, there is likely to be poor outcomes, as shown by the HSG. One of our recipes is not to systematically replace rapid-acting human insulins by fast-acting analogues. Because the multicenter studies of the HSG, performed in developed countries without financial restriction, show that treatment of childhood diabetes is inadequate in general and that levels of HbA1c are very different, diabetes treatment teams should individually explore the reasons for failure, without any prejudice or bias. Any dogmatism must be avoided. Treatment cost *vs* results must also be taken into account.

Key words: Type 1 diabetes mellitus; Insulin regimen; Diabetic children; Glycated hemoglobin; Conventional treatment; Intensive treatment

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Core tip: Four international comparisons of the Glycated hemoglobin levels (HbA1c) levels (1995, 1998, 2005, 2009) have been performed by the Hvidoere Study Group on childhood diabetes in about twenty pediatric diabetology centers from about twenty industrialized countries in Europe, North America, Japan and Australia. The one center that has consistently had the lowest HbA1c values (approximate 7.3% or 56.3 mmol/mol) is my center in Brussels. This is more often obtained with a twice-daily free-mixed insulin regimen. The so-called "Dorchy's recipes" are summarized.

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COMMENTARY ON HOT TOPICS

Diabetes Control and Complications Trial research group

The principal aims of therapeutic management of the child, adolescent and adult with type 1 diabetes are to allow good quality of life^[1,2] and to avoid long-term complications (retinopathy, neuropathy, nephropathy, cardiovascular disease, etc.) by maintaining blood glucose concentrations close to the normal level^[3,4] while always minimizing hypoglycemia. Glycated hemoglobin levels (HbA1c) provide a good criterion of overall glycemic control. According to the diabetes control and complications trial research group (DCCT), they must be, in adults, under 7% (53 mmol/mol), if the upper normal limit is about 6% (42 mmol/mol)^[3]. The DCCT obtained such results utilizing targeted blood glucose (BG) treatment decisions usually with multidose insulin (MDI) (≥ 4 shots/d) and/or insulin pump treatment compared to a relatively fixed insulin dose schedule in the control group^[3]. Such MDI and continuous subcutaneous insulin infusion (CSII) treatment was subsequently known as intensive treatment when it really should have been defined as the targeted BG intent to reach BG goals and HbA1c goals safely that was "intensive treatment". However, in our experience, this is possible even in diabetic children and adolescents with the twice-daily free mixing insulin regimen as well as with the basal-bolus regimen, as we have shown since the 90 s^[5-7].

Hvidoere Study Group on childhood diabetes

After the publication of the conclusions of the DCCT and of my own results, causing some debate about "Dorchy's recipes"^[6], The Hvidoere Study Group on childhood diabetes (HSG) evolved in 1994, during a

workshop, to discuss strategies that could be important in improving quality of pediatric and adolescent diabetes care and, therefore good HbA1c levels. Four international comparisons of metabolic control (1995, 1998, 2005, 2009) have been performed in about twenty pediatric diabetology centers from about twenty industrialized countries in Europe, North America, Japan and Australia^[2,8-10]. A capillary blood sample was provided by participants and analyzed centrally at the Steno Diabetes Center in Denmark. HbA1c was DCCT aligned: normal range 4.4%-6.3% or 25-45 mmol/mol, mean 5.4% or 35.5 mmol/mol. The mean is 0.3% higher than the DCCT laboratory level: normal range 4.05%-6.05%, mean 5.05%.

Cameron *et al*^[11] have reviewed the major studies of the HSG, both cross-sectional and longitudinal, and summarized the body of work published in 28 peer reviewed medical and scientific journals (Table 1). The authors note that "The one center that has consistently had the lowest HbA1c values from 1995 to 2009" is my center in Brussels. They comment: "The Hvidoere member in question is highly charismatic and has a very prescriptive, 'recipe'-based approach to managing diabetes in his clinic. He prescribes mostly twice-daily free mixing injections of insulin and eschews, a flexible approach to dietary intake. This does not appear to be at the expense of either hypoglycemia or QOL in his patient group. Although many aspects of his practice are shared by other Hvidoere members, it has proved very difficult to translate this total approach into other contexts for a variety of reasons. However, this experience is emblematic that consistently excellent outcomes can be achieved by simple, 'non-intensive' insulin regimens that are underpinned by a strong philosophy of care"^[11].

Cameron *et al*^[11] conclude in their review: "Therapeutic strategies in and of themselves are not enough to obtain desired clinical outcomes. While all clinical regimens have some clinical utility, it is the underlying therapeutic philosophy based on a qualified common training for all team members delivering diabetes care and education to the families that drives improvement. The clinical aphorism of 'Ask for mediocrity and you will receive' holds true. Thus, it appears that the best results will be obtained by physicians who are target-driven and teams and families that have unanimity of purpose. Perhaps the conclusions relating the best clinical practice drawn from the entire body of work of the Hvidoere studies can be best summarized as -be dogmatic about outcome but flexible in approach".

In the studies of the HSG, the different insulin regimens were: "conventional" twice daily (CT), CTpremix, CTfreemix, CTfreemix + (used only in center number one, *i.e.*, in my center in Brussels), basal-bolus injections (MDI), CSII. In the 4th study^[10], there was confusion between CTfreemix and CTfreemix +, fortunately corrected by an erratum^[10]. The lowest HbA1c levels were found in the CTfreemix+ group, 7.3% \pm 0.5% (56.3 mmol/mol); approximately the same values were obtained in the three preceding studies by the Brussels team. In 2007, after three HSG studies, de Beaufort *et al*^[9] noted:

Table 1 HbA1c comparisons in the 4 studies by the Hvidoere international study group on childhood diabetes (1995-2009)

Hvidoere studies	Number of countries of pediatric centers	Number of subjects	Age (yr)	Mean HbA1c (%) \pm SD (mean DCCT aligned)	Spread in center mean HbA1C (%) (DCCT aligned)	Conclusions
1995 ^[8]	18 (Europe, North America, Japan) 22	2873	0-18	8.6 \pm 1.7 (8.3)	7.6-10.2 (7.3-9.9)	No difference in glycemic control was found among adolescents treated with two, three, and four or more daily injections. Girls on 4 injections had higher BMI
1998 ^[2]	17 (Europe, North America, Japan) 21	2101	11-18	8.7 \pm 1.7 (8.4)	7.7-10.1 (7.4-9.8)	The differences between centers were not explicable by differences in insulin regimens. The centers with the lowest mean HbA1c also had lowest rates of severe hypoglycemia and reported better QOL
2005 ^[9]	19 (Europe, North America, Japan, Australia) 21	2093	11-18	8.6 \pm 1.7 (8.2)	7.7-9.5 (7.4-9.2)	Intensified insulin regimens (MDI and CSII) showed no lower HbA1c compared with twice daily free-mixing (lowest HbA1c)
2009 ^[10]	17 (Europe, North America, Japan, Australia) 18	1113	< 11	8.3 \pm 1.3 (8.0)	7.6-9.2 (7.3-8.9)	despite major changes in management (> 99% on analog insulins and 33% with CSII), the lowest HbA1c levels were found in the twice daily free-mixing insulin regimen in Brussels (7.3% \pm 0.5%)

MDI: Multidose insulin; CSII: Continuous subcutaneous insulin infusion.

"The management of children and adolescents with type 1 diabetes has undergone many changes over the past decade, aiming to improve glycemic control and reduce risks of vascular complications, without sacrificing quality of life. These have included increased usage of insulin analogues, basal-bolus regimens, and CSII. Despite these substantial changes, it has been difficult to demonstrate significant improvements in metabolic outcome. This study in 21 international centers was initiated to investigate the impact of treatment changes on glycemic control and to establish whether the previously reported differences between centers were diminishing. The results confirm that there has been no improvement in glycemic control over a decade, with mean A1c levels of 8.6% or 70.5 mmol/mol (1995), 8.7% or 71.6 mmol/mol (1998), and 8.6% or 70.5 mmol/mol (2005), and the substantial differences between centers have remained stable." That means that more expensive and technically complicated treatments have nearly no impact on HbA1c. Only two centers showed a significant reduction ($\geq 0.5\%$) in A1c from 1998 to 2005 and one center had a significant increase in A1c. The conclusion is that, in countries unable to afford a sophisticated and expensive treatment, it is possible to obtain good results without necessarily using expensive insulins and pumps.

As my team has consistently had the lowest HbA1c values during the comparisons of the HSG from 1995 to 2009, I think that I am allowed to summarize the so-called "Dorchy's recipes..."^[6,12-15].

Dorchy's recipes

Two daily insulin free-mixed regimen in children or even teenagers: Two daily insulin free-mixed regimen with an human rapid-acting insulin or a fast-

acting analogue and NPH (*i.e.*, 4 insulins per day as in the basal-bolus regimen) in children < 15-16 years is easy and effective in countries where the meal schedule allows correct allocation of diet. The first injection (and insulin dose alteration) is done before going to school and the second injection (and insulin dose alteration) after returning from school, before dinner, with the facultative help of the parents. Diabetic children have to eat a snack in the middle of the morning and afternoon periods with their friends, without the need to give an additional insulin injection or to measure blood glucose. This reduces the risk of insulin omission. The doses of the 4 insulins are adjusted according to the results of 4 daily blood glucose measurements of the preceding days (retroactive analysis) and not only to the present glycemia (reactive responsivity). A third injection with a fast-acting analogue may be done to allow a greater snack or to correct hyperglycemia (= CTfreemix +).

Basal-bolus regimen in adolescents but more complicated: Basal-bolus regimen in adolescents: increased flexibility in daily life and dietary freedom, but more complicated; no simplistic sliding scales according to the present glycemia; insulin dose alteration must be triple: (1) retrospective, according to previous BG analysis, trial and error experiments, in order to enjoy more freedom for meals, sports, *etc*; (2) prospective according to programmed changes in meals and sports (*i.e.*, add more insulin if overeating or temporarily reduce insulin dose to prevent activity-related expected hypoglycemia); and (3) with only a "touch" of compensatory adaptation (reactive dose changes) according to the present glycemia. This needs psychological maturity and ongoing education support and teaching of child, adolescent and family

members, otherwise the multiple injection system lead to anarchy, "cheating" and obesity, especially but not only in adolescent girls. Before or after the meals, there is an injection of rapid human insulin or fast-acting analogue, and before sleeping, an injection of a stable long-acting analogue^[16]. The doses of the 4 insulin injections are adjusted according to analysis of the results of at least 4 daily blood glucose measurements (if three meals; otherwise more blood glucose determinations and injections) of the preceding days and not only to the present BG level.

CSII is very rarely recommended: In our experience, CSII is very rarely recommended and used in about 1% of our patients at their request. We do not promote use of expensive insulin pump regimens and believe that patients and their family members can do as well with pens and syringes. Pumps in children and adolescents have not been associated with significant improvements in daily BG results or in A1c according to results of the HSG^[17] and also by the PedPump study in 30 centers of 17 countries^[17]. In that study, the use of less than 6.7 daily boluses was a significant predictor of an HbA1c level > 7.5% or 58.5 mmol/mol, despite increasing blood glucose measurements and the added expense that this entails. We suspect this reflects insufficient education and motivation and inconsistent team target goal setting as the explanation. In a Belgian retrospective cross-sectional study among 12 pediatric centers, A1c actually was higher among patients with insulin pump therapy^[18].

No rejection of non-analogue older human insulins: No systematic (automatic or dogmatic) replacement of rapid-acting human insulins by fast-acting analogues such as more expensive aspart, lispro or glulisine^[14]. In the two daily insulin free-mixed regimen as well as in the basal-bolus regimen, the choice of a fast-acting analogue is made if the time period between the injection and the following glycemia, allowing to judge the insulin injected before, is less than 3 or 4 h, *i.e.*, the duration of action of the fast-acting analogue. Otherwise, we use a human rapid-acting insulin whose duration of action reaches 6 to 8 h rather than the newer and more expensive fast-acting analogues.

No carbohydrate counting: The dietician never gives rigid meal plans or exchange lists. "Diet" is never prescribed. No carbohydrate counting is recommended because there is no linear correlation between the metabolism of X grams of glucose by Y units of insulin^[12,13,15]. The dietician builds up a picture of the family's and child or teen's usual habits and life style. When possible, the family is encouraged to adopt a similar and normal eating pattern so that the child and adolescent with diabetes does not have to eat specially prepared meals. The main problem with the twice-daily insulin regimen is the allocation of carbohydrates in 6 meals according to the cumulated action of the insulins.

The dietician must know perfectly the actions of the insulins and their adjustment. While being criticized for this being too difficult, our glycemic control and A1c results certainly prove that this is feasible to accomplish with large numbers of children, adolescents and young adults. In addition, all members of the professional diabetes team must have the same treatment philosophy, as promulgated by the DCCT, to provide the same message and same target BG and A1c goals^[19].

Screening for subclinical complications: Screening for subclinical and asymptomatic complications by sensitive methods from puberty in order to increase the motivation of both the patient and the doctor^[20]. After age 13 and/or 3 years of diabetes duration, we perform every year: retinal fluorescein angiography (rather than just direct ophthalmoscopy), measurement of motor and sensitive conduction velocities (which is different from a painful electromyography), sympathetic cutaneous response or heart rate variability and dosage of microalbuminuria. It is important to do a diagnosis at the stage of functional and reversible abnormalities before the installation of irreversible lesions. It is important to be able to say to the patient, for example, "you have no complaint, but as you can see on this photograph, there are two leakages of fluorescein in your left eye; it is reversible if you improve your HbA1c; otherwise, that will become an irreversible lesion leading later to overt complications". The same message for the slowing of conduction velocity or the presence of abnormal microalbuminuria. Every year, we also perform lipid analyses, thyroid and celiac screenings, measurement of blood pressure, *etc.*,^[21,22]. Early identification of such abnormalities allows potential early treatment, *i.e.*, medication to control hypertension or early nephropathy, lower lipids, *etc.*

Knowledge of HbA1c target: One hundred percent of our patients and/or their parents as well as the members of the multidisciplinary team know the HbA1c target, *i.e.*, less than 7% or 53 mmol/mol, and one hundred percent of our patients and/or their families know the result of their HbA1c results obtained, on average, every two months before the consultation. This is strongly associated with HbA1c outcome as shown by the HSG^[23].

Friendly and personal contacts: Friendly and personal contacts with a large dose of psychological support are indispensable in the long-term relationship of a patient with a chronic disease and diabetes is perhaps the best example because of the multitude of daily behavioral decisions that must be acknowledged and accomplished. The diabetologist must know the whole story of the life of his or her patient, and must adapt his or her psychology to the psychology of the patients and of their families, and not the reverse. The diabetologist is not interchangeable as it can be the case for some other pediatric specialties or practices and this too may help explain our consistent excellent A1c results compared to

others in Hvidoere.

Observers are not allowed during consultations: In the office of the diabetologist, at the outpatient clinic, we do not allow observers: temporary assistants or students. This is most important especially with adolescents, in an effort not to disrupt the mutual trust and to preserve privacy. Patients are not undressed (except to search for lipodystrophies)... They are not ill... It is important on a psychological point of view, mainly with adolescents and with Muslims.

Education is made-to-measure: Nearly 50% of our patients are immigrants and mainly of Moghrabin origin (especially from Morocco). Because of the such cultural, economic and social differences, the education we offer must be adapted to the family and their food choices with individualized teaching modules and concepts but with the same overall glycemic goals in mind. Education must be made-to-measure.

High frequency of long-duration consultations: The duration of the medical consultation varies between 30 to 60 min and is preceded by a consultation with a nurse specialized in pediatrics as well as in diabetology. If necessary further consultation takes place with the dietician, the psychologist or the social worker.

Treatment is nearly costless; the nurses are allowed to go to schools: Care is provided in a specialized pediatric and adolescent/young adult diabetology clinic of a high ranking Belgian university public hospital, recognized by the Belgian Social Security ministry in an official manner. Medical and paramedical (nurse, dietician, psychologist, social worker) "consultations" and material necessary for treatment are nearly costless. Nurses are also allowed and compensated for time to do home and school visits and this is especially helpful for those in poor financial, psychological or immigrant circumstances where more teaching time is required to reach goals and sustain them. We believe that such multidisciplinary ongoing care helps explain our good results even among otherwise potentially more problematic patient populations.

We follow our patients into adulthood: We follow our patients into adulthood and do not automatically suggest their transition to adult physicians after an arbitrary age of 18 years. We believe this allows us to assist with transition through adolescence and into better self-care behaviors as young adults. We also believe that this allows our professional team to be better aware of the actual complications that may only occur after longer duration of diabetes. At the onset of 2014, we followed 527 children and adolescents with diabetes aged < 18 year and 495 aged ≥ 18 year.

Influence of family characteristics and alexithymia on HbA1c: The HSG has shown that family factors,

particularly dynamic and communication factors such as parental over-involvement and adolescent-parent concordance on responsibility for diabetes care appear be important determinants of metabolic outcomes in adolescents with diabetes^[24]. We tried to determine the family characteristics and the psychological factors influencing A1c. The maternal perception of family cohesiveness and maternal alexithymia predict on glycemic control in children and adolescents with diabetes^[25]. We showed, for the first time, that children who have difficulties in expressing their feelings to others are more at risk of poor glycemic control. In future, it will be useful to identify the diabetic young people who have such difficulties and to consider interventions designed specifically for them^[26].

Confusion between conventional and intensive therapy

The team that I have created in Brussels believes it is inappropriate to automatically designate the term "intensive treatment" only to imply insulin pumps or multidose insulin regimens when, in fact, it is the goals of glycemic control and A1c achievement that should define intensified treatment not the manner or number of insulin doses each day. It is inadequate to systematically assign the multiple injection regimen, or the pump therapy, to "intensive" treatment, and some forms of the twice-daily injection regimen abusively called "conventional" (there are different strategies as shown by the HSG, premix insulins giving the worst results and the CTfreemix + obtaining the best HbA1c levels) to a non-intensified therapeutic category of insulin therapy. Indeed, a multiple injection regimen, or the use of pumps, not associated with a good intensified and complete education, as well with the application of the consecutive knowledge, may have deleterious effects on HbA1c, as shown by the HSG. The confusion between "conventional therapy" and "intensive therapy" was born from misinterpretation of how the DCCT was structured in 1993^[3]. In their "conventional group" with one or two daily injections, there was no insulin adjustment except in order to avoid clinical symptoms such as polyuria and polydipsia with hyperglycemia or symptoms and signs reflecting excessive hypoglycemia and there were very few consultation visits except for every three months follow-up assessments for overall monitoring and lab work. There was no blood glucose target established for the conventional treatment group, no specific amount of monitoring and minimal education sessions that took place; all of this was designed to mimic the type of "non-intensive" usual treatment at that time^[3]. Bolli^[27] wrote: "One concept should be clear. The difference between intensive and non-intensive therapy is limited to the glycemic targets (mean glycemia < 150 mg/dL which gives an HbA1c < 7%). Non-intensive therapy is defined as a model of insulin treatment (2 or ≥ 4 injections, CSII, diet, HBGM, education, *etc.*) giving a mean blood glucose concentration and % of HbA1c above the values indicated by the DCCT".

General conclusion

Because recent multicenter studies, even those performed in developed countries without financial restriction, show that treatment of childhood, adolescent and young adults diabetes is inadequate in general and that levels of HbA1c are very different, diabetes treatment teams should individually explore the reasons for failure, without any prejudice or bias, in their own centers especially when center average A1c results are over 8%. The number of daily insulin injections, 2 or ≥ 4 or the use of pumps, by itself does not necessarily give better results. Merely increasing the number of daily insulin injections or encouraging insulin pump treatment does not automatically produce better results although may offer greater flexibility for the patient and family. Key remains unified education by a team of diabetes professionals who know their patient and his/her family, work to educate and re-educate and mutually sets goals known and agreed upon by not only the entire team providing such care but also the patient and his or her family. Any dogmatism must be avoided. Treatment cost *vs* results must also be taken into account.

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WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Insulin sensitivity and complications in type 1 diabetes: New insights

Petter Bjornstad, Janet K Snell-Bergeon, Kristen J Nadeau, David M Maahs

Petter Bjornstad, Janet K Snell-Bergeon, Kristen J Nadeau, David M Maahs, Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO 80045, United States
Petter Bjornstad, Janet K Snell-Bergeon, Kristen J Nadeau, David M Maahs, Barbara Davis Center for Diabetes, University of Colorado School of Medicine, Aurora, CO 80045, United States

Petter Bjornstad, Janet K Snell-Bergeon, Kristen J Nadeau, David M Maahs, Department of Pediatrics, Division of Pediatric Endocrinology, University of Colorado School of Medicine, Aurora, CO 80045, United States

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Correspondence to: Petter Bjornstad, MD, Barbara Davis Center for Diabetes, University of Colorado School of Medicine, 13123 East 16th Avenue, Aurora, CO 80045, United States. petter.bjornstad@childrenscolorado.org

Telephone: +1-720-7771234

Fax: +1-720-7777301

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pressure control, vascular complications remain the most important cause of morbidity and mortality in patients with type 1 diabetes. For that reason, there is a need to identify additional risk factors to utilize in clinical practice or translate to novel therapies to prevent vascular complications. Reduced insulin sensitivity is an increasingly recognized component of type 1 diabetes that has been linked with the development and progression of both micro- and macrovascular complications. Adolescents and adults with type 1 diabetes have reduced insulin sensitivity, even when compared to their non-diabetic counterparts of similar adiposity, serum triglycerides, high-density lipoprotein cholesterol, level of habitual physical activity, and in adolescents, pubertal stage. Reduced insulin sensitivity is thought to contribute both to the initiation and progression of macro- and microvascular complications in type 1 diabetes. There are currently clinical trials underway examining the benefits of improving insulin sensitivity with regards to vascular complications in type 1 diabetes. Reduced insulin sensitivity is an increasingly recognized component of type 1 diabetes, is implicated in the pathogenesis of vascular complications and is potentially an important therapeutic target to prevent vascular complications. In this review, we will focus on the pathophysiologic contribution of insulin sensitivity to vascular complications and summarize related ongoing clinical trials.

Key words: Type 1 diabetes; Insulin sensitivity; Vascular complications; Hyperfiltration; Cystatin C; Creatinine; Glomerular filtration rate

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Core tip: Adolescents and adults with type 1 diabetes have reduced insulin sensitivity compared to their non-diabetic counterparts. Reduced insulin sensitivity is implicated in the development and progression of micro and macrovascular complications in type 1 diabetes. Clinical trials are underway investigating

Abstract

Despite improvements in glucose, lipids and blood

insulin sensitivity as a therapeutic target to prevent vascular complications in type 1 diabetes. Methods are needed to identify which patients with type 1 diabetes would benefit from treatment of insulin resistance and translation of this to clinical practice.

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INTRODUCTION

The public health burden of type 1 diabetes (T1D), a disease affecting approximately 1.4 million people in the United States and 30 million globally^[1], is progressively increasing, largely due to the prevalence of the associated macro- and microvascular complications^[1-3]. Macrovascular disease in the form of coronary artery disease (CAD) is the major cause of morbidity and mortality in patients with T1D^[3-7]. Annually, up to 1%-2% of young adults (28-38 years of age) with T1D develop CAD^[3-5,8,9]. By their mid-forties, over 70% of men and 50% of women with T1D develop measureable coronary artery calcification (CAC) by computed tomography (CT) scan^[3-5,10,11] - a marker of significant atherosclerotic plaque burden. In addition to macrovascular disease, microvascular disease continues to cause morbidity and mortality; for example, diabetic nephropathy remains the leading cause of end-stage renal disease in the United States^[12-14], and diabetic retinopathy, another form of microvascular disease, is the single most common cause of new-onset blindness^[15].

Despite significant improvements in conventional risk factors (*e.g.*, blood pressure, glucose and lipid control) during the past two decades, vascular complications remain a major concern in T1D^[14,16-19]. There is a need for improved methods to identify people at risk for, and prevent the development and progression of, these complications.

Adolescents and adults with T1D demonstrate reduced insulin sensitivity, even when compared to non-diabetic counterparts of similar adiposity, body fat distribution, serum triglycerides, high-density lipoprotein cholesterol, level of habitual physical activity, and in adolescents, pubertal stage^[17,20-25]. In addition to the insulin resistance documented historically in adolescents with very poor glycemic control^[22], more recently significant insulin resistance has been documented in adolescents and adults with T1D, despite modern advances in technology and better glycemic control^[20]. Increasing rates of obesity in T1D most likely also contribute to impaired insulin sensitivity^[26]. Moreover, a subset of participants in The Diabetes Control and Complications Trial (DCCT) in the intensive treatment arm experienced greater weight gain and worse CVD profiles, which may suggest that lowering

HbA1c is not without potential negative effects^[27].

The role of insulin sensitivity in the development and progression of macro-^[28-30] and microvascular complications^[31] in T1D is increasingly recognized. Prospective studies are needed to test the hypothesis that reduced insulin sensitivity is a unifying risk factor for the development of both micro- and macrovascular complications. However, there is increasing evidence implicating reduced insulin sensitivity in the pathogenesis of vascular complications in T1D^[26,32]. It is therefore important to better understand the role of insulin sensitivity in the development of micro- and macrovascular complications in T1D to enable us to intervene early in the pathophysiologic course to halt or slow progression. Accordingly, in this review, we examine the current evidence addressing insulin sensitivity and vascular complications in T1D.

PATHOPHYSIOLOGY OF REDUCED INSULIN SENSITIVITY IN T1D

Historically, when glycemic control was poorer, reduced insulin sensitivity in people with T1D was thought to be solely related to adiposity and high HbA1c^[22,33], but recent data have challenged this assumption and suggest that reduced insulin sensitivity cannot simply be explained by excess weight or poor glycemic control^[20,21]. In fact, insulin resistance in multiple tissues has recently been documented in T1D subjects with glycemic control much improved from the pre-DCCT era ($7.5\% \pm 0.9\%$ and $8.6\% \pm 1.6\%$ in adults and adolescents) and with BMI similar to that of non-diabetic individuals^[20,21]. These data suggest that resistance to insulin's action on glucose utilization, hepatic glucose release and non-esterified fatty acid suppression is also mediated by factors beyond prevailing adiposity or glycemia^[20,21].

The exact mechanism of reduced insulin sensitivity in T1D is poorly understood. Various hypotheses exist to explain reduced insulin sensitivity in T1D^[34,35], including prolonged peripheral exposure to supraphysiologic levels of exogenous insulin, weight gain in part caused by intensive insulin therapy, and genetic and environmental factors that contribute to type 2 diabetes (T2D)^[26,34,35] (Figure 1). Another proposed mechanism includes lack of delivery of insulin to the portal circulation, causing reduced insulin delivery to the liver, and subsequent lower hepatic IGF-1 production, and lower feedback inhibition to the pituitary leading to higher growth hormone levels, which are known to induce insulin resistance^[36] (Figure 1). Abnormal glucagon regulation leading to increased hepatic glucose output has also been implicated in insulin resistance^[36]. Another possible mechanistic pathway linking reduced insulin sensitivity to vascular complications in T1D is *via* insulin's effects on overall non-essential fatty acid (NEFA) exposure and lipotoxicity in development of macro- and microangiopathy^[20,34,35,37]. Finally, ectopically accumulated fat and its catabolites have been proposed to induce insulin resistance *via* the effects of various signaling

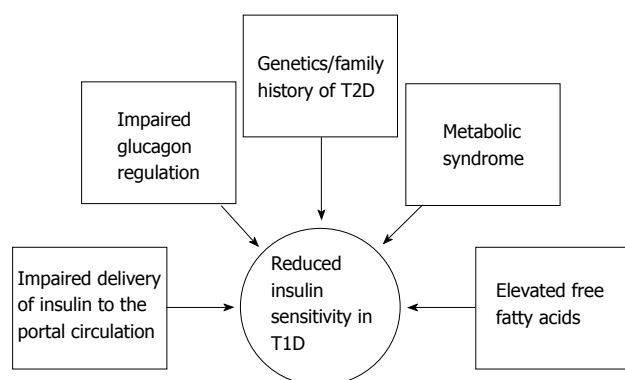


Figure 1 Possible mechanisms of reduced insulin sensitivity in type 1 diabetes. T1D: Type 1 diabetes; T2D: Type 2 diabetes.

pathways including MAPK, protein kinase C, I κ B kinases, S6 kinases and ER stress on GLUT4, although the role of ectopic fat remains controversial^[38-40] (Figure 1).

There are also robust data demonstrating that subjects with T1D with greater degrees of insulin resistance and/or family history of T2D are at greater risk of micro- and macrovascular complications^[41-44] (Figure 1). Furthermore, a high prevalence of metabolic syndrome (38% in men and 40% in women) has been reported in adults with T1D^[45]. A close relation between serum uric acid (SUA), insulin sensitivity and metabolic syndrome in non-diabetic subjects has been shown^[46]. Conversely, in adolescents and adults with T1D we demonstrated very weak associations between SUA and estimated insulin sensitivity^[47].

CURRENT METHODS OF MEASURING AND ESTIMATING INSULIN SENSITIVITY IN T1D

Although insulin resistance is recognized as an important component of vascular complications in T1D, none of the guidelines make specific recommendations about when or how to test for insulin resistance. The insulinopenic nature of T1D prohibits the use of the IV glucose tolerance test (IVGTT) and oral glucose tolerance test (OGTT)-based methods to estimate insulin sensitivity. The gold standard measurement of insulin sensitivity in T1D, glucose disposal rate (GDR) from a hyperinsulinemic-euglycemic clamp, is too cumbersome for use in routine clinical care, but newer insulin sensitivity estimation equations (estimated GDR, eGDR), demonstrate strong agreement with measured insulin sensitivity and offer promise in the clinical setting^[11]. Insulin sensitivity prediction equations from the SEARCH Study (eIS-SEARCH)^[11], the Pittsburgh Epidemiology of Diabetes Complications Study (eIS-EDC)^[48], and the Coronary Artery Calcification in Type 1 diabetes study (eIS-CACTI)^[20,31,47] are available, with others currently under development. The eIS-CACTI model includes waist circumference, daily insulin dose per kg body weight, triglycerides and DBP: $\exp(4.1075 -$

$0.01299 \times \text{waist}(\text{cm}) - 1.05819 \times \text{insulin dose [daily units per kg]} - 0.00354 \times \text{triglycerides (mg/dL)} - 0.00802 \times \text{DBP (mm Hg)}$). The eIS-CACTI explains 63% of the variance in the GDR in hyperinsulinemic-euglycemic clamp studies, and has been validated in adolescent and adult cohorts with and without T1D^[20,31,47]. There remains a need for a commonly accepted measure to estimate insulin sensitivity to be used in the clinical setting to identify patients who would benefit from therapies to improve insulin sensitivity.

INSULIN SENSITIVITY AND MICROVASCULAR COMPLICATIONS

The association between insulin sensitivity and microvascular complications is increasingly recognized, but it is not a recent discovery. In 1968, Martin *et al.*^[49] showed that microvascular complications were associated with insulin resistance in long-standing T1D subjects. In 1993, Yip *et al.*^[50] explored insulin resistance as an underlying factor in T1D and found reduced insulin sensitivity, measured by GDR, in a small group with microalbuminuria, while Orchard *et al.*^[48] later demonstrated that eGDR predicted overt nephropathy in T1D subjects in the EDC cohort. Finally, we and others have reported that higher estimated insulin sensitivity at baseline predicts lower odds of developing diabetic retinopathy, proliferative diabetic retinopathy and diabetic neuropathy independent of other established risk factors^[32,48,51]. We have also previously demonstrated that reduced estimated insulin sensitivity predicted incident microalbuminuria and rapid glomerular filtration decline by cystatin C over 6 years^[31] and that increased insulin sensitivity predicted regression of albuminuria in adults with T1D^[32], similar to data in the EDC study^[48,52].

Mechanisms underlying insulin sensitivity and renal pathology remain unclear, but the association between reduced insulin sensitivity and hemodynamic changes in the kidney is increasingly recognized^[13]. A growing body of data demonstrates that insulin resistance is associated with an elevation of glomerular hydrostatic pressure causing increased renal vascular permeability and ultimately glomerular hyperfiltration^[13,53-60]. Another possible mechanistic pathway linking insulin resistance to diabetic nephropathy is *via* effects on overall non-esterified fatty acid exposure and lipotoxicity, leading to the development of angiopathy^[36].

Markers of reduced insulin sensitivity in adults with T1D have also been linked with increased risk of diabetic retinopathy and proliferative diabetic retinopathy in the EURODIAB Prospective Complications Study^[61]. However, the investigators in EURODIAB included surrogates for insulin sensitivity including triglycerides and waist-to-hip ratio rather than measured or validated estimates of insulin sensitivity^[61]. In the DCCT/EDIC study, higher estimated insulin sensitivity using the Pittsburgh eGDR equation was associated with a lower risk for retinopathy in both the conventionally treated and intensively treated groups, though not independent of HbA1c^[30]. Some

studies in adults with T2D demonstrate an association between reduced measured insulin sensitivity and diabetic retinopathy^[62,63], but there is a need for more data in T1D. There are also limited data on insulin sensitivity and diabetic neuropathy in T1D, with only a single small study showing an association between estimated insulin sensitivity and diabetic neuropathy in adults with T1D^[64]. In contrast, the association between reduced insulin sensitivity and diabetic neuropathy is well recognized in adults with T2D^[65-67].

INSULIN SENSITIVITY AND MACROVASCULAR COMPLICATIONS

In the general population, insulin resistance has been implicated as an important contributor to accelerated atherosclerosis^[68,69]. In the CACTI study, a longitudinal cohort study of adults with T1D designed to investigate the determinants of early and accelerated atherosclerosis in T1D, insulin sensitivity independently predicted CAC^[20,70]. Data from EDC also demonstrated strong associations between insulin sensitivity and coronary artery disease in adults with T1D^[30]. In the DCCT, excess weight gain was associated with insulin resistance, as well as more extensive atherosclerosis during EDIC^[43]. Moreover, we have also previously shown associations between insulin resistance and cardiac and exercise dysfunction in adolescents with T1D^[21]. Renal health, which is known to be associated with better insulin sensitivity, is also strongly associated with higher peak exercise capacity in adolescents with T1D^[52]. Finally, we have shown that higher estimated insulin sensitivity in adolescents with T1D is inversely associated with CVD risk factors^[44].

CLINICAL TRIALS

Modification of insulin sensitivity therefore holds promise as a therapeutic target to reduce vascular complications in T1D, since both life style changes (diet and exercise) and drugs such as metformin can improve insulin sensitivity in other populations. Metformin is an inexpensive and well tolerated medication. The most common adverse effects associated with metformin are gastrointestinal, with anemia due to vitamin B12 malabsorption and lactic acidosis being rare events when used in T2D^[71]. In T2D, metformin is widely considered to protect against cardiovascular complications^[71,72]. In contrast, metformin is not advocated in any major national or international guidelines for the management of T1D^[72]. In a recent meta-analysis of randomized trials, Vella *et al.*^[72] found that metformin therapy in T1D was associated with reduced insulin-dose requirements but no clear evidence for an improvement in glycemic control was demonstrated^[72]. Moreover, Nadeau *et al.*^[73] recently showed that low-dose metformin decreased total daily insulin doses in adolescents with T1D, likely representing improvement in insulin sensitivity^[73]. Metformin is also associated with reduced LDL cholesterol^[74], precursors of advanced

glycation end production^[75,76] and a decrease in blood pressure^[77,78] in adults with T1D and T2D. However, there are currently insufficient data on vascular outcomes and their surrogates to routinely recommend metformin therapy in adolescents and adults with T1D for improving cardiovascular outcomes. For that reason, there is a need for well-designed randomized clinical trials of sufficient size and duration to show clinical benefit of metformin. Another important consideration is the significant variation observed in adults with T2D in response to metformin^[71]. The inter-individual variation in metformin may also apply to patients with T1D. Genetic variation may be one of the important determinants of this inter-individual variation, and improved understanding of underlying genes and pathways has the potential to improve the effect of metformin on insulin sensitivity^[71].

The BARI-2D study showed no benefit of an insulin sensitizing strategy compared to insulin provision on diabetic nephropathy in older adults with coronary artery disease T2D^[79]. In contrast to most contemporary cohorts with T1D, the BARI-2D trial included older adults with T2D, with most participants already having both hypertension and hyperlipidemia. It is plausible that the vascular injuries in older adults with T2D and longstanding pathology may not be responsive to changes in insulin sensitivity. A hypothesis is that early intervention prior to establishment of vascular lesions may result in significant delay of clinical pathology as suggested by the concept of “metabolic memory” in the DCCT-EDIC study^[80-84]. Also, clinical cardiovascular disease typically does not manifest until older ages; for example, it took 17 years of follow-up for the benefits of intensive management to manifest in DCCT^[27]. Improvements in outcomes due to adjunctive therapy in the era of intensive management may be more subtle. Furthermore, long-term studies in children are lacking^[85].

The REducing With MetfOrmin Vascular Adverse Lesions in Type 1 Diabetes (REMOVAL, NCT01483560) study is an ongoing double-blind randomized clinical trial with metformin to improve insulin sensitivity in adults with T1D in an attempt to prevent vascular complications, and the Effects of Metformin on Cardiovascular Function in Adolescents With Type 1 Diabetes (EMERALD, NCT01808690) is an ongoing double-blind randomized clinical trial with metformin to evaluate if metformin will improve tissue-specific insulin resistance in T1D adolescents using the hyperinsulinemic-euglycemic clamp technique, as well as improve vascular, cardiac, exercise and muscle mitochondrial function (Table 1). The effect of Metformin on Vascular and Mitochondrial Function in Type 1 Diabetes (MeT1, NCT01813929) study is also underway which seeks to measure the effect of improving insulin sensitivity on vascular function and compliance, and mitochondrial function in adults with T1D. Metformin Therapy for Overweight Adolescents with Type 1 Diabetes (NCT01881828) being performed in the T1D Exchange clinic network seeks to evaluate the efficacy and safety of the use of metformin in

Table 1 Clinical trials investigating insulin sensitivity as a therapeutic target in type 1 diabetes

Clinical trial name(s)	Description
Metformin Vascular Adverse Lesions in Type 1 Diabetes (REMOVAL) trial (NCT01483560)	Double-blind RCT with metformin to improve insulin sensitivity in subjects with T1D in an attempt to prevent vascular complications
Effects of Metformin On Cardiovascular Function In Adolescents with Type 1 Diabetes (EMERALD) study (NCT01808690)	Double-blind RCT with metformin to evaluate if metformin will improve tissue-specific insulin resistance in T1D adolescents using the hyperinsulinemic-euglycemic clamp technique, as well as improve vascular, cardiac, exercise and muscle mitochondrial function
Insulin Clamp Ancillary Study for Assessment of Insulin Resistance (NCT02045290)	Assess if metformin will improve tissue-specific insulin resistance in type 1 diabetes using hyperinsulinemic euglycemic clamps
Metformin Therapy for Overweight Adolescents With Type 1 Diabetes (NCT01881828)	Evaluate the efficacy and safety of the use of metformin in addition to standard insulin therapy in overweight and obese children and adolescents, age 12 - < 20 yr, with type 1 diabetes for at least 1 yr
Effect of Metformin on Vascular and Mitochondrial Function in Type 1 Diabetes (MeT1, NCT01813929)	Measure the effect of insulin sensitivity vascular function and compliance, and mitochondrial function in T1D

RCT: Randomized clinical trial; T1D: Type 1 diabetes.

addition to standard insulin therapy in overweight and obese children and adolescents, age 12 - < 20 years, with type 1 diabetes for at least 1 year. Furthermore, the insulin Clamp Ancillary Study for Assessment of Insulin Resistance (NCT02045290) is an associated study underway to evaluate if metformin will improve tissue-specific insulin resistance in obese T1D adolescents using hyperinsulinemic-euglycemic clamp technique (Table 1). Smaller metformin trials are also underway. Additional studies are also needed to assess the impact of other drugs that influence insulin sensitivity in T2D, including glucagon-like peptide-1 analogues, dipeptidyl peptidase-4 inhibitor, sodium glucose co-transporter 2 inhibitors, thiazolidinediones and bromocriptine, which may have similarly beneficial effects in T1D.

DIET AND EXERCISE

Diet and exercise are important modifiable risk factors in the development of insulin resistance and vascular complications in T1D^[85]. We have previously reported that adults with T1D consume higher levels of fat and saturated fat than their non-diabetic peers, and that the high intake of fat is associated with risk factors for coronary heart disease^[86,87]. Although studies suggest that nutrition influences vascular complications in adults with T1D^[85,88], it remains inconclusive whether insulin resistance or specific nutrients are responsible for this association. The ADA and ISPAD both emphasize incorporation of fruits, vegetables, whole grains, and low-fat food choices^[89,90], but further studies related to insulin sensitivity are required. Adolescents with T1D also appear to be more sedentary and less fit than their non-diabetic counterparts^[91]. Higher physical fitness among youth with T1D is associated with lower HbA1c^[85,91]. In a study of overweight and sedentary nondiabetic children, 3 mo of aerobic training provided dose-response benefits for insulin resistance compared with usual physical activity^[92]. Moreover, small T1D studies have demonstrated increased fitness and decreased total daily insulin dosage with aerobic and strength training,

compared with normal daily activities^[93]. Finally, higher levels of fitness reduced the mortality risk associated with diabetes and CVD in older adult men with diabetes, but those with T1D *vs* T2D were not analyzed separately^[94]. Moreover, higher levels of energy expenditure (due to a more active lifestyle) were found to be associated with increased cardiorespiratory fitness in a small study of T1D adults, but not necessarily better glycemic control^[95]. Therefore, while physical activity appears very promising to improve insulin resistance and reduce CVD, definitive trials in T1D are still required.

CONCLUSION

One of the major challenges in preventing vascular complications in T1D relates to the accurate identification of high risk patients at an early stage when pathology may be amenable to intervention. A promising potential therapeutic target is insulin sensitivity. Reduced insulin sensitivity is well documented in both adolescents and adults with T1D, and is thought to contribute both to the initiation and progression of macro- and microvascular complications. Novel insulin sensitivity equations derived from easily identified risk factors (*e.g.*, waist circumference and insulin dose) may allow the clinician to estimate insulin sensitivity in the clinical setting. Interventions to improve insulin sensitivity, including diet, exercise and insulin sensitizing medications, are potential therapies to supplement conventional therapies in reducing vascular complications in T1D, but further study is required. Finally, translation of insulin sensitivity into clinical practice as a therapeutic target to reduce vascular complications requires investment in adequately powered clinical trials designed to capture important long-term vascular outcomes.

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WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Utility of different glycemic control metrics for optimizing management of diabetes

Klaus-Dieter Kohnert, Peter Heinke, Lutz Vogt, Eckhard Salzsieder

Klaus-Dieter Kohnert, Peter Heinke, Eckhard Salzsieder,
 Institute of Diabetes "Gerhardt Katsch", D-17495 Karlsburg,
 Germany

Lutz Vogt, Diabetes Service Center, D-17495 Karlsburg,
 Germany

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Correspondence to: Klaus-Dieter Kohnert, MD, PhD, Institute of Diabetes "Gerhardt Katsch", Greifswalder Str. 11a, D-17495 Karlsburg, Germany. kohnert@diabetes-karlsburg.de

Telephone: +49-383-5568406

Fax: +49-383-5568444

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complications, recent studies have revealed that this metric has some limitations; it conveys a rather complex message, which has to be taken into consideration for diabetes screening and treatment. On the basis of recent clinical trials, the relationship between HbA1c and cardiovascular outcomes in long-standing diabetes has been called into question. It becomes obvious that other surrogate and biomarkers are needed to better predict cardiovascular diabetes complications and assess efficiency of therapy. Glycated albumin, fructosamin, and 1,5-anhydroglucitol have received growing interest as alternative markers of glycemic control. In addition to measures of hyperglycemia, advanced glucose monitoring methods became available. An indispensable adjunct to HbA1c in routine diabetes care is self-monitoring of blood glucose. This monitoring method is now widely used, as it provides immediate feedback to patients on short-term changes, involving fasting, preprandial, and postprandial glucose levels. Beyond the traditional metrics, glycemic variability has been identified as a predictor of hypoglycemia, and it might also be implicated in the pathogenesis of vascular diabetes complications. Assessment of glycemic variability is thus important, but exact quantification requires frequently sampled glucose measurements. In order to optimize diabetes treatment, there is a need for both key metrics of glycemic control on a day-to-day basis and for more advanced, user-friendly monitoring methods. In addition to traditional discontinuous glucose testing, continuous glucose sensing has become a useful tool to reveal insufficient glycemic management. This new technology is particularly effective in patients with complicated diabetes and provides the opportunity to characterize glucose dynamics. Several continuous glucose monitoring (CGM) systems, which have shown usefulness in clinical practice, are presently on the market. They can broadly be divided into systems providing retrospective or real-time information on glucose patterns. The widespread clinical application of CGM is still hampered by the lack of generally

Abstract

The benchmark for assessing quality of long-term glycemic control and adjustment of therapy is currently glycated hemoglobin (HbA1c). Despite its importance as an indicator for the development of diabetic

accepted measures for assessment of glucose profiles and standardized reporting of glucose data. In this article, we will discuss advantages and limitations of various metrics for glycemic control as well as possibilities for evaluation of glucose data with the special focus on glycemic variability and application of CGM to improve individual diabetes management.

Key words: Markers of glycemic control; Hemoglobin A1c; Postprandial glucose; Risk of hyperglycemia and hypoglycemia; Continuous glucose monitoring; Glycemic variability; Glucose dynamics; Standardization; Diabetes mellitus

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Core tip: Hemoglobin A1c is the gold standard to assess glycemic control and a surrogate for diabetes-associated complications. Self-monitoring of blood glucose complements daily diabetes management but is insufficient in providing complete information on short-term changes in glucose levels induced by effects of food or antidiabetic medication. Key metrics beyond HbA1c are needed for glycemic control on a day-to-day basis as well as more advanced monitoring methods. Herein, we will review advantages and limitations of different metrics for glycemic control as well as possibilities for characterization of glucose dynamics with the special focus on glycemic variability and continuous glucose monitoring.

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INTRODUCTION

Since landmark studies have provided evidence that glycated hemoglobin (HbA1c) is linked to vascular complications of diabetes^[1,2], this biomarker of glycemia emerged as the benchmark for current diabetes management. Thus, optimal diabetes control aims to restore levels of HbA1c to as normal as possible to reduce or prevent diabetic complications. However, HbA1c has some important limitations and is a rather complex measure of hyperglycemia. It represents an indicator for overall glucose exposure, integrating fasting, preprandial as well as postprandial hyperglycemia, but their relative contribution varies with the quality of glycemic control^[3]. Apart from several medical conditions that can cause inaccurate test results, HbA1c neither captures glucose fluctuations nor does it provide any information on glucose dynamics.

Chronic sustained hyperglycemia is well known to increase the risk for micro- and macrovascular compli-

cations in type 1 as well as in type 2 diabetes. Especially postprandial/postchallenge hyperglycemia, independent of HbA1c or fasting glucose, has been associated with cardiovascular disease^[4], and this could be confirmed very recently in a post-hoc analysis of the “Effects of prandial vs fasting glycemia on cardiovascular outcomes in type 2 diabetes (HEART2D)” study^[5].

As generally accepted and laid down in the American Diabetes Association (ADA) and International Diabetes Federation (IDF) guidelines, strict glycemic control, implicating comprehensive diabetes evaluation, is needed to prevent or delay diabetes complications. Nevertheless, the outcomes of the ACCORD^[6] and ADVANCE^[7] trials have taught us that HbA1c levels should be tailored to the patients’ health status-older age and extensive comorbid conditions require less stringent targets. In the overwhelming majority of large clinical trials, HbA1c has been used to predict long-term outcomes related to morbidity and mortality in people with type 1 and type 2 diabetes, but the strength of association with macrovascular end points was weaker than with microvascular end points. Furthermore, it remains still unclear how various measures of glycemia predict diabetes complications and whether a combination of several markers might even be more strongly related to adverse outcomes than a single biomarker. A recent analysis of data from the Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complication Study by Nathan *et al*^[8] supports the suggestion of using two glycemic markers to strengthen risk prediction. Thus, it would not be surprising if in the near future a combination of shorter and longer term glycemic markers could be used to predict cardiovascular outcomes more precisely. Now, we believe that time has come to move from measurement of HbA1c to other markers, allowing for assessment of short-time and intermediate-time changes in glycemia.

Although self-monitoring of blood glucose (SMBG) is still the predominant mode of glucose monitoring, the use of advanced technology, such as continuous glucose monitoring (CGM) has shown remarkable benefits and expanded significantly during recent years. One of the major problems in utilization such systems are appropriate evaluation of the great amount of data provided by CGM and the lack of standardization.

The purpose of the present review is to give an insight into the problems of choosing the most relevant markers of glycemic control and how to evaluate CGM data properly to optimize management of diabetes in order to avoid long-term complications.

MARKERS OF GLYCEMIC CONTROL

Glycemic markers are indispensable in routine practice as well as in clinical trials to guide therapy and to investigate the efficacy of medications on patients’ glycemic control. A summary of useful glucose measures is shown in Table 1. As discussed in the following, not only do these markers cover different timeframes of glycemic control,

Table 1 Traditional and alternative markers of glycemic control

Marker	Time span of glycemic control	Ref.
Hemoglobin A1c	1-3 mo	Cohen ^[15] , 2007
Glycated serum proteins	2-3 wk	Takahashi <i>et al</i> ^[33] , 2007
1,5-Anhydroglucitol	1-2 wk	Dungan <i>et al</i> ^[43] , 2008
Glycemic variability indices	24-72 h	Rodbard ^[54] , 2009
Mean plasma glucose	24-72 h	Bergental <i>et al</i> ^[30] , 2013
Fasting plasma glucose	8-10 h	Monami <i>et al</i> ^[22] , 2013
Postprandial plasma glucose	2-4 h	Standl <i>et al</i> ^[23] , 2011

they also provide different information on glucose metabolism and may reflect different pathways.

HbA1c

HbA1c is formed by nonenzymatic glycation as adduct of glucose and the hemoglobin molecule. The HbA1c value reflects average glucose over 1-3 mo. The National Glycohemoglobin Standardization Program is the organization that evaluates, sets standards for accuracy, and certifies methods for measurement of HbA1c. Besides laboratory tests, even home monitors for patients have been approved, *e.g.*, Bayer A1cNow Selfcheck At-HomeA1c System or BioRad's Micromat™ II Hemoglobin Instrument.

HbA1c has been used as a biomarker for more than three decades as universally accepted means for monitoring glycemic control and as clinical surrogate endpoint in diabetes. In both patients with type 1 and type 2 diabetes, it is well documented that HbA1c predicts the occurrence of diabetes complications. A review by Khaw *et al*^[9] examined HbA1c as a risk predictor for cardiovascular disease and found that a 1% increment in absolute concentration of HbA1c was associated with about 10%-20% increase in cardiovascular risk^[9]. Elley *et al*^[10] confirmed in a large prospective cohort study of 48444 people with type 2 diabetes that increased HbA1c is an independent risk factor for cardiovascular disease, after adjusting for traditional risk factors. This is consistent with work by Ma *et al*^[11] who suggested from data of a retrospective study in older patients with diabetes that elevated HbA1c values are an independent predictor of complex coronary lesions. However, a very recent analysis in subjects without diabetes and cardiovascular disease obtained little additional benefit for prediction of first-onset cardiovascular disease^[12]. Prior to the Emerging Risk Factors Collaboration study^[12] large trials, such as ACCORD^[6] and ADVANCE^[7], also failed to demonstrate the ability to alter cardiovascular outcomes upon lowering HbA1c values in patients with long-standing diabetes. This is in contrast to the effects of tight glycemic control in reducing microvascular complications. As a corollary, the uncertainty around HbA1c results in relation to clinical outcomes was augmented. Moreover, deeper

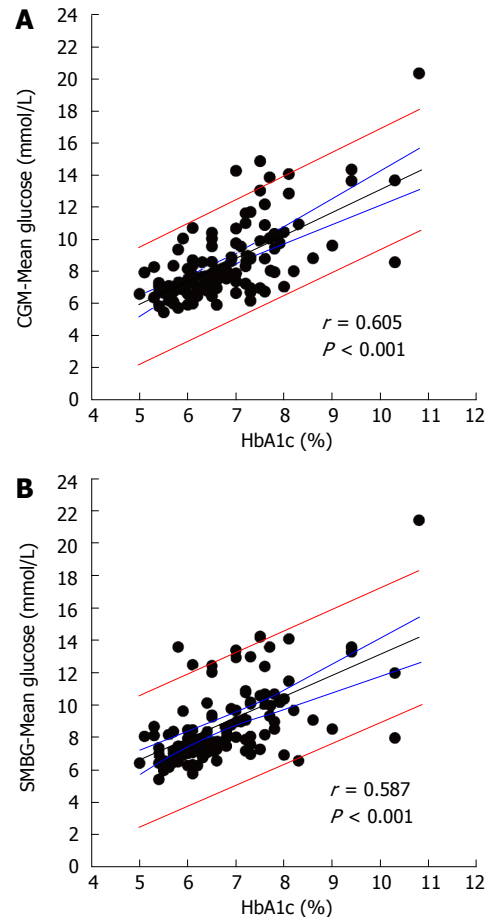


Figure 1 Relationship between hemoglobin A1c and mean glucose obtained from (A) continuous glucose monitoring and (B) self-monitoring of blood glucose in a cohort of 114 non-insulin treated type 2 diabetic patients. Medians (25th-75th percentile) for age, diabetes duration, and HbA1c were 59.0-68.0 yr, 2.0-10.0 yr, and 6.0%-7.3% (42-56 mmol/mol), respectively. The lines denote the regression lines (black), 95%CI (blue), and prediction intervals (red) (Kohnert *et al*, Unpublished data). CGM: Continuous glucose monitoring; HbA1c: Hemoglobin A1c.

insight into the pathogenesis of diabetes has disclosed important limitations of HbA1c measurement. For example, early analyses recognized that diabetic patients with identical HbA1c values can have different mean glucose concentrations^[13,14]. The regression analysis shown in Figure 1 for a cohort of our type 2 diabetic patients demonstrates that although the regression coefficients between HbA1c and mean glucose obtained either from CGM or concurrent SMBG measurements are similar (Kohnert *et al*, Unpublished); wide variations in the relationship among and within the patients can be seen. In a minority of patients such mismatch might partly be explained by unequal temporal distribution of glucose sampling, but more importantly, there are studies to provide evidence that this observation is due to differences in intracellular glycation rates^[15]. It appears that glycation of hemoglobin is not simply a concentration-dependent process, and factors other than glucose are likely to be involved. Moreover, conditions that could interfere with HbA1c measurement, causing erroneous values, are high red cell turnover, hemolytic anemia, blood

transfusion, chronic renal or liver disease^[16], and drug treatment. Under these circumstances, HbA1c cannot be used as a glucose control measure; and alternative markers should be considered. The most important limitation of HbA1c is its inability to predict hypoglycemia and to capture short-term changes of glycemia. Furthermore, we have previously shown that in well-controlled patients with type 2 diabetes, HbA1c is mainly determined by chronic sustained hyperglycemia; glycemic fluctuations go undetected^[17]. However, this is critical for safe and timely adjustment of insulin administration and clinical decision making. Thus, there has been increasing interest in additional markers for better glycemic control over shorter timeframes. The markers in question, however, may have specific characteristics and are not equally suited for diabetes management.

Fasting glucose, postprandial glucose, and mean glucose

In contrast to HbA1c, estimation of glucose exposure for specific time periods overnight or 2 to 4 h postprandial may be useful in monitoring effects of food, exercise, or antidiabetic medications. Thus, fasting glucose (FPG) and postprandial glucose (PPG) provide an acute assessment of glycemia. However, in their original work on the relationship between FPG and PPG, Monnier *et al.*^[9] have shown that the relative contribution of these measures changes with increasing HbA1c values^[3] and worsening of the metabolic situation is indicated by loss of postprandial glycemic control^[18]. According to the ADA Standards of medical care in diabetes - 2014^[19], FPG values of ≥ 7.0 mmol/L and 2-h plasma glucose (2hPG) of ≥ 11.1 mmol/L are considered criteria for the diagnosis of diabetes. Among a number of studies, which have examined the relationship of FPG or 2hPG to mortality, data from the Baltimore Longitudinal Study on Aging showed that FPG levels, exceeding 7.0 mmol/L increased the risk of mortality and the 2hPG added predictive power to that of FPG alone^[20]. Impaired fasting glucose emerged also as independent predictor of cardiovascular mortality in the Australian Diabetes, Obesity and Lifestyle Study^[21]. A recent meta-analysis suggested that reduction of FPG was related to a decrease of cardiovascular mortality with data on PPG pointing in the same direction^[22].

Standl *et al.*^[23] have listed 14 long-term observational studies showing that elevated PPG levels increase the risk of cardiovascular disease or the occurrence of a cardiovascular event approximately threefold. By contrast, data from prospective studies on the association between PPG and cardiovascular risk in established diabetes are limited. The Diabetes Intervention study^[24] has revealed the harmful link between PPG levels >10 mmol/L and increased risk of cardiovascular events and reported that reduction below this level decreased myocardial infarction and death in type 2 diabetes. Cavelot *et al.*^[25] confirmed this association in their follow-up study, demonstrating that PPG was a stronger predictor of cardiovascular

events than fasting glucose. Data obtained from a study conducted by Esposito *et al.*^[26] showed that postmeal incremental glucose values > 2.78 mmol/L, found in two thirds of study participants, were correlated with carotid intima-media thickness. Further support for the concept of treating elevated PPG came from a post-hoc analysis of the HEART2D study^[27]. Although all these studies could not clarify, whether PPG is a real marker of cardiovascular events or a surrogate of complex metabolic processes taking place in the postprandial phase^[28], this measure appears to be helpful for assessing the meal-induced glucose excursion and efficacy of diabetes treatment. In order to reduce the risk of cardiovascular events, the ADA^[19] and IDF^[29] recommend PPG values ≤ 10.0 and ≤ 9.0 mmol/L, respectively.

When considering glucose exposure, mean glucose is the metric with which the quality of diabetes management can be judged by clinicians as well as patients at shorter intervals and more easily than with HbA1c. For this reason, an expert panel of diabetes specialists recommended mean glucose/median glucose of all readings as one of the helpful glucose metrics^[30].

Fructosamin and glycated serum proteins

In recent years, fructosamin and serum glycated proteins with shorter half-lives (14-21 and 17-20 d, respectively) than hemoglobin have been evaluated as markers of glycemia. Fructosamin is formed by attachment of the molecule primarily to albumin *via* a nonenzymatic reaction. The fructosamin assay uses a colorimetric method, is rapid, inexpensive and specific, and can be applied to measure glycation of serum proteins, principally albumin^[31]; however, there is little standardization of this test. Several studies showed good correlations between fructosamin and HbA1c and glycated albumin^[32]. Glycated albumin (GA) is a ketoamine that is formed *via* nonenzymatic glycation and has been reported to be a useful marker of shorter-term glycemic control in diabetes^[33]. It is a more rapidly responding indicator than hemoglobin, although the glycation rate for both proteins is comparable^[34]. Various methods to quantify GA are available but have not been consistently standardized-most common are affinity chromatography and enzymatic assays. Two cross-sectional studies, a Japanese and an American one, involving diabetic patients on hemodialysis^[35,36], suggested that GA is a better marker of glycemic control than HbA1c. The consistent finding of significantly lower % GA/HbA1c ratios in diabetic patients without nephropathy compared to those on dialysis indicates that HbA1c underestimates glycemic control under these circumstances. It is likely that factors such as reduced survival of red blood cells and transfusions contribute to lowering of HbA1c levels in diabetic patients on hemodialysis. GA has been found useful in neonatal and gestational diabetes to detect short-term changes in glycemia^[37,38]. Since glycated albumin was shown to be an independent variable of maximum glucose levels, it appears to be a more sensitive marker than HbA1c for glycemic excursions, as they occur during postprandial

times^[39]. This is important because postprandial glucose excursions are known risk factors for diabetic micro- and macrovascular diabetes complications. More recently, it was found that serum GA levels are higher in relation to HbA1c in diabetes patients with reduced basal pancreatic β -cell function^[40]. If in the state of postprandial hyperglycemia, indicating postprandial β -cell dysfunction, serum concentrations were found to be increased, then GA could be a useful surrogate marker for cardiovascular risk^[41]. This has not yet been confirmed by clinical trials, although the finding of elevated GA, but not HbA1c levels in patient with coronary artery stenosis points out such a relationship^[42].

1,5-Anhydroglucitol

Another analyte, 1,5-anhydroglucitol (1,5-AG), has been suggested for use as intermediate marker of glycemia to complement HbA1c measurements^[43]. It is a naturally occurring inert polyol, which represents a six-carbon chain monosaccharide with a structure similar to glucose. An automated assay named GlycoMark™ is commercially available. 1,5-AG competes with glucose for tubular re-absorption and can hence not be used as a marker for glycemic control in patients with impaired kidney function. Furthermore, it should be noted that glucose levels exceeding the renal threshold for glycosuria, *i.e.*, 10 mmol/L (180 mg/dL), lead to a rapid reduction in serum concentration of 1,5-AG^[44]. Poor glycemic control, indicated by high HbA1c values ($> 9.0\%$, > 75 mmol/mol), is therefore associated with lower not higher levels of 1,5-AG. Although this marker responds sensitively and rapidly to daily glucose excursions in patients with near or at goal HbA1c levels^[45], it can not identify hypoglycemia. Dungan *et al.*^[46] have reported that 1,5-AG varied markedly in diabetes patients despite similar HbA1c and showed that this was mainly attributable to different postprandial glucose excursions. This makes 1,5-AG superior compared to HbA1c or GA (serum fructosamine) measurements as a marker for identifying postprandial hyperglycemia. Consequently, 1,5-AG has been used to evaluate drug strategies on postprandial glycemia. Studies, including exenatide^[47], sitagliptin^[48] or biphasic insulin^[49], for example, support the usefulness of 1,5-AG as a marker to identify treatment effects on postprandial glycemic excursions that would have otherwise been missed. However, it must be emphasized that 1,5-AG is not able to determine glycemic variability.

Metrics of glycemic variability

Clinical observations in patients with type 1 and type 2 diabetes have revealed that glucose profiles can greatly differ even if patients are well-controlled. While in some patients small or moderate glucose excursions and rare hypoglycemia occur, there are marked postprandial increases with frequent hypoglycemic episodes in others. Such ups and downs in glucose levels over time, either measured within 24 h or from day to day at the same time point, reflect glycemic variability (GV) classified as

within-day and between-day variability, respectively^[50]. It was Monnier *et al.*^[51] who suggested that GV is one of the important components of dysglycemia in diabetes.

With the advent of CGM, quantification of GV gained considerable clinical importance^[52]. Numerous indices for evaluation of various aspects of GV are currently available, which have been carefully characterized by Rodbard^[53,54] and Cameron *et al.*^[55]. Although they can principally be calculated from frequently sampled SMBG data, *i.e.*, seven- or eight-point glucose profiles, it is advisable to use CGM datasets, because capturing relevant glucose peaks and nadirs requires sampling frequencies of 1-5 min. It is thus not unexpected that several studies found the magnitude of GV to depend on the sampling frequency^[56,57]. Furthermore, it is very important to clearly differentiate between indices of GV and indices of the quality of glycemic control. Measures of GV quantify short-term changes in glycemia and reflect different and specific aspects of glycemic control but should not be interchanged. Validated indices such as mean amplitude of glycemic excursions (MAGE), mean of daily difference, continuous overall net glycemic action are often used in clinical research, but they are not easy to calculate. Several computer programs have recently been developed for better handling of sampled glucose data. We previously developed a computer program to calculate MAGE^[58], and meanwhile, there is other software available, such as GlyCulator^[59] and EasyGV (www.easygv.co.uk) for computing glycemic variability indices. In order to standardize measures of glycemia and glucose data reporting, an expert panel of diabetes specialists recommended for the ease of use, familiarity, and correlation with other factors of glycemic control, the following three measures of GV: SD around the mean glucose (SD), coefficient of variation (CV), and interquartile range (IQR)^[30]. Especially, if CGM data are collected, IQR is the most reliable aggregate measure of GV, as the panel announced. Normative values for GV indices have been published by Hill *et al.*^[60] and Zhou *et al.*^[61].

In regard to the clinical relevance, it remains controversial whether GV is an independent causative or contributing factor to diabetes complications^[62]. Nevertheless, there are a few studies in patients with type 1 diabetes to suggest GV to impact on the development of microvascular complications^[63,64]. In an 11-year follow-up study, Bragd *et al.*^[65] found that GV measured by SD of blood glucose was a predictor of the prevalence of peripheral neuropathy. Moreover, Snell-Bergeron reported subclinical atherosclerosis to be associated with glucose levels and glucose SD in men with type 1 diabetes^[66]. The potential importance of GV for the development of microvascular complications has been corroborated by Soupal *et al.*^[67] in a recent cross-sectional study of type 1 diabetes patients. This study showed significantly increased values for GV indices, such as SD, CV, and MAGE, for patients with microvascular complications as compared to those without complications. In this context, it should be

noted that analysis of data from the Diabetes Control and Complications Trial showed that long-term fluctuations in glycemia expressed as SDs of HbA1c independently relate to the development of retinopathy and nephropathy^[68]. With respect to type 2 diabetes, there are more study data available than for type 1 diabetes, demonstrating close associations between GV and vascular complications^[69]. In patients with well-controlled glycemia, Zhou *et al*^[70] reported that increased MAGE is one of the risk factors for microalbuminuria. Vaduva *et al*^[71] observed increased values for several GV indices in type 2 diabetic patients with chronic kidney disease compared to those without kidney damage; and Mirani *et al*^[72] noticed glucose profiles with higher GV in insulin-treated diabetes patients on hemodialysis than in the hemodialysis-free intervals. One retrospective long-term follow-up study showed that fasting glucose variability was a risk factor for diabetic retinopathy independent of mean fasting glucose or HbA1c^[73]. Regarding macrovascular complications, Chen *et al*^[74] obtained data from a case-control study to suggest a significant association between GV and progression of atherosclerosis, as determined by measurement of carotid intima-media thickness. These latter data are consistent with the value of MAGE in predicting better than HbA1c major adverse cardiac events^[75], coronary artery disease in newly diagnosed diabetes^[76] and its severity in established type 2 diabetes^[77]. A strong argument was presented for the role of GV by the recent analysis of the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled Evaluation) trial that revealed a clear association between SD of glucose and macro- as well as microvascular events in type 2 diabetes^[78]. It should further be noted that MAGE, has been found by Rizzo *et al*^[79] to be associated with impairment of cognitive function independent from the main markers of glycemia (HbA1c, FPG, PPG); and Penckofer *et al*^[80] reported an impact of GV on mood and life quality in women with type 2 diabetes.

Finally, experimental findings and clinical observations suggesting that GV more than sustained chronic hyperglycemia induces increased oxidative stress^[81] provide sure indications that GV is involved in the development of vascular disease. Because traditional measures of GV, with the exception of % CV, are closely correlated with mean glucose, it remains difficult to define an independent role for GV in the development of diabetes complications. Nevertheless, in clinical practice, minimizing GV is important to achieve acceptable glycemic stability without increasing the risk of hypoglycemia^[82-84].

Metrics of glycemic risk

Essentially two indices, such as the average daily risk range (ADRR)^[85] and the glycemic risk assessment diabetes equation (GRADE)^[86] have been developed to grade the quality of glycemic control and to complement clinical assessment of diabetes treatment. These metrics are calculated by converting glucose values obtained from SMBG or CGM into risk scores, *i.e.*, they quantify the risk

for glycemic extremes, both hyper- and hypoglycemia. They do not measure GV *per se*, rather its consequences. Nevertheless, ADRR scores correlate with several GV indices^[60,87] and were further shown to correlate with patients' insulin sensitivity, epinephrine release^[88], and weakly with basal β -cell function (HOMA%B)^[89]. The ADRR includes the high blood glucose index and the low blood glucose index (LBGI), which quantify the risk for hyperglycemia and hypoglycemia. Among the advantages of ADRR that should be emphasized are the equal sensitivity to predict excessive hyperglycemic as well as hypoglycemic episodes and the possibility to use either SMBG or CGM data for its calculation^[90]. On the other hand, ADRR has been considered as apparently less sensitive to therapeutic effects^[87]. Nonetheless, with regard to our own research (Kohnert *et al*, unpublished data) we were able to differentiate between treatment modalities, as depicted in Figure 2. ADRR is usually reported as cutoff scores based on risk categories^[90]. Even glucose meter software programs for automatic calculation are meanwhile available. Treatment studies that have used ADRR as outcome measure are still limited in number. Patton and coauthors^[91] published a comprehensive review article on the use of ADRR in assessment research and treatment outcomes research, suggesting that adults and youths with diabetes could well benefit from monitoring their ADRR scores. However, as these authors stated, it is currently unknown to which extent ADRR is used in routine diabetes control.

GRADE has been introduced by Hill *et al*^[86]. The GRADE score is an expression of the mean GRADE value derived from any glucose profile. The percentage of time spent in a specified range can be given as %GRADE_{hypoglycemia}, %GRADE_{euglycemia}, %GRADE_{hyperglycemia}. There have been only a few studies that have used GRADE scores, mainly in comparison with GV indices. One study has shown that GRADE was significantly improved in response to unmasking of CGM glucose values^[87]; another study found GRADE scores to be reduced concomitant with lowering of GV after adjustment of therapy in patients with type 2 diabetes^[84]. Although both ADRR and GRADE indicate increased glycemic risk, it should be noted that they are only moderately correlated with one another^[87]. Nevertheless, as shown in Table 2, our data suggest that among the above metrics GRADE_{hypoglycemia} and LBGI derived from CGM data are superior in estimating the risk of hypoglycemia (Kohnert *et al*, unpublished data).

Metrics of glucose dynamics

Regulation of glucose concentration is a complex process that is linked with several ultradian rhythms. Even though certain aspects of the failing glucoregulation observed in the development and progression of diabetes may be assessed by classical indices of GV, they do not include a time component^[92]. The metrics of GV described above may thus give information about the extent of excursions, yet information about glucose dynamics is not

Table 2 Linear regression relating hypoglycemia as dependent variable with measures of glycemic control as independent variables in type 2 diabetes

Asymptomatic hypoglycemia	Measure	R ²	P value
Time (h/d) spent < 3.9 mmol/L	GRADE _{HYP}	0.734	< 0.001
	LBGI	0.471	< 0.001
	% CV	0.293	< 0.001
	HbA1c	0.048	0.02

Data analyzed from 114 patients treated with diet and oral antidiabetic drugs. GRADE_{HYP}: Glycemic risk assessment diabetes equation hypoglycemia; LBGI: Low blood glucose index; % CV: Percent coefficient of variation (Kohnert *et al*, Unpublished data).

sufficiently provided, *i.e.*, how the glucoregulatory system moves from one state to another over time. In other words, GV indices are not suitable to gain deeper insight into regulatory dynamics. Various analytical methods have been used for indicating the range of glycemic dynamics in nondiabetic and diabetic patients associated with typical disease conditions. Time-series analysis techniques provide an approach to discover changes in glucose dynamics. Thus, autocorrelation function has been applied to glucose time series analysis in nondiabetic and type 1 diabetic individuals^[93], but is difficult to exploit in type 2 diabetes due to the largely nonstationary data sequence. Utilizing detrended fluctuation analysis (DFA), Churrua *et al*^[94] and Yamamoto *et al*^[95] observed a loss of glucose profile complexity, as detected by the short- and long-term scaling exponent α_1 and α_2 , in the progression from normoglycemia to impaired glucose tolerance to overt diabetes. Ogata *et al*^[96] have reported that increasing long-range DFA scaling exponents reflect abnormalities in glycemic control. Interestingly, they found that the MAGE was correlated only to the DFA long-range scaling exponent α_2 in patients with diabetes. According to Khovanova *et al*^[97], glucose profile dynamics can be defined by three complementary characteristics: nonstationarity (DFA exponent α), linear predictability (autocorrelation coefficient γ), and amplitude of variation (SD of glucose). Kovatchev *et al*^[98] and Molnár *et al*^[99] introduced the Poincaré plot time series analysis tool to acquire temporal glycemic variability from CGM data. The primary method defines short-term and long-term variability, corresponding to the length of the minor SD1 and major SD2 axes of the plot. In his recent work, Crenier^[100] extended Poincaré plot quantification by introducing and validating new partial Poincaré plot metrics, *e.g.*, area and shape of the fitting ellipse calculated at specific time points. While the majority of these metrics closely correlated with classical indices of GV, the shape index did not, indicating that the Poincaré plot captures many types of variability. One may speculate that in order to solve the question of whether GV is an independent contributor to the development of diabetes complications, analysis at multiple time scales would provide a better approach than use of classical indices. Indeed, in a recent cross-sectional, observational study,

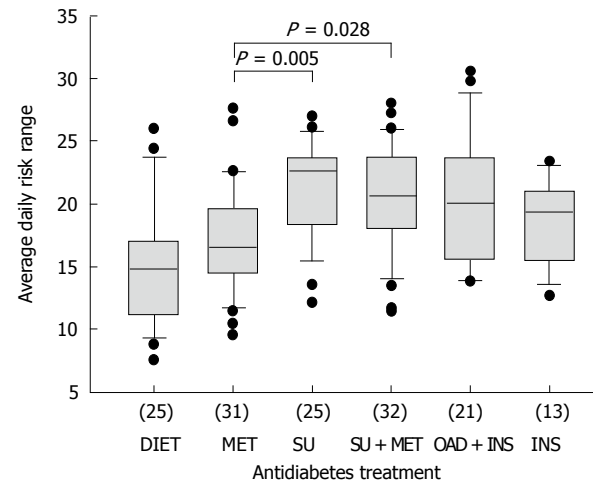


Figure 2 Differentiation between treatment groups of type 2 diabetic patients using the average daily risk range scores. Sample size for each group is given in parenthesis. Between-treatment group differences were evaluated by one-way analysis of variance and are statistically significant ($P < 0.001$). MET: Metformin; SU: Sulfonylurea; OAD: Oral antidiabetic drugs; INS: Insulin (Kohnert *et al*, Unpublished data).

Cui *et al*^[101] introduced Multi-Scale glycemic variability for analysis of CGM data at multiple time scales. They identified five unique ultradian GV cycles that modulate glucose over time ranges of 0.5 to 12 h and showed that greater GV within these cycles was associated with detrimental changes in brain morphology and function.

Biomarkers and surrogate biomarkers for diabetes complications

It is agreed upon that chronic sustained hyperglycemia represents one of the today's most important surrogate biomarker for development of microvascular diabetes complications. In addition to markers of glycemia, several novel biomarkers have been identified, capable of predicting onset or progression of nephropathy in type 2 diabetes. In a recent systematic review, Hellemons *et al*^[102] assessed the validity of such biomarkers and found, for example, that serum interleukin 18, urinary ceruloplasmin, immunoglobulin G, and transferrin were valid markers to predict onset of diabetic nephropathy. Vascular cell adhesion molecule 1, interleukin 6, von Willebrand factor, and intercellular adhesion molecule 1 were identified as markers for progression of nephropathy. Although a number of circulating (*e.g.*, high sensitive C-reactive protein, brain natriuretic peptide), genetic, and imaging biomarkers (*e.g.*, carotid intima-media thickness) are significantly related with cardiovascular risk, their predictive power for individuals is restricted. The relationship of hyperglycemia with macrovascular disease is not as clear as with microvascular complications. Since large clinical trials^[6,7] failed to provide convincing evidence that HbA1c is a reliable surrogate, adequate markers for cardiovascular outcomes in diabetic individuals with longer disease duration are not yet available^[103]. The uncertainty related to cardiovascular

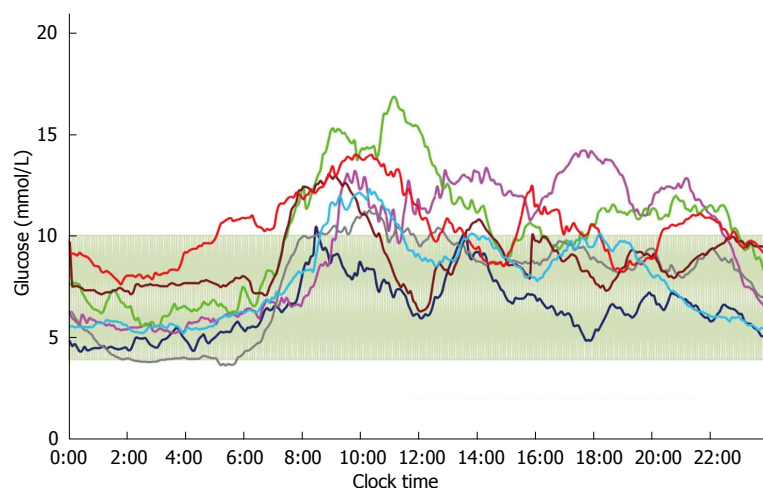


Figure 3 Continuous glucose monitoring traces from seven patients with an HbA1c value of 6.5% selected from the type 2 diabetes cohort treated with oral antidiabetes drugs. Average 24-h glucose profiles are shown. Shading indicates the glucose target range 3.9-10.0 mmol/L (modified from Kohnert *et al*, Bull Karaganda University 2013; 72: 6-15).

disease led to the release of the new recommendations on evaluating cardiovascular risk in drugs intended to treat type 2 diabetes^[104] by the United Kingdom Food and Drug Administration. Given the complexity of diabetes, it is conceivable that no single biomarker can indicate the risk of complications or disease progression. New technologies, including metabolomics, proteomics, and genomics have the potential to unravel the pathogenesis of diabetes and put forward new concepts for the development of biomarkers beyond impaired glucose regulation.

GLUCOSE MONITORING

The development of hand-held blood glucose meters some decades ago made it possible for diabetes patients to monitor their own blood glucose levels at any time in a convenient way and enabled adjustment of therapy. With the universal availability of glucose meters, SMBG found broad application for management of glycemic control. However, this traditional monitoring usually measures single glucose values at a time point, which is determined by the user; it provides only a snapshot of the whole glucose picture and rapid changes occurring between single measurements escape detection. Introduction of the CGM technology presented a great step forward toward modern diabetes management, because it overcomes limitations of traditional SMBG by producing glucose profiles instead of distinct measurements over several days, real-time glucose values, glucose trends and warnings when glucose values approach dangerously low or high levels. CGM recordings also provided evidence that diurnal glucose patterns may considerably differ in individual patients, even at identical HbA1c levels—a fact overlooked in the past. Figure 3 depicts individual average CGM profiles from a subsample of type 2 diabetes patients with identical HbA1c values. As can be seen, the profiles are quite different in that: (1) most of them exceed the target range (5%-23% glucose values above 10 mmol/L); (2) they show marked glycemic excursions (%CV 20.6-38.1); and (3) the glucose complexity long-range DFA scaling exponent α_2 varies between 1.32

and 1.54. It is conceivable that frequent use of CGM and careful pattern analysis is able to improve glycemic control by uncovering such trouble points.

Clinical study outcomes and data obtained from every-day diabetes management have shown that the use of CGM can consistently improve glycemic control^[105]. Notwithstanding that those with unstable diabetes who are prone to hypoglycemia and hypoglycemia unawareness will benefit most, the majority of diabetes patients can achieve their glucose targets when using CGM^[106]. Two variants of CGM based on sensor technology are available: retrospective and real-time glucose monitoring^[107,108]. While CGM systems such as CGMS Gold, Guardian T, Glucoday, and iPro2 were mainly designed as a tool for health care providers to collect glucose data over a sensing period of 3-7 d during which the data were masked to patients, provide real-time glucose monitors like Guardian RT, Dexcom Seven Plus, and Navigator real-time glucose values, trends, and alarms if glucose levels become high or low. The latter CGMs enable immediate therapeutic action, but require training experience for both health care practitioners and patients. However, all the above systems measure glucose subcutaneously, whereby the kinetics of the sensing process is defined by the physiology of the subcutaneous space. Glucose sensing in the peritoneal space, as recently shown, has the potential to optimize glucose monitoring because of faster intraperitoneal than subcutaneous kinetics^[109].

Even though application of CGMs has convincingly demonstrated practical utility in diabetes management, *i.e.*, food response^[110], reduction of glucose variability, time spent in hypo-/hyperglycemia, and improvement of HbA1c levels, this technology is still underutilized for a number of reasons^[30]. One of the main problems is the lack of standardized metrics and a more user-friendly presentation of data. There are currently several well-established clinical and research measures that have shown to be useful in analyzing and characterizing CGM profiles. An expert panel of diabetes specialists identified time in range as one of the key metrics for guiding diabetes treatment^[30]. This metric can be expressed either as “% of glucose readings” or “hours per day”. As the

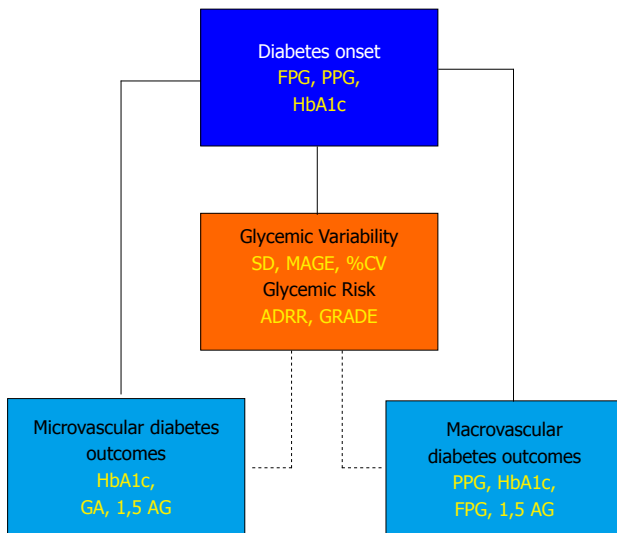


Figure 4 Glycemic Markers and Risk of Diabetic Complications. The solid lines show established relationships of the glycemic markers with microvascular and macrovascular diabetes complications; dotted lines represent possible relations with glycemic variability. FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; HbA1c: Hemoglobin A1c; GA: Glycated albumin; 1,5 AG: 1,5 Anhydroglucitol; SD: Standard deviation of plasma glucose values; MAGE: Mean amplitude of glycemic excursions; CV: Coefficient of variation; ADRR: Average daily risk range; GRADE: Glycemic risk assessment diabetes equation.

default target range, 3.9-10.0 mmol/L (70-180 mg/dL) was selected. Although this is not a “normal” range, it is commonly considered as acceptable in clinical practice. Individual targets closer to the physiological range can be defined, depending on age, comorbidities or patient compliance.

CONCLUSION

For the time being, HbA1c will remain the most important metric of long-term glycemic control, but may be supplanted by other parameters with advancing glucose monitoring technologies. Alternative metrics, such as GA and 1,5-AG can be clinically useful to assess medium- or short-term glycemic control, and in certain conditions that could interfere with HbA1c measurement. In view of the fact that many diabetes patients with apparently good glycemic control (HbA1c < 7%, < 53 mmol/mol) have high postmeal incremental glucose values, it seems warranted to integrate measurement of PPG into daily diabetes control. GV is one of the most important parameters that must not be neglected in order to optimize diabetes management. Since the known GV metrics are highly intercorrelated, any validated index can be used for evaluation of glucose fluctuations. MAGE and SD of glucose have been most commonly used; however, % CV is correlated to hypoglycemia and independent of mean glucose. ADRR as well as GRADE estimate the risk induced by high variability of glucose values and weigh low and high glucose equally. They can thus be helpful in patient care for assessments of glycemic quality. Based on our experience, we would recommend, in addition to the long-term measure HbA1c,

mean glucose and PPG as shorter-term indicators, and ADRR or GRADE for the quality of glycemic control. We would further recommend SD around the mean glucose, MAGE, and %CV as metrics of GV. Since these measures do not consider a time component Poincaré plot metrics might attract more attention to quantify short-and long-term GV and their relationship to the development of diabetes complications. For practical reasons and according to specific needs, a combination of shorter and longer term glycemic markers should be used for assessment of diabetes control to predict vascular outcomes more precisely. Finally, the control of glucose concentration is incomplete without dynamic measurements. Because of the limited available data, the utility of current metrics of glucose dynamics can not yet be judged, but they have shown promising potential to provide deeper insight into the glucoregulatory system hitherto not achieved with currently used metrics.

Since this article brings into focus metrics of glycemic control, the schematic representation in Figure 4 depicts which of these metrics may be predictive of micro- and macrovascular outcomes in diabetes. Nevertheless, it remains unclear whether glycemic variability and/or changes in glucose dynamics are implicated, but to achieve optimal glycemic control one should be aware that other factors than simply high blood glucose levels are likely to contribute to complications of diabetes. The discovery of new markers as reliable surrogates for clinical outcomes rather than simply glycemic control will advance the ability to assess the risk of complications and target treatment of diabetes.

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WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Causative anti-diabetic drugs and the underlying clinical factors for hypoglycemia in patients with diabetes

Hidekatsu Yanai, Hiroki Adachi, Hisayuki Katsuyama, Sumie Moriyama, Hidetaka Hamasaki, Akahito Sako

Hidekatsu Yanai, Hiroki Adachi, Hisayuki Katsuyama, Sumie Moriyama, Hidetaka Hamasaki, Akahito Sako, Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, Chiba 272-8516, Japan

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Correspondence to: Hidekatsu Yanai, MD, PhD, FACP, Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, 1-7-1 Kohnodai, Chiba 272-8516, Japan. dyanai@hospk.ncgm.go.jp

Telephone: +81-47-3723501

Fax: +81-47-3721858

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Abstract

Recent clinical trials indicated that the intensive glycemic control do not reduce cardiovascular disease mortality among diabetic patients, challenging a significance of the strict glycemic control in diabetes management. Furthermore, retrospective analysis of the Action to Control Cardiovascular Risk in Diabetes study demonstrated a significant association between

hypoglycemia and mortality. Here, we systematically reviewed the drug-induced hypoglycemia, and also the underlying clinical factors for hypoglycemia in patients with diabetes. The sulfonylurea use is significantly associated with severe hypoglycemia in patients with type 2 diabetes. The use of biguanide (approximately 45%-76%) and thiazolidinediones (approximately 15%-34%) are also highly associated with the development of severe hypoglycemia. In patients treated with insulin, the intensified insulin therapy is more frequently associated with severe hypoglycemia than the conventional insulin therapy and continuous subcutaneous insulin infusion. Among the underlying clinical factors for development of severe hypoglycemia, low socioeconomic status, aging, longer duration of diabetes, high HbA1c and low body mass index, comorbidities are precipitating factors for severe hypoglycemia. Poor cognitive and mental functions are also associated with severe hypoglycemia.

Key words: Comorbidity; Hypoglycemia; Insulin; Oral anti-diabetic drugs

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Core tip: The use of sulfonylurea is significantly associated with severe hypoglycemia in patients with type 2 diabetes. Biguanide and thiazolidinediones use are also highly associated with severe hypoglycemia. The intensified insulin therapy is more frequently associated with severe hypoglycemia compared with other insulin therapies. Low socioeconomic status, aging, longer duration of diabetes, high HbA1c and low body mass index, comorbidities, poor cognitive and mental function are precipitating factors for severe hypoglycemia.

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INTRODUCTION

The Diabetes Controls and Complication Trial and the United Kingdom Prospective Diabetes Study lead us to consider the strict glycemic control to prevent micro- and macro-vascular complications^[1,2]. Recent clinical trials such as Action to Control Cardiovascular Risk in Diabetes (ACCORD) presented that cardiovascular disease mortality did not decrease by the intensive glycemic control in diabetic patients^[3-5], challenging a significance of the strict glycemic control in diabetes management.

In retrospective analysis of the ACCORD study, the annual mortality among patients in the intensive and standard glucose control arms were significantly higher in patients with severe hypoglycemia (2.8% and 3.7%, respectively) than those with no episodes (1.2% and 1.0%, respectively)^[6].

Patients with diabetes treated with insulin and hypoglycemic drugs are at a greater risk of developing hypoglycemia than patients treated with only diet and exercise^[7-9]. Drug-induced hypoglycemia causes substantial morbidity and mortality, and compromises physiological and behavioral defenses against subsequent hypoglycemia, and also precludes the maintenance of glycemic control^[10-26].

Here we systematically reviewed drug-induced hypoglycemia, and the underlying clinical factors for the development in diabetic patients.

CAUSATIVE ANTI-DIABETIC DRUGS FOR HYPOGLYCEMIA

The list of published articles about the drug-induced hypoglycemia is shown in Table 1. Kim *et al.*^[27] analyzed subjects with severe hypoglycemia who were brought to the Emergency Departments (ED) between January 1, 2004 and December 30, 2009. Fifty three percent of subjects were treated by insulin. Among patients with severe hypoglycemia due to sulfonylurea (SU), the glimepiride use increased from 2004 to 2009, while the glizalazide use decreased. Among patients treated with insulin, the treatment by using long-acting insulin analogues and premixed insulin increased, while the treatment by neutral protamine Hagedorn (NPH)-insulin and regular insulin (RI) decreased. According to the accumulated data between 2004 and 2009, glimepiride (24.2%) and NPH/RI (38.3%) use were frequently associated with severe hypoglycemia.

A retrospective cohort study showed that severe hypoglycemia in patients with type 1 diabetes was almost due to

insulin, and 42.3% and 51.1% of type 2 diabetic patients were due to SU and insulin, respectively^[28]. Signorovitch *et al.*^[29] showed that the use of SU (38.2%), biguanide (56.3%) and thiazolidinediones (TZD) (14.5%) were highly associated with the development of severe hypoglycemia. Although this study did not reveal whether monotherapy or combination therapy by using biguanide induced severe hypoglycemia, this study showed that the number of patients treated with biguanide was greater than those with SU. To understand the burden of severe hypoglycemia among new users of insulin and oral anti-diabetic drugs (OAD), Moisan *et al.*^[30] conducted an inception cohort study using the databases of the Quebec health insurance board and the Quebec registry of hospitalizations between January 1, 2000 and December 31, 2008. A total of 188659 new users of anti-diabetic treatment were included. A total of 3575 (1.9%) individuals had at least 1 hypoglycemia-related ED visit. This study also showed the greater use of metformin (45.0%) as compared with SU (32.1%).

Hsu *et al.*^[31] showed that the number of insulin and SU user was significantly greater in patients with severe hypoglycemia (24.2% for insulin, 67.8% for SU) than in patients without hypoglycemia (4.35% and 54.95%, respectively).

Holstein *et al.*^[32] compared the incidences of severe hypoglycemia between 2007-2010 and 1997-2000. Severe hypoglycemia among all emergency admissions significantly increased from 0.68% in 1997-2000 to 0.83% in 2007-2010, which was associated with the intensification of anti-hyperglycemic therapy. In type 1 diabetes, severe hypoglycemia increased from 11.5/100000 inhabitants to 23.4/100000 inhabitants for ten years, and also increased in type 2 diabetes from 18.5/100000 inhabitants to 32.6/100000 inhabitants. The number of drugs had increased in type 1 and type 2 diabetes. In patients with type 1 diabetes, the number of incidence of severe hypoglycemia due to the intensified insulin therapy (IIT) increased from 64 in 1997-2000 to 96 in 2007-2010, and severe hypoglycemia due to IIT (79.3%) was more frequent compared with the conventional (6.6%) or continuous subcutaneous insulin infusion (CSII) (13.2%), in 2007-2010. In type 2 diabetes, the frequency of IIT significantly increased in 2007-2010 as compared with those in 1997-2000. Severe hypoglycemia due to SU monotherapy increased from 45 cases to 67 cases. Severe hypoglycemia due to glimepiride ($n = 65$) occurred fourfold more frequently than severe hypoglycemia due to glibenclamide ($n = 16$). Ha *et al.*^[33] also reported that glimepiride was the most frequently prescribed drug in patients with severe hypoglycemia in South Korea.

In the survey by Geller *et al.*^[34], in an estimated 22.9% of ED visits for insulin-related hypoglycemia, more than 1 type of insulin product was documented. Long-acting (32.9%) and rapid-acting (26.4%) products were the most commonly documented insulin product types. Metformin and SU were the most commonly documented concomitant OAD, identified in 50.9% (95%CI: 47.6%-54.2%) and 39.2% (95%CI: 34.8%-43.6%),

Table 1 Published articles about the drug-induced hypoglycemia in patients with diabetes

Ref.	Subjects	Year	Nation	Setting	OAD	Insulin	Combination
Kim <i>et al</i> ^[27]	Type 2 (n = 298)	2004-2009	South Korea	The Emergency Department of two general hospitals	Glimepiride (24.2%) Gliclazide (5.4%) Glibenclamide (8.4%)	NPH/RI (38.3%) Premixed (11.1%) Glargine/Detemir (13.1%) Insulin (100%)	
Tsujimoto <i>et al</i> ^[28]	Type 1 (n = 85)	2006-2012	Japan	Retrospective cohort study in one medical center		Insulin (51.1%)	
Signorovitch <i>et al</i> ^[29]	Type 2 not treated with insulin (n = 5582)	1998-2010	United States	US-based employer claims database	SU (42.3%) Others (6.6%) SU (38.2%) Biguanides (56.3%) a-GI (0.9%) Sitagliptin (1.0%) Incretin mimetics (0.5%) TZD (14.9%)		
Moisan <i>et al</i> ^[30]	Not determined (n = 3575)	2000-2008	Canada	Inception cohort study using the database of the Quebec health insurance board and the Quebec registry of hospitalizations	SU (32.1%) Metformin (45.0%) SU + Metformin (12.3%) Others (2.1%)	Insulin (8.5%)	
Hsu <i>et al</i> ^[31]	Type 2 (n = 500)	1998-2009	Taiwan	A nationwide population-based study using the National Health Insurance Research Database	SU (67.8%) Others (61.4%)	Insulin (24.2%)	
Holstein <i>et al</i> ^[32]	Type 1 (n = 92) Type 1 (n = 121) Type 2 (n = 148) Type 2 (n = 225)	1997-2000 2007-2010 1997-2000 2007-2010	German	A longitudinal population-based study	 SU (30.4%) SU (29.8%) Metformin (0.9%)	Conventional (27.2%) Intensified (69.6%) CSII (3.3%) Conventional (6.6%) Intensified (79.3%) CSII (13.2%) Conventional (52.7%) Intensified (0%) CSII (0%) Conventional (40.8%) Intensified (21.8%) CSII (0%)	 SU + Insulin (16.9%) SU + Insulin (6.7%)
Ha <i>et al</i> ^[33]	Not determined (n = 320)	2006-2009	South Korea	Retrospective analysis of hypoglycemic patients presented to emergency room of Uijeongbu St. Mary's Hospital	Glimepiride (29.7%) Glibenclamide (4.7%) Gliclazide (4.7%) Gliquidone (1.3%) Glipizide (0.9%) Others (24.7%)	Insulin (29.1%)	SU + Insulin (5.0%)
Geller <i>et al</i> ^[34]	Not determined (n = 8100)	2007-2011	United States	Nationally representative public health surveillance of adverse drug events among insulin-treated patients seeking emergency department care		Insulin (83.4%)	Insulin + Biguanide (8.5%) SU (6.6%) TZD (3.6%) DPP-4 inhibitors (1.3%) GLP-1 analogues (0.2%) Others (0.9%)
Ben-Ami <i>et al</i> ^[35]	Type 1 and 2 (n = 99)	1986-1992	Israel	Retrospective analysis of the medical record in Rambam Medical Center	Glyburide (51.5%) Glyburide + Metformin (10.2%)	Insulin (23.2%)	Insulin + Glyburide (13.1%) Insulin + Metformin (2.0%)
Quilliam <i>et al</i> ^[36]	Type 2 (n = 536581)	2004-2008	United States	Retrospective cohort designed to assess the rate and costs of hypoglycemia among working-age patients with type 2 diabetes in the MarketScan database	SU (42.3%) Metformin (75.7%) TZD (33.3%) Other oral agents (4.4%)	Insulin (6.0%) Other injectable agents (2.7%)	

Parsaik <i>et al.</i> ^[37]	Type 1 (n = 210)	2003-2009	United States	Population-based study	Simple insulin (10.0%) MDI (67.0%) CSII(18.0%)	OAD + Insulin (1.0%)
	Type 2 (n = 503)				Simple insulin (27.0%) MDI (37.0%) CSII (1.0%)	OAD + Insulin (11.0%)

a-GI: a-glucosidase inhibitors; CSII: Continuous subcutaneous insulin infusion; DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; MDI: Multiple daily insulin injection; NPH: Neutral protamine Hagedorn; OAD: Oral anti-diabetic drug; RI: Regular insulin; SU: Sulfonylurea; TZD: Thiazolidinediones.

respectively, of estimated ED visits for insulin-related hypoglycemia.

Ben-Ami *et al.*^[35] found that the glyburide use as mono-therapy (51.5%) and as combination therapy with metformin was the most frequently used drug in patients with hypoglycemic coma. Quilliam *et al.*^[36] estimated the rate and costs of hypoglycemia in patients with type 2 diabetes, by using a retrospective cohort design to assess the rate and costs of hypoglycemia among working-age patients in the 2004-2008 MarketScan database. The use of SU (42.3%), metformin (75.7%) and TZD (33.3%) were highly associated with the development of hypoglycemia. In the study among patients with type 1 diabetes by Parsaik *et al.*^[37], multiple daily insulin injection (MDI) (67.0%) was more frequently associated with severe hypoglycemia as compared with simple insulin (10.0%) and CSII (18.0%). In type 2 diabetes, MDI was also more frequently associated with severe hypoglycemia than simple insulin (27.0%), CSII (1.0%) and combination therapy with OAD (11.0%).

UNDERLYING CLINICAL FACTORS FOR HYPOGLYCEMIA

According to “Evaluation and Management of Adult Hypoglycemia Disorders: An Endocrine Society Clinical Practice Guideline”, the causes of hypoglycemia in ill or medicated adult individuals include hypoglycemia due to anti-diabetic drugs (insulin or insulin secretagogue), alcohol and drugs other than anti-diabetic agents and alcohol; critical illness (hepatic, renal and heart failure), sepsis and inanition; deficiency of cortisol, glucagon and epinephrine; non-islet cell tumor^[38]. These can also be the causes of hypoglycemia in diabetic patients. Conventional risk factors include excessive anti-diabetic drugs doses, ill-timed, or of the wrong type; decreased exogenous glucose delivery; increased glucose utilization; decreased endogenous glucose production; increased insulin sensitivity; decreased insulin clearance^[38].

Hypoglycemia occurs due to relative or absolute insulin excess and compromised physiological defenses against decrease in plasma glucose^[38-42]. The physiological defenses against decrease in plasma glucose include: reduction of insulin secretion; enhancement of glucagon and epinephrine secretion^[39,43,44], which are compromised in patients with type 1 diabetes and also patients with long duration of type 2 diabetes^[39,40,45,46]. Defective glucose counter-regulation is associated with the risk of

severe hypoglycemia^[47,48].

The list of published articles about the underlying clinical factors for hypoglycemia is shown in Table 2. Yaffe *et al.*^[49] reported that black race and low education level were significantly associated with severe hypoglycemia. Punthakee *et al.*^[50] also reported that significant associations of race and education level with severe hypoglycemia. Leese *et al.*^[51] indicated older age, a longer duration of diabetes, and a higher HbA1c as underlying clinical factors for hypoglycemic patients, which was also reported by Punthakee *et al.*^[50]. Yaffe *et al.*^[49] also suggested a significant association between severe hypoglycemia and a higher HbA1c. A lower body mass index (BMI) was also associated with the development of severe hypoglycemia^[50,51].

Punthakee *et al.*^[50] studied the association between severe hypoglycemia and cognitive function, and showed poor cognitive function is associated with severe hypoglycemia in type 2 diabetic patients. Yaffe *et al.*^[49], Hsu *et al.*^[31] and Signorovitch *et al.*^[29] also reported a significant association between mental disorders and severe hypoglycemia. Neurological disorders such as stroke and epilepsy which influence mental and cognitive functions were also associated with development of severe hypoglycemia^[29,31,50].

Heart, liver and renal functions affect pharmacokinetics and clearance of insulin and OAD. Liver cirrhosis, renal disease including diabetic nephropathy, heart diseases including cardiovascular diseases are significantly associated with severe hypoglycemia^[29,31,50]. Hsu *et al.*^[31] performed a nationwide cohort study, and suggested that comorbidities such as hypertension and renal disease are associated with hypoglycemic episodes. Signorovitch *et al.*^[29] also indicated a significant associations of hypoglycemia with comorbidities such as mental disorders and stroke. In their study, patients with hypoglycemia showed a higher Charlson comorbidity index than those without hypoglycemia.

Neuropathy is also associated with hypoglycemia^[50]. In neuropathy, especially, hypoglycemia-associated autonomic failure (HAAF) is significantly associated with the development of severe hypoglycemia^[46,52]. In patients with HAAF, in the absence of reduction of insulin secretion and enhancement of glucagon secretion, the defective glucose counter-regulation by epinephrine induces hypoglycemia unawareness by reducing the sympathetic neural activity and neurogenic symptoms^[39,40,45]. According to “Evaluation and Management of Adult Hypoglycemia Disorders: An Endocrine Society Clinical Practice

Table 2 Published articles about the underlying clinical factors for the development of hypoglycemia in patients with diabetes

Ref.	Clinical factors	Hypoglycemia	No hypoglycemia	P value
Yaffe <i>et al</i> ^[49]	Black race/ethnicity (%)	72.1	44.9	< 0.01
	Education (< high school education) (%)	36.1	24.0	0.04
	Glycated hemoglobin level (%)	8.0	7.2	< 0.01
	Prevalent diabetes mellitus (%)	85.2	47.9	< 0.01
Hsu <i>et al</i> ^[31]	MMSE score [mean (SD)]	89.6 (5.7)	91.5 (5.2)	< 0.01
	Hypertension (%)	63.6	51.2	< 0.0001
	Liver cirrhosis (%)	3.0	1.3	0.0074
	Renal disease (%)	17.4	5.2	< 0.0001
	Mental disease (%)	21.4	12.5	< 0.0001
	Cancer (%)	8.0	2.4	< 0.0001
	Stroke (%)	15.0	4.0	< 0.0001
	Heart disease (%)	13.2	3.6	< 0.0001
	Age (mean, yr)			
Leese <i>et al</i> ^[51]	Type 1 treated with insulin	37.7	32.8	0.009
	Type 2 treated with insulin	66.6	63.2	0.038
	Diabetes duration (mean, years)			
	Type 1 treated with insulin	20.7	16.7	0.013
Signorovitch <i>et al</i> ^[29]	BMI (mean, kg/m ²)			
	Type 2 treated with insulin	26.7	30.1	< 0.001
	Mental disorders (%)	15.2	11.4	< 0.001
	Neurological disorders (%)	17.2	10.7	< 0.001
	Cardiovascular disorders (%)	60.4	59.0	0.05
	Renal disorders (%)	16.5	12.3	< 0.001
	Epilepsy (%)	1.2	0.7	< 0.001
	Stroke (%)	4.9	2.9	< 0.001
	CCI [mean (SD)]	1.42 (1.70)	1.3	< 0.001
Punthakee <i>et al</i> ^[50]	Age [yr, mean (SD)]	63.91 (6.41)	62.41 (5.77)	0.002
	Female (%)	55.6	46.1	0.019
	Race			< 0.0001
	Non-Hispanic white (%)	60.0	70.9	
	African American (%)	30.0	15.4	
	Hispanic (%)	6.3	7.1	
	Others (%)	3.8	6.6	
	Education			0.01
	Less than high school (%)	16.3	12.8	
	High school graduate (%)	35.0	25.2	
	Some college (%)	26.9	35.1	
	College graduate (%)	21.9	26.9	
	BMI [mean (SD), kg/m ²]	32.08 (5.64)	33.03 (5.33)	0.029
	Diabetes duration [mean (SD) of years]	14.13 (8.74)	10.18 (7.22)	< 0.0001
	HbA1c (%)	8.46 (1.06)	8.27 (1.05)	0.021
	History of stroke (%)	11.3	4.6	0.0002
	History of cardiovascular disease (%)	41.9	28.4	0.0003
	Neuropathy score [mean (SD)]	0.53 (0.50)	0.45 (0.50)	0.049
	UACR (mg/mmol)			< 0.0001
	< 30 (%)	58.8	72.4	
	30-300 (%)	27.5	21.9	
	> 300 (%)	13.8	5.7	
	DSST score [mean (SD)]	46.45 (17.01)	52.89 (15.76)	< 0.0001
	RAVLT score [mean (SD)]	6.90 (2.72)	7.55 (2.53)	0.002
	Stroop score [mean (SD)]	37.69 (22.02)	31.66 (16.25)	< 0.0001
	MMSE score [mean (SD)]	26.83 (2.80)	27.45 (2.49)	0.002

BMI: Body mass index; CCI: Charlson comorbidity index; DSST: Digit Symbol Substitution Test; MMSE: Mini-Mental Status Exam; RAVLT: Rey Auditory Verbal Learning Test; UACR: Urinary albumin creatinine ratio.

Guideline”, risk factors for HAAF include absolute deficiency of endogenous insulin secretion; a history of severe hypoglycemia, and hypoglycemia unawareness^[38].

CONCLUSION

The use of SU is significantly associated with severe

hypoglycemia in patients with type 2 diabetes. Especially, the glimepiride-induced severe hypoglycemia (approximately 20%-30%) occurred more frequently as compared with other SU. The use of biguanide (approximately 45%-76%) and TZD (approximately 15%-34%) are also highly associated with the development of severe hypoglycemia. The study that investigated insulin product types and

Table 3 Summary of the underlying clinical factors for the development of hypoglycemia in patients with diabetes

- 1 Socioeconomic status (education, race)
- 2 Aging
- 3 State of diabetes (duration, HbA1c, body mass index)
- 4 Cognitive and mental function
- 5 Comorbidity
- 6 Failure of organ which influence on clearance of insulin and oral anti-diabetic drugs (Heart, liver, renal failure)
- 7 Hypoglycemia-associated autonomic failure

hypoglycemia is very limited. In one study in Korea, NPH/RI was more frequently associated with severe hypoglycemia as compared with premixed insulin and glargine/detemir. In diabetic patients treated with insulin, IIT is more frequently associated with severe hypoglycemia compared with conventional insulin therapy and CSII.

Summary of the underlying clinical factors for hypoglycemia is shown in Table 3. Low socioeconomic status, aging, longer duration of diabetes, high HbA1c and low BMI are precipitating factors for severe hypoglycemia. Poor cognitive and mental functions are also associated with the development of severe hypoglycemia. Comorbidities including heart, liver, renal failures are likely to induce severe hypoglycemia. We should also pay attention to HAAF which leads to very serious hypoglycemia.

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WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Literature review on the management of diabetic foot ulcer

Leila Yazdanpanah, Morteza Nasiri, Sara Adarvishi

Leila Yazdanpanah, Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 6135715794, Iran

Morteza Nasiri, Sara Adarvishi, Nursing and Midwifery School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 7541886547, Iran

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Correspondence to: Morteza Nasiri, BSc, MSc, Nursing and Midwifery School, Ahvaz Jundishapur University of Medical Sciences, Golestan road, Khozestan, Ahvaz 7541886547, Iran. mortezanasiri.or87@yahoo.com

Telephone: +98-772-6225292

Fax: +98-772-6223012

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Based on National Institute for Health and Clinical Excellence strategies, early effective management of DFU can reduce the severity of complications such as preventable amputations and possible mortality, and also can improve overall quality of life. The management of DFU should be optimized by using a multidisciplinary team, due to a holistic approach to wound management is required. Based on studies, blood sugar control, wound debridement, advanced dressings and offloading modalities should always be a part of DFU management. Furthermore, surgery to heal chronic ulcer and prevent recurrence should be considered as an essential component of management in some cases. Also, hyperbaric oxygen therapy, electrical stimulation, negative pressure wound therapy, bio-engineered skin and growth factors could be used as adjunct therapies for rapid healing of DFU. So, it's suggested that with appropriate patient education encourages them to regular foot care in order to prevent DFU and its complications.

Key words: Diabetes mellitus; Wound management; Diabetic foot ulcer; Amputation; Foot care

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Core tip: Diabetic foot ulcer (DFU) is the most common complication of diabetes mellitus that usually fail to heal, and leading to lower limb amputation. Early effective management of DFU as follows: education, blood sugar control, wound debridement, advanced dressing, offloading, advance therapies and in some cases surgery, can reduce the severity of complications, and also can improve overall quality of life of patients especially by using a multidisciplinary team approach.

Abstract

Diabetic foot ulcer (DFU) is the most costly and devastating complication of diabetes mellitus, which affect 15% of diabetic patients during their lifetime.

Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. *World J Diabetes* 2015; 6(1): 37-53 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i1/37.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i1.37>

INTRODUCTION

Diabetes mellitus (DM) is one of the main problems in health systems and a global public health threat that has increased dramatically over the past 2 decades^[1,2]. According to epidemiological studies, the number of patients with DM increased from about 30 million cases in 1985, 177 million in 2000, 285 million in 2010, and estimated if the situation continues, more than 360 million people by 2030 will have DM^[3,4].

Patients with DM are prone to multiple complications such as diabetic foot ulcer (DFU). DFU is a common complication of DM that has shown an increasing trend over previous decades^[5-7]. In total, it is estimated that 15% of patients with diabetes will suffer from DFU during their lifetime^[8]. Although accurate figures are difficult to obtain for the prevalence of DFU, the prevalence of this complication ranges from 4%-27%^[9-11].

To date, DFU is considered as a major source of morbidity and a leading cause of hospitalization in patients with diabetes^[1,5,12,13]. It is estimated that approximately 20% of hospital admissions among patients with DM are the result of DFU^[14]. Indeed, DFU can lead to infection, gangrene, amputation, and even death if necessary care is not provided^[14]. On the other hand, once DFU has developed, there is an increased risk of ulcer progression that may ultimately lead to amputation. Overall, the rate of lower limb amputation in patients with DM is 15 times higher than patients without diabetes^[8]. It is estimated that approximately 50%-70% of all lower limb amputations are due to DFU^[8]. In addition, it is reported that every 30 s one leg is amputated due to DFU in worldwide^[9]. Furthermore, DFU is responsible for substantial emotional and physical distress as well as productivity and financial losses that lower the quality of life^[15]. The previous literature indicates that healing of a single ulcer costs approximately \$17500 (1998 United States Dollars). In cases where lower extremity amputation is required, health care is even more expensive at \$30000-33500^[16]. These costs do not represent the total economic burden, because indirect costs related to losses of productivity, preventive efforts, rehabilitation, and home care should be considered. When all this is considered, 7%-20% of the total expenditure on diabetes in North America and Europe might be attributable to DFU^[17].

ETIOLOGY OF DFU

Recent studies have indicated multiple risk factors associated with the development of DFU^[18-21]. These risk factors are as follows: gender (male), duration of diabetes longer than 10 years, advanced age of patients, high Body Mass Index, and other comorbidities such as retinopathy, diabetic peripheral neuropathy, peripheral vascular disease, glycated hemoglobin level (HbA_{1c}), foot deformity, high plantar pressure, infections, and inappropriate foot self-care habits^[1,12,20-22] (Figure 1).

Although the literature has identified a number of

diabetes related risk factors that contribute to lower-extremity ulceration and amputation, to date most DFU has been caused by ischemic, neuropathic or combined neuroischemic abnormalities^[6,17] (Figure 2). Pure ischemic ulcers probably represent only 10% of DFU and 90% are caused by neuropathy, alone or with ischemia. In recent years, the incidence of neuroischemic problems has increased and neuroischemic ulcers are the most common ulcers seen in most United Kingdom diabetic foot clinics now^[23].

In total, the most common pathway to develop foot problems in patients with diabetes is peripheral sensorimotor and autonomic neuropathy that leads to high foot pressure, foot deformities, and gait instability, which increases the risks of developing ulcers^[24-26]. Today, numerous investigations have shown that elevated plantar pressures are associated with foot ulceration^[27-29]. Additionally, it has been demonstrated that foot deformities and gait instability increases plantar pressure, which can result in foot ulceration^[24,30].

MANAGEMENT OF DFU

Unfortunately, often patients are in denial of their disease and fail to take ownership of their illness along with the necessary steps to prevent complication and to deal with the many challenges associated with the management of DFU. However, numerous studies have shown that proper management of DFU can greatly reduce, delay, or prevent complications such as infection, gangrene, amputation, and even death^[6,31,32].

The primary management goals for DFU are to obtain wound closure as expeditiously as possible^[33,34]. As diabetes is a multi-organ systemic disease, all comorbidities that affect wound healing must be managed by a multidisciplinary team for optimal outcomes with DFU^[35-38]. Based on National Institute for Health and Clinical Excellence strategies, the management of DFU should be done immediately with a multidisciplinary team that consists of a general practitioner, a nurse, an educator, an orthotic specialist, a podiatrist, and consultations with other specialists such as vascular surgeons, infectious disease specialists, dermatologists, endocrinologists, dieticians, and orthopedic specialists^[39]. Today, numerous studies have shown that a multidisciplinary team can reduce amputation rates, lower costs, and leads to better quality of life for patients with DFU^[39-41]. The American Diabetes Association has concluded that a preventive care team, defined as a multidisciplinary team, can decrease the risks associated with DFU and amputation by 50%-85%^[42]. It's suggested that with applying this approach take appropriate strategies for management of DFU to consequently reduce the severity of complications, improve overall quality of life, and increase the life expectancy of patients^[30]. In this article, we review available evidence on the management of DFU as follows: education, blood sugar control, wound debridement, advanced dressing,

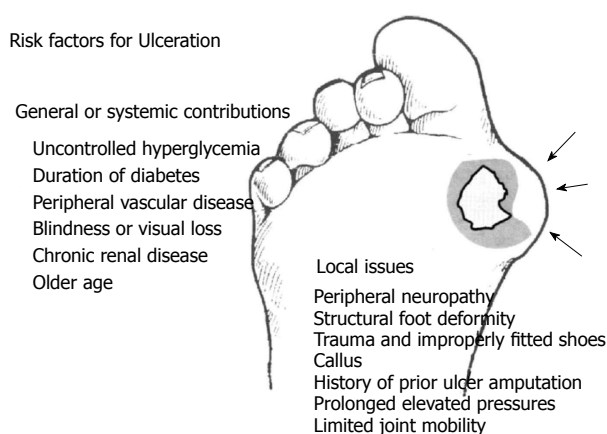


Figure 1 The risk factors for diabetic foot ulcer. Ulcers may be distinguished by general or systemic considerations vs those localized to the foot and its pathology. (Data adapted from Frykberg *et al*^[18]).

offloading, surgery, and advanced therapies that are used clinically.

RESEARCH

In this review article, we searched for articles published between March 1, 1980 and July 28, 2014 in the following five electronic databases: PubMed, Science Direct, Embase, Web of Science, and Scopus, for both English and non-English language articles with the following keywords: “diabetic foot ulcer”, “amputations”, “wound management”, “debridement”, “advanced dressings”, “offloading modalities”, “hyperbaric oxygen therapy”, “electrical stimulation”, “negative pressure wound therapy”, “bio-engineered skin”, “growth factors”, and “foot care” as the medical subject heading (MeSH). Study designs that were included were randomized controlled trials (RCTs), case-control studies, cohort studies, prospective and retrospective uncontrolled studies, cross-sectional studies, and review studies. Case reports and case series were excluded. We searched bibliographies for all retrieved and relevant publications to identify other studies.

Education

It has been shown that up to 50% of DFU cases can be prevented by effective education. In fact, educating patients on foot self-management is considered the cornerstone to prevent DFU^[12,43-45].

Patient education programs need to emphasize patient responsibility for their own health and well-being. The ultimate aim of foot care education for people with diabetes is to prevent foot ulcers and amputation. Currently, a wide range and combinations of patient educational interventions have been evaluated for the prevention of DFU that vary from brief education to intensive education including demonstration and hands-on teaching^[46]. Patients with DFU should be educated about risk factors and the importance of foot care,

including the need for self-inspection, monitoring foot temperature, appropriate daily foot hygiene, use of proper footwear, and blood sugar control^[47]. However, education is better when combined with other care strategies, because previous reviews on patient education has suggested that when these methods were combined with a comprehensive approach, these methods can reduce the frequency and morbidity of the limb threatening complications caused by DFU^[48].

Blood sugar control

In patients with DFU, glucose control is the most important metabolic factor. In fact, it is reported inadequate control of blood sugar is the primary cause of DFU^[6,49,50].

The best indicator of glucose control over a period of time is HbA_{1c} level. This test measures the average blood sugar concentration over a 90-d span of the average red blood cell in peripheral circulation. The higher the HbA_{1c} level, the more glycosylation of hemoglobin in red blood cells will occur. Studies have shown that blood glucose levels > 11.1 mmol/L (equivalent to > 310 mg/mL or an HbA_{1c} level of > 12) is associated with decreased neutrophil function, including leukocyte chemotaxis^[50]. Indeed, a greater elevation of blood glucose level has been associated with a higher potential for suppressing inflammatory responses and decreasing host response to an infection^[6]. Pomposelli *et al*^[51] has indicated that a single blood glucose level > 220 mg/dL on the first postoperative day was a sensitive (87.5%) predictor of postoperative infection. Furthermore, the authors found that patients with blood glucose values > 220 mg/dL had infection rates that were 2.7 times higher than for patients with lower blood glucose values (31.3% *vs* 11.5%, respectively)^[51]. In addition, it's indicated that a 1% mean reduction in HbA_{1c} was associated with a 25% reduction in micro vascular complications, including neuropathy^[47]. Investigations have found that poor glucose control accelerated the manifestation of Peripheral Arterial Disease (PAD). It has been shown that for every 1% increase in HbA_{1c}, there is an increase of 25%-28% in the relative risk of PAD, which is a primary cause of DFU^[52]. However, to date, no RCT has been performed to determine whether improved glucose control has benefits after a foot ulcer has developed.

Debridement

Debridement is the removal of necrotic and senescent tissues as well as foreign and infected materials from a wound, which is considered as the first and the most important therapeutic step leading to wound closure and a decrease in the possibility of limb amputation in patients with DFU^[53-56]. Debridement seems to decrease bacterial counts and stimulates production of local growth factors. This method also reduces pressure, evaluates the wound bed, and facilitates wound drainage^[32,57].

There are different kinds of debridement including surgical, enzymatic, autolytic, mechanical, and biological^[58] (Table 1). Among these methods, surgical debridement has

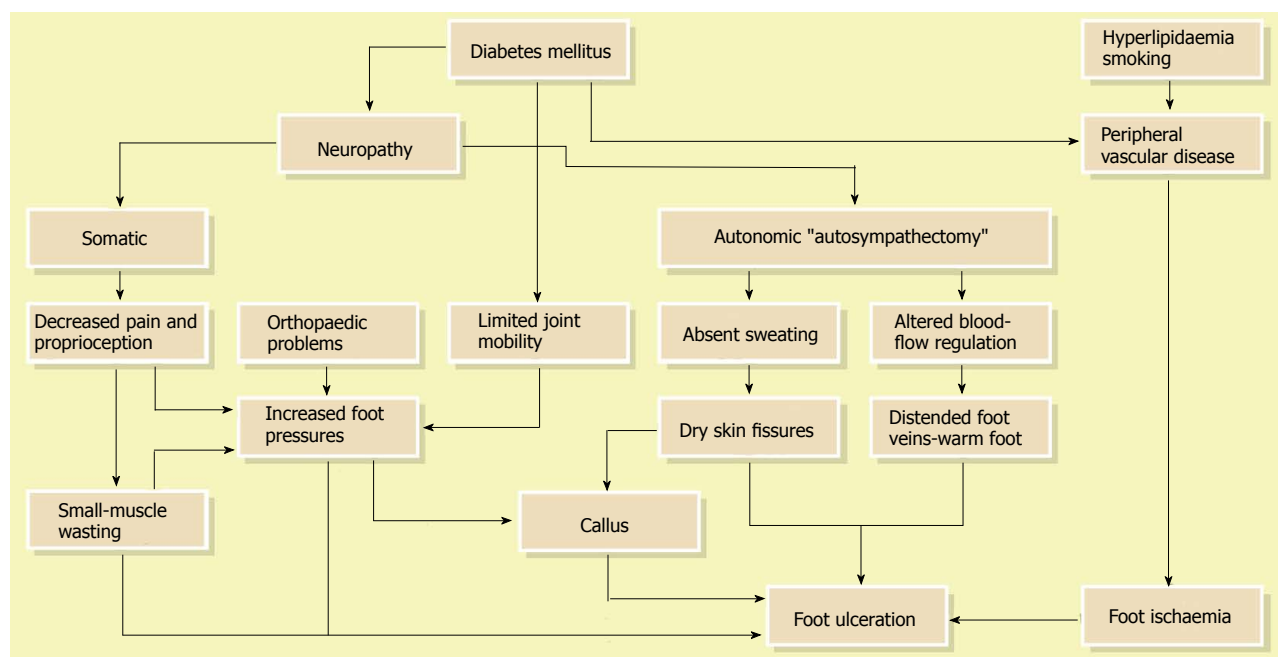


Figure 2 Etiology of diabetic foot ulcer. (Data adapted from Boulton *et al*^[17]).

Table 1 Different kind of debridement for patients with diabetic foot ulcer

Method	Explanation	Advantages	Disadvantages
Surgical or Sharp	Callus and all nonviable soft tissues and bone remove from the open wound with a scalpel, tissue nippers, curettes, and curved scissors. Excision of necrotic tissues should extend as deeply and proximally as necessary until healthy, bleeding soft tissues and bone are encountered ^[59]	Only requires sterile scissors or a scalpel, so is cost-effective ^[55]	Requires a certain amount of skill to prevent enlarging the wound ^[55]
Mechanical	This method includes wet to dry dressings, high pressure irrigation, pulsed lavage and hydrotherapy ^[76] , and commonly used to clean wounds prior to surgical or sharp debridement ^[76]	Allows removal of hardened necrosis	It is not discriminating and may remove granulating tissue It may be painful for the patients ^[55]
Autolytic	This method occurs naturally in a healthy, moist wound environment when arterial perfusion and venous drainage are maintained ^[18]	It's cost-effective ^[55] It is suitable for an extremely painful wound ^[18]	It's time consuming and may require an equivocal time for treatment ^[18]
Enzymatic	The only formulation available in the United Kingdom contains Streptokinase and Streptodornase (Varidase Topical® Wyeth Laboratories). This enzyme aggressively digests the proteins fibrin, collagen and elastin, which are commonly found in the necrotic exudate of a wound ^[77,78]	They can be applied directly into the necrotic area ^[55]	Streptokinase can be systemically absorbed and is therefore contraindicated in patients at risk of an MI It's expensive ^[55]
Biological	Sterile maggots of the green bottle fly (<i>Lucilia sericata</i>) are placed directly into the affected area and held in place by a close net dressing. The larvae have a ferocious appetite for necrotic material while actively avoiding newly formed healthy tissue ^[79,80]	They discriminate between the necrotic and the granulating tissue ^[79]	There may be a reluctance to use this treatment by patients and clinicians It's expensive ^[79,80]

been shown to be more effective in DFU healing^[54,59-62]. Surgical or sharp debridement involves cutting away dead and infected tissues followed by daily application of saline moistened cotton gauze^[53]. The main purpose of this type of debridement is to turn a chronic ulcer into an acute one. Surgical debridement should be repeated as often as needed if new necrotic tissue continues to form^[63]. It has been reported that regular (weekly) sharp debridement is associated with the rapid healing of ulcers than for less frequent debridement^[59,64-66]. In a retrospective cohort study, Wilcox *et al*^[60] indicated that frequent debridement healed more wounds in a shorter time ($P < 0.001$). In fact,

the more frequent the debridement, the better the healing outcome.

The method of debridement depends on characteristics, preferences, and practitioner level of expertise^[54]. When surgical or sharp debridement is not indicated, then other types of debridement could be used.

An older debridement type that is categorized as biological debridement is maggot debridement therapy (MDT), which is also known as maggot therapy or larval therapy. In this method, sterile and live forms of the *Lucilia sericata* larvae are applied to the wound to achieve debridement, disinfection, and ultimately wound

Table 2 Common offloading techniques

Technique	Casting techniques	Footwear related techniques	Surgical offloading techniques	Other techniques
Examples	TCC (Figure 3) iTCC (Figure 5)	Shoes or half shoes (Figure 7) Sandals	ATL Liquid silicone injections/tissue augmentation Callus debridement	Bed rest Crutches/Canes/Wheelchairs
	RCW (Figure 4)	Insoles		Bracing (patella tendon bearing, ankle-foot orthoses)
	Scotch-cast boots (Figure 6)	In-shoe orthoses	Metatarsal head resection osteotomy/arthroplasty/os ectomy/ exostectomy	Walkers
	Windowed casts	Socks	External fixation	Offloading dressings
	Custom splints			Felted foam/padding Plugs

Data adapted from Armstrong *et al*^[82]. TCC: Total contact cast; iTCC: Instant TCC; RCW: Removable cast walkers; ATL: Achilles tendon lengthening.

healing^[67-69]. Indeed, larvae secrete a powerful autolytic enzyme that liquefies necrotic tissues, stimulates the healing processes, and destroys bacterial biofilms^[70-72]. This technique is indicated for open wounds and ulcers that contain gangrenous or necrotic tissues with or without infection^[72]. To date, paucity of RCTs show efficacy of this method with DFU; however, some of retrospective^[71,73]; and prospective^[74] studies have shown MDT as a clinically effective treatment for DFU. These studies reported that MDT can significantly diminish wound odor and bacterial count, including *Methicillin-Resistant Staphylococcus Aurous*, prevent hospital admission, and decrease the number of outpatient visits among patients with DFU^[71,73-75].

Despite the advantages of debridement, adequate debridement must always precede the application of topical wound healing agents, dressings, or wound closure procedures, which may be expensive.

Offloading

The use of offloading techniques, commonly known as pressure modulation, is considered the most important component for the management of neuropathic ulcers in patients with diabetes^[81,82]. Recent studies have provided evidence indicating that proper offloading promotes DFU healing^[83-85].

Although many offloading modalities are currently in use (Table 2), only a few studies describe the frequency and rate of wound healing with some of the methods frequently used clinically. The choice of these methods is determined by patient physical characteristics and abilities to comply with the treatment along with the location and severity of the ulcer^[82].

The most effective offloading technique for the treatment of neuropathic DFU is total contact casts (TCC)^[82,86,87]. TCC is minimally padded and molded carefully to the shape of the foot with a heel for walking (Figure 3). The cast is designed to relieve pressure from the ulcer and distribute pressure over the entire surface of the foot; thus, protecting the site of the wound^[82]. Mueller *et al*^[87] conducted an RCT that showed TCC healed a higher percentage of plantar ulcers at a faster rate when compared with the standard treatment. In

addition, a histologic examination of ulcer specimens has shown that patients treated with TCC before debridement had better healing as indicated by angiogenesis with the formation of granulation tissue than for patients treated with debridement alone as indicated by a predominance of inflammatory elements^[88]. The contributory factors to the efficacy of TCC treatment are likely to be due to pressure redistribution and offloading from the ulcer area. In addition, the patient is unable to remove the cast, which thereby forces compliance, reduces activity levels, and consequently improves wound healing^[84]. However, the frequency of side effects referred to in the literature and minimal patient acceptance make this approach inappropriate for wide applications^[89,90]. Fife *et al*^[91] has shown that TCC is vastly underutilized for DFU wound care in the United States. Based on this study, only 16% of patients with DFU used TCC as their offloading modalities. The main disadvantage of TCC was the need for expertise in its application. Most centers do not have a physician or cast technician available with adequate training or experience to safely apply TCC. In addition, improper cast application can cause skin irritation and in some cases even frank ulceration. Also, the expense of time and materials (the device should be replaced weekly), limitations on daily activities (*e.g.*, bathing), and the potential of a rigid cast to injure the insensate neuropathic foot are considered other disadvantages. Furthermore, TCC does not allow daily assessment of the foot or wound, which is often contraindicated in cases of soft tissue or bone infections^[36,32,83]. In some cases, it is suggested to use other kinds of offloading techniques such as a removable cast walker (RCW) or Instant TCC (iTCC).

An RCW is cast-like device that is easily removable to allow for self-inspection of the wound and application of topical therapies that require frequent administration^[82,90] (Figure 4). The application of this method allows for bathing and comfortable sleep. In addition, because RCW is removable, they can be used for infected wounds as well as for superficial ulcers^[82]. However, in a study that compared the effectiveness of TCC, RCW, and half-shoe, this method did not show equivalent healing time (mean healing time: 33.5, 50.4, and 61.1 d, respectively), and a



Figure 3 Total contact cast for patients with diabetic foot ulcer. (Data adapted from Armstrong *et al*^[82]).



Figure 5 Instant total contact cast for patients with diabetic foot ulcers. The removable cast walker shown in Figure 5 has now been rendered irremovable by the application of bands of casting. (Data adapted from Rathur *et al*^[86]).



Figure 4 Removable cast walker (DH Walker) for patients with diabetic foot ulcer. (Data adapted from Rathur *et al*^[86]).

significantly higher proportion of people with DFU were healed after 12 wk wearing a TCC compared with the two other widely used offloading modalities^[81].

iTCC, which involves simply wrapping a RCW with a single layer of cohesive bandage, Elastoplast or casting tape (Figure 5), is another offloading technique that is shown to be more effective than TCC^[92] and RCW^[93]. This technique forces the patient to adhere to advice to immobilize the foot while allowing for ease of application and examination of the ulcer as needed. A preliminary randomized trial of TCC *vs* iTCC (Figure 6) in the management of plantar neuropathic foot ulcers has confirmed equivalent efficacy of the two devices and that iTCC is cheaper, quicker to apply, and has fewer adverse effects than traditional TCC^[93]. As this device does not require a skilled technician to apply it, it could revolutionize the future management of plantar neuropathic ulcers. It has been suggested that iTCC will dramatically change the treatment of non-ischemic, neuropathic, diabetic plantar ulcers, and has the potential to replace TCC as the gold standard for offloading plantar neuropathic ulcers^[92].

Regardless of the modality selected, patients should return to an unmodified shoe until complete healing of the ulcer has occurred (Figure 7). Furthermore, any shoe that resulted in the formation of an ulcer should not be worn again^[94].

Advanced dressing

A major breakthrough for DFU management over the last decades was the demonstration of novel dressings^[13,95]. Ideally, dressings should confer moisture balance, protease sequestration, growth factor stimulation, antimicrobial activity, oxygen permeability, and the capacity to promote autolytic debridement that facilitates the production of granulation tissues and the re-epithelialization process. In addition, it should have a prolonged time of action, high efficiency, and improved sustained drug release in the case of medicated therapies^[95,96]. Hence, no single dressing fulfills all the requirements of a diabetic patient with a foot ulcer. The choice of dressing is largely determined by the causes of DFU, wound location, depth, amount of scar or slough, exudates, condition of wound margins, presence of infection and pain, need for adhesiveness, and conformability of the dressing^[13].

Wound dressing can be categorized as passive, active, or interactive^[97]. Passive dressings are used as protective functions and for acute wounds because they absorb reasonable amounts of exudates and ensure good protection. Active and interactive dressings are capable of modifying the physiology of a wound by stimulating cellular activity and growth factors release. In addition, they are normally used for chronic wounds because they adapt to wounds easily and maintain a moist environment that can stimulate the healing process^[95,98]. The main categories of dressings used for DFU are as follows: films, hydrogels, hydrocolloids, alginates, foams, and silver-impregnated (Table 3).

Today, all dressings are commonly used in clinical practice, while the efficacy of these products has been a challenge for researchers and clinicians, and there are controversial results regarding their use^[36,99]. However, dressings are used based on DFU characteristics (Figure 8), hydrogels have been found to be the most popular choice of dressing for all DFU types^[96]. Some studies dealing with the incorporation of these products show great potential in the treatment of DFU^[100,101]. However, these findings do not represent a practical option since the application of these compounds is expensive and

Table 3 Classification of advanced wound dressings used for diabetic foot ulcers healing

Type	Example	Explanation	Advantages	Disadvantages
Hydrocolloids	Duoderm (Convatec) Granuflex (Convatec) Comfeel (Coloplast)	These kind of dressings usually composed of a hydrocolloid matrix bonded onto a vapor permeable film or foam backing. When in contact with the wound surface this matrix forms a gel to provide a moist environment ^[102]	Absorbent Can be left for several days Aid autolysis ^[96]	Concerns about use for infected wounds May cause maceration Unpleasant odor ^[96]
Hydrogels	Aquaform (Maersk Medical) Intrasite Gel (Smith and Nephew) Aquaflor (Covidien)	These dressings consist of cross-linked insoluble polymers (<i>i.e.</i> , starch or carboxymethylcellulose) and up to 96% water. These dressings are designed to absorb wound exudate or rehydrate a wound depending on the wound moisture levels. They are supplied in either flat sheets, an amorphous hydrogel or as beads ^[96]	Absorbent Donate liquid Aid autolysis ^[96]	Concerns about use for infected wounds May cause maceration using for highly exudative wounds ^[96]
Foams	Allevyn (Smith and Nephew) Cavicare (Smith and Nephew) Biatain (Coloplast) Tegaderm (3M)	These dressings normally contain hydrophilic polyurethane foam and are designed to absorb wound exudate and maintain a moist wound surface ^[103]	Highly absorbent and protective Manipulate easily ^[96] Can be left up to seven days Thermal insulation ^[96]	Occasional dermatitis with adhesive ^[96] Bulky ^[6] May macerate surrounding skin ^[6]
Films	Tegaderm (3M) Opsite (Smith and Nephew)	Film dressings often form part of the construction of other dressings such as hydrocolloids, foams, hydrogel sheets and composite dressings, which are made up of several materials with the film being used as the outer layer ^[107,108]	Cheap Manipulate easily Permeable to water vapor and oxygen but not to water microorganisms ^[95]	May need wetting before removal ^[96] Aren't suitable for infected wounds ^[107,108] Nonabsorbent If fluid collects under film it must be drained or the film replaced ^[6] May need wetting before removal ^[96]
Alginates	Calcium Alginate Dressing (Smith and Nephew Inc., Australia) Kaltostat (ConvaTec) Sorbagon (Hartman United States, Inc.) Medihoney (Derma Sciences Inc., Canada)	The alginate forms a gel when in contact with the wound surface which can be lifted off with dressing removal or rinsed away with sterile saline. Bonding to a secondary viscose pad increases absorbency ^[104]	Highly absorbent Bacteriostatic Hemostatic Useful in cavities ^[96]	
Silver-impregnated	Acticoat (Smith and Nephew) Urgosorb Silver (Urgo)	These dressing used to treat infected wounds as silver ions are thought to have antimicrobial properties ^[109]	Antiseptic Absorbent ^[96] Reduce odor Improved pain-related symptoms Decrease wound exudates Have a prolonged dressing wear time ^[112]	High cost ^[96]

**Figure 6** Scotch-cast boot for off-loading pressure from the foot of a diabetic patient with foot ulcer. (Data adapted from Armstrong *et al*^[82]).**Figure 7** Half shoe for off-loading pressure from the foot of a diabetic patient with foot ulcer. (Data adapted from Armstrong *et al*^[82]).

difficult to regulate^[1102-105]. Nevertheless, they have longer

wear times, greater absorbency, may be less painful, and

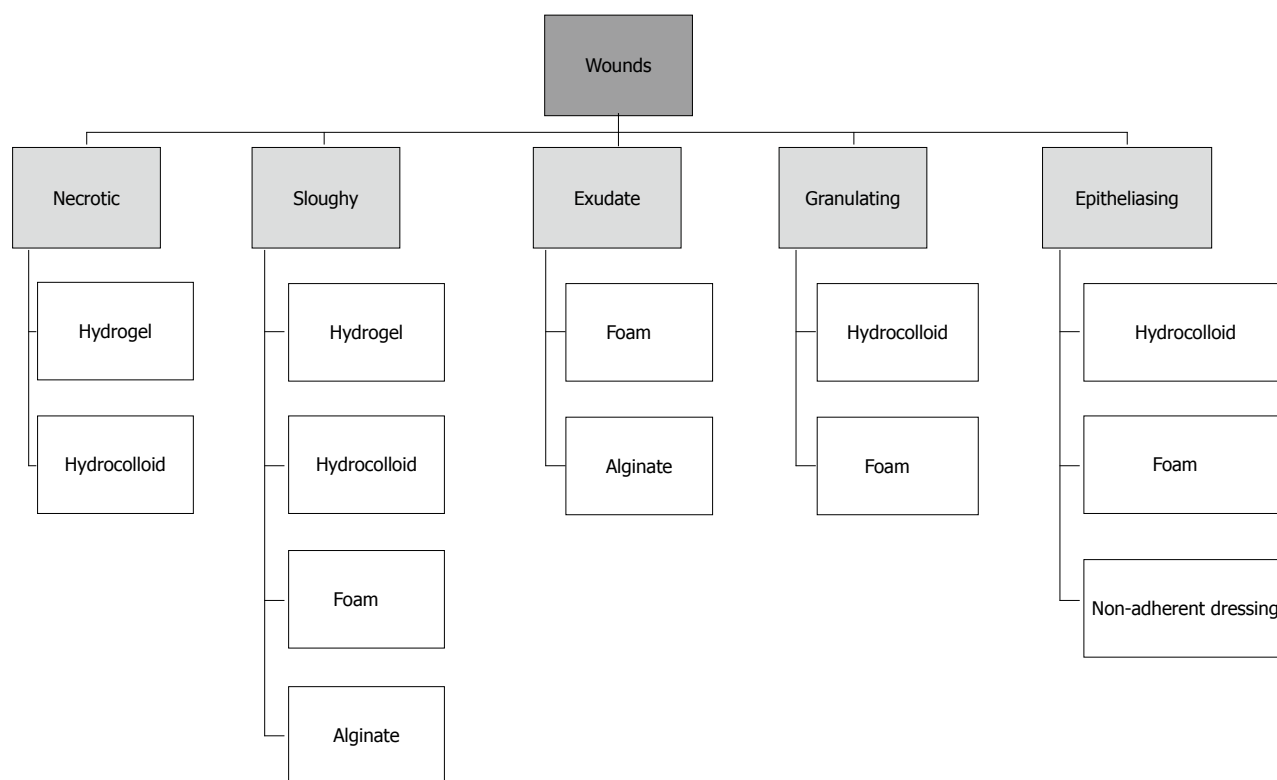


Figure 8 Classification of the different advanced dressing types usually used in diabetic foot ulcer treatment. (Data adapted from Moura *et al*^[95]).

are typically less traumatic when removed. Moreover, in certain patients, they are cost effective because of the lowered frequency of dressing changes and not requiring extensive nursing time^[106].

Surgery

Diabetic foot surgery plays an essential role in the prevention and management of DFU^[110], and has been on the increase over the past 2 decades^[111,112]. Although surgical interventions for patients with DFU are not without risk, the selective correction of persistent foot ulcers can improve outcomes^[113].

In general, surgery for DFU healing includes non-vascular foot surgery, vascular foot surgery, and in some cases amputation. Nonvascular foot surgery is divided into elective, prophylactic, curative, and emergent surgeries that aim to correct deformities that increase plantar pressure^[114] (Table 4). Today, a few studies have reported long-term outcomes for diabetic foot surgery in RCTs^[60,115,116]. In one study conducted by Mueller *et al*^[115], subjects were randomized into two groups of Achilles Tendon-Lengthening (ATL) group, who received treatment of ATL and TCC, and a group who received TCC only. Their results showed that all ulcers healed in the ATL group and the risk for ulcer recurrence was 75% less at seven months and 52% less at two years than for the TCC group^[115].

Vascular foot surgery such as bypass grafts from femoral to pedal arteries and peripheral angioplasty to improve blood flow for an ischemic foot have been recently developed^[117]. While studies have shown that these

procedures help to heal ischemic ulcers^[118-120], no RCT has been shown to reduce DFU.

While the primary goal of DFU management focuses on limb salvage, in some cases amputation may offer a better functional outcome, although this is often not clearly defined^[41]. This decision is individualized and multifactorial to match patient lifestyle, medical, physical, and psychological comorbidities^[121]. In general, amputation is considered as an urgent or curative surgery and should be the last resort after all other salvage techniques have been explored, and the patient must be in agreement^[122]. Indications for an amputation include the removal of infected or gangrenous tissues, control of infection, and creation of a functional foot or stump that can accommodate footwear or prosthesis^[123].

ADVANCED THERAPIES

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBOT) has shown promise in the treatment of serious cases of non-healing DFU, which are resistant to other therapeutic methods^[124-127]. HBOT involves intermittent administration of 100% oxygen, usually in daily sessions^[128]. During each session, patients breathed pure oxygen at 1.4-3.0 absolute atmospheres during 3 periods of 30 min (overall 90 min) intercalated by 5 min intervals in a hyperbaric chamber^[124,129] (Figure 9).

Today, RCTs have reported beneficial effects from HBOT in numerous studies^[130-134]. A recent double-blind RCT conducted by Löndahl *et al*^[134] demonstrated a significantly improved outcome in the intervention

Table 4 Different types of nonvascular diabetic foot surgery

Type	Explanation
Elective	The main goal of this surgery is to relieve the pain associated with particular deformities such as hammertoes, bunions, and bone spurs in patients without peripheral sensory neuropathy and at low risk for ulceration
Prophylactic	These procedures are indicated to prevent ulceration from occurring or recurring in patients with neuropathy, including those with a past history of ulceration (but without active ulceration)
Curative	These procedures are performed to effect healing of a non-healing ulcer or a chronically recurring ulcer when offloading and standard wound care techniques are not effective. These include multiple surgical procedures aimed at removing areas of chronically increased peak pressure as well as procedures for resecting infected bone or joints as an alternative to partial foot amputation
Emergent	These procedures are performed to arrest or limit progression of acute infection

Data adapted from Frykberg *et al*^[18].

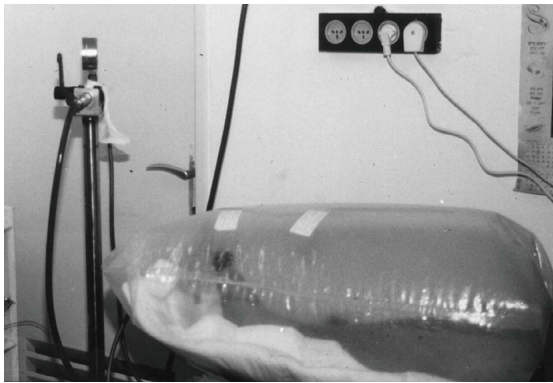


Figure 9 The polyethylene hyperbaric chamber. Oxygen in a concentration of 100% was pumped into the bag through a regular car wheel valve. The open end of the bag was sealed by an elastic bandage to the leg above the knee. Oxygen was allowed to leak around the bandage, and the pressure in the chamber was kept to between 20 and 30 mmHg (1.02-1.03 atm) above atmospheric pressure. (Data adapted from Landau^[127]).

group as the treated patients were more likely to heal within 12 mo [25.48 (52%) *vs* 12.42 (29%); $P = 0.03$]. In addition, Kranke *et al*^[135], in a systematic review, revealed that treatment with HBOT resulted in a significantly higher proportion of healed DFU when compared with treatment without HBO (relative risk, 5.20; 95%CI: 1.25-21.66; $P = 0.02$). However, in another systematic review conducted by O'Reilly *et al*^[136], no significant effects on amputation rates were found in the RCT evidence and in the high quality studies, no difference was found between HBOT group compared to standard wound care group.

The exact mechanism of HBOT remains poorly understood. Some studies have reported that HBOT improved wound tissue hypoxia, enhanced perfusion, reduced edema, down regulated inflammatory cytokines, and promoted fibroblast proliferation, collagen production, and angiogenesis^[137-140]. In addition, it was demonstrated that HBOT stimulated vasculogenic stem cell mobilization from bone marrow and recruited them to the skin wound^[139].

Despite reports of increased healing rates and decreased amputation rates with using HBOT, adjuvant use of this method in DFU remains a controversial issue. HBOT does not substitute for antibiotic therapy, local humid

therapy, or surgical wound debridement. Furthermore, HBOT is available in only a minority of communities as it is expensive [a full course of treatment in the United States typically costs \$50000 (Medicare) to \$200000 (private pay)] and is time-consuming (an average of 60 total hours in the chamber)^[5,6].

Electrical stimulation

Electrical stimulation (ES) has been reported as a perfect adjunctive therapy for DFU healing in recent literature. Currently, there is a substantial body of work that supports the effectiveness of ES for DFU healing^[141-144]. In a randomized, double-blind, placebo-controlled trial study conducted by Peters *et al*^[141] on 40 patients with DFU, significant differences in number of healed ulcers (65% in treatment group *vs* 35% in control group) were found at 12 wk.

Based on the literature review, it is suggested that ES could improve common deficiencies that have been associated with faulty wound healing in DFU, such as poor blood flow, infection, and deficient cellular responses^[141,145]. This therapy is a safe, inexpensive, and a simple intervention to improve wound healings in patients with DFU^[145,146].

Negative pressure wound therapy

Negative pressure wound therapy (NPWT) is a non-invasive wound closure system that uses controlled, localized negative pressure to help heal chronic and acute wounds. This system uses latex-free and sterile polyurethane or polyvinyl alcohol foam dressing that is fitted at the bedside to the appropriate size for every wound, and then covered with an adhesive drape to create an airtight seal. Most commonly, 80-125 mmHg of negative pressure is used, either continuously or in cycles. The fluid suctioned from the wound is collected into a container in the control unit^[147,148] (Figure 10).

It seems that NPWT removes edema and chronic exudate, reduces bacterial colonization, enhances formation of new blood vessels, increases cellular proliferation, and improves wound oxygenation as the result of applied mechanical force^[149-151].

This method has been advocated by numerous RCTs as a safe and effective adjunctive modality in the treatment of DFU. Studies have shown that wound

Table 5 Brief description of commonly used bioengineered tissue products

Type	Explanation	Use	RCT studies
Apligraf (Advanced Biohealing Inc., La Jolla, CA)	A bilayered living-skin construct containing an outer layer of live allogeneic human keratinocytes and a second layer of live allogeneic fibroblasts on type 1 collagen dispersed in a dermal layer matrix. Both cell layers are grown from infant fore skin and looks and feels like human skin ^[164,165]	It's used for full-thickness neuropathic DFU of greater than 3 wk duration, resistant to standard therapy (also without tendon, muscle, capsule, or bone exposure) and is contraindicated in infected ulcers ^[167]	Veves <i>et al.</i> ^[168] Falanga <i>et al.</i> ^[169] Edmonds ^[170] Steinberg <i>et al.</i> ^[171]
Dermagraft (Organogenesis Inc, Canton, Mass)	An allogeneic living-dermis equivalent and includes neonatal fibroblasts from human fore skin cultured on a polyglactin scaffold ^[164,165]	It's used for DFU of greater than 6 wk duration, full thickness in depth but without tendon, muscle, joint, or bone exposure and is contraindicated in infected ulcers ^[164,167]	Marston <i>et al.</i> ^[172] Gentzkow <i>et al.</i> ^[173]
Oasis (Cook Biotech, West Lafayette, IN)	An acellular biomaterial derived from porcine small intestine submucosa, contains numerous crucial dermal components including collagen, glycosaminoglycans (hyaluronic acid), proteoglycans, fibronectin, and bioactive growth factors such as fibroblast growth factor-2, transforming growth factor β 1, and VEGF ^[164,165]	It's used for full-thickness DFU ^[174]	Niezigoda <i>et al.</i> ^[174]

DFU: Diabetic foot ulcer; VEGF: Vascular endothelial growth factor.

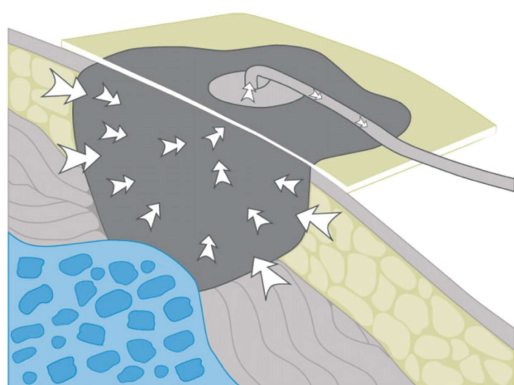


Figure 10 Schematic drawing of the negative pressure wound treatment. (Data adapted from Vikatmaa *et al.*^[147]).

healing with this approach results in a higher proportion of healed wounds, faster time for wound closure, a more rapid and robust granulation tissue response, and a potential trend towards reduced risk for a second amputation than for the control treatment^[148,152-156]. In addition, meta-analysis studies have indicated that NPWT significantly reduces healing times and increases the number of healed wounds^[147,157,158].

While the evidence for NPWT in DFU patients is promising, this method does not replace surgical wound debridement to improve blood circulation in all DFU patients. Investigations have shown that when NPWT is initiated, there must be no significant infection or gangrene in the wound^[147,158]. Also, RCTs have shown significantly higher mean material expenses for wounds treated with NPWT when compared to conventional therapy (moist gauze) in the management of full-thickness wounds requiring surgical closure^[159,160].

Bioengineered skin

Bio-engineered skin (BES) has been used during the last decades as a new therapeutic method to treat DFU^[161-164]. This method replaces the degraded and destructive milieu

of extra cellular matrix (ECM) with the introduction of a new ground substance matrix with cellular components to start a new healing trajectory^[165]. Currently, three kinds of BES products approved in the United States are available to use for DFU including Derma graft (Advanced Bio healing Inc., La Jolla, CA), Apligraf (Organogenesis Inc., Canton, Mass), and, more recently, Oasis (Cook Biotech, West Lafayette, IN)^[164,166]; and numerous RCT studies shown their efficacy in DFUs healing (Table 5).

BES product cells are seeded into the scaffolds and cultured *in vitro*. *In vitro* incubation establishes the cells and allows the cell-secreted ECM and growth factors to accumulate in the scaffold. The cells within live cell scaffolds are believed to accelerate DFU healing by actively secreting growth factors during the repair process^[164,165]. In addition, it seems that BES can provide the cellular substrate and molecular components necessary to accelerate wound healing and angiogenesis. They act as biologic dressings and as delivery systems for growth factors and ECM components through the activity of live human fibroblasts contained in the dermal elements^[162,163,170].

Despite the advantages of BES, they cannot be used in isolation to treat DFU. Peripheral ischemia, which is one of the pathological characteristics of DFU, is a critical contributing factor that affects BES transplantation. Hence, surgical revascularization and decompression as well as wound bed preparation are considered as essential prerequisites for BES applications. In addition, this method needs control of the infection^[77,175]. Therefore, the above-mentioned points may result in high long-term costs and cause major concern for use of this treatment^[176].

Growth factors

DFU has demonstrated the benefits from growth factors (GFs) such as platelet derived growth factor (PDGF), fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factors (IGF1, IGF2), epidermal growth factor, and transforming growth factor β ^[177]. Among the aforementioned GFs, only recombinant human PDGF

(rhPDGF) (Becaplermin or Regranex), which is a hydrogel that contains 0.01% of PDGF-BB (rhPDGF-BB), has demonstrated increased healing rates when compared with controls in a number of clinical trials^[178-181] and has shown sufficient DFU repair efficacy to earn Food and Drug Administration (FDA) approval^[182]. In one randomized placebo controlled trial involving patients with full thickness DFU, Becaplermin demonstrated a 43% increase in complete closure *vs* placebo gel (50% *vs* 35%)^[183]. In another randomized placebo-controlled trial, Sibbald *et al.*^[184] demonstrated that patients with infection-free chronic foot ulcers treated with the best clinical care and once-daily applications of 100 µg/g Becaplermin gel had a significantly greater chance of 100% ulcer closure by 20 wk than those receiving the best clinical care plus placebo (vehicle gel) alone.

GFs have been shown to stimulate chemotaxis and mitogenesis of neutrophils, fibroblasts, monocytes, and other components that form the cellular basis of wound healing^[178,185]. Despite FDA approval and other reviewed studies, the clinical use of Becaplermin remains limited because of its high cost^[186] and uncertain patient-specific clinical benefits^[187,188]. Some studies have indicated that endogenous PDGF stimulates tumor infiltrating fibroblasts found in human melanoma cells and is overexpressed at all stages of human astrocytoma growth^[164]. So, it would be biologically possible that topical administration of recombinant PDGF could promote cancer.

CONCLUSION

Foot ulcers in patients with diabetes is common, and frequently leads to lower limb amputation unless a prompt, rational, multidisciplinary approach to therapy is taken. The main components of management that can ensure successful and rapid healing of DFU include education, blood sugar control, wound debridement, advanced dressing, offloading, surgery, and advanced therapies, which are used clinically. These approaches should be used whenever feasible to reduce high morbidity and risk of serious complications resulting from foot ulcers.

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WJD 5th Anniversary Special Issues (4): Diabetes-related complications

Pathogenesis of diabetic cerebral vascular disease complication

Ren-Shi Xu

Ren-Shi Xu, Department of Neurology, the First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

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Correspondence to: Ren-Shi Xu, MD, PhD, Department of Neurology, the First Affiliated Hospital of Nanchang University, No. 17 of Yongwaizheng St., Nanchang 330006, Jiangxi Province, China. xurenshi@yahoo.com
 Telephone: +86-791-88603798

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vascular smooth muscle dysfunction, oxidative stress, and the downregulation of miRs participated in vessel generation and recovery as well as the balance of endotheliocytes. In turn, these abnormalities, mainly *via* phosphatidylinositol 3 kinase, mitogen-activated protein kinase, polyol, hexosamine, protein kinase C activation, and increased generation of advanced glycosylation end products pathway, play an important role in inducing diabetic CVD complication. A deeper comprehension of pathogenesis producing diabetic CVD could offer base for developing new therapeutic ways preventing diabetic CVD complications, therefore, in the paper we mainly reviewed present information about the possible pathogenesis of diabetic CVD complication.

Key words: Complication; Diabetes mellitus; Cerebral vascular disease; Pathway; Pathogenesis

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Core tip: A better understanding pathogenesis of diabetic cerebral vascular disease (CVD) could provide the basis for developing novel therapeutic strategies against diabetic CVD complication. Our article highlights the pathogenesis as some promising options to prevent CVD complications in diabetes, including metabolic and vascular changes and main pathways are involved in diabetic CVD complication.

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Abstract

Diabetes mellitus is one of the most potent independent risk factors for the development of diabetic cerebral vascular disease (CVD). Many evidences suggested that hyperglycemia caused excess free fatty acids, the loss of endothelium-derived nitric oxide, insulin resistance, the prothrombotic state, endothelial dysfunction, the abnormal release of endothelial vasoactivators,

INTRODUCTION

Diabetic mellitus (DM) is a chronic disease leading to a

fault of insulin due to pancreas dysfunction, which causes hyperglycemia with metabolic imbalances in carbohydrate, fat and protein^[1]. Morbidities of DM significantly elevate in late decades, which are primarily due to alter in life style, an elevation in the incidence of obesity and longevity. Current projections estimate that the number of people with DM will nearly double by 2025^[2,3].

About 100 million populations suffer from DM in the world^[4]. Among them, five to ten percent are type 1 DM of insulin dependence and ninety to ninety-five percent are type 2 DM (T2MD) of non insulin dependence. The current evidences demonstrated that morbidities of T2MD would increase owing to life styles leading to obesity^[5]. T2DM is a very common disease, which has an asymptomatic period between the actual onset of diabetic hyperglycemia and clinical diagnosis. This stage has been evaluated to sustain at the fewest 4-7 years, and 30%-50% patients of T2DM are still unknown. This leads to the development of chronic complications of diabetes, which remain the chief problems in diabetic care, and which cause a lack of fitness to work, disability, and premature death^[6,7].

Among the chronic complications of diabetes, the vasculopathy is the first serious complication. The vasculopathy related to DM was traditionally divided into two major parts. Firstly, diabetic complications associated micrangium including retina, nephridium and neural system lesion; secondly, the atherothrombotic complications related to macro-arteries like myocardial infarction, hypertension, peripheral artery lesion^[8].

DM is one of the well known risk factor for cerebrovascular accident^[9,10]. Prolonged untreated DM contributes to micrangium lesion, hypoxic and ischemic damages of tissues, which elevates the danger of apoplexy and aggravates cerebral lesion caused by blood insufficiency^[10,11]. Its incidence in DM patients is 2-6 times more than non DM^[12-14] and ultimately its complications and subsequent prevalence is higher yet. Patients demonstrate a progressive atherosclerosis in cerebral arteries and increased a vascular reaction to vascular constrictors, a deregulated reaction to vascular dilators and damaged automatic regulation of brain blood stream. Changed endothelium function of small arteries and a damaged vascular motor function of resistance vessel can lead to change mediation of local blood stream and deficient perfusion of tissue in diabetic patients^[10,15,16].

Many studies^[8,17-19] stress the strong link between the cerebral vascular disease (CVD) complications and DM and describe a close association between CVD microvascular complications and DM, suggest that excess free fatty acids (FFAs), the loss of endothelium-derived nitric oxide (NO), insulin resistance, the prothrombotic state, endothelial dysfunction, the abnormal release of endothelial vasoactivators, vascular smooth muscle (VSM) dysfunction, oxidative stress and the miRs downregulation participated in vessel generation, vessel recovery as well as endothelium balance generate diabetic CVD complications by these major mechanisms of phosphatidylinositol 3 kinase, mitogen-activated protein

kinase, polyol, hexosamine, generation of advanced glycosylation end products (AGEs) and protein kinase C (PKC) pathways activation^[20,21]. The aim of this review is to review the possible pathogenesis of diabetic CVD complication.

SUPERFLUOUS FREE FATTY ACIDS

DM facilitates lipolysis, reduces uptaking of skeletal muscle, which result in superfluous concentrations of FFAs. Moreover, elevates the flux of FFAs into liver causes the stimulation of triglycerides synthesis, assembly and secretion of very low-density lipoprotein (VLDL) particles. Hypertriglyceridemia and the decreased high-density lipoprotein (HDL) cholesterin as the transportation of cholesterin from HDL to VLDL have been determined to be strongly relative to atherosclerosis. It also is likely that FFAs generation promotes reactive oxygen species (ROS) and PKC. PKC elevation and phosphatidylinositol 3 kinase (PI3-K) downregulation may cause endothelial impairment.

Recycling concentrations of FFAs rise in DM because of the superfluous releasion derived from adipose tissue as well as the reducing uptake of skeletal muscle^[22-24]. FFAs can damage endothelial function *via* a series of mechanisms such as elevating oxygen-mediated free radicals generation, PKC activation and dyslipidemia aggravation^[25-27]. FFAs levels increase activates PKC, reduces insulin receptor substrate-1 associated PI3-K activity^[25,28]. Increased triglyceride levels decreases HDL through facilitating cholesterin transportation from HDL to VLDL^[29]. These disturbances alter LDL configuration, elevating the quantity of the more LDL of small density contributing to atherosclerosis^[30,31]. Hypertriglyceridemia and decreased HDL are suggested to be relative to endothelial dysfunction^[21,32-34].

THE LOSS OF ENDOTHELIUM-DERIVED NITRIC OXIDE

Endothelial and vascular smooth muscle cells (VSMCs) dysfunction and an inclination of thrombus formation result in atherogenesis as well as the relative complications. Because endothelial cells (ECs) mediate the vascular function and structure, they take on an important anatomical location on interaction of circulatory blood and vascular wall. In normal conditions, ECs active substances, synthesize and release vascular activators to preserve vessel balance, to ensure a normal blood stream and nutritious transportation while avoiding thrombus formation as well as white blood cell permeation^[35]. One of key molecules produced by ECs is NO, it is generated by an endothelial NO synthase (eNOS) *via* a 5 electrons oxidation of the guanidine nitrogen terminal of L-arginine^[36].

The NO biologic availability is a vital element in vessel abnormality, which results in vascular dilation activated guanylyl cyclase in VSMCs^[36]. Furthermore, NO prevents vasculum from internal lesion like atherogenesis-mediated

molecule signal that stops platelet and leukocyte interacting with vessel wall and inhibits VSMCs proliferation and migration^[37,38]. Contrarily, ECs reduction-mediated NO induces elevated pro-inflammatory transcription factor nuclear factor kappa B (NFκB) activity which causes a leukocyte adhesion molecules expression, chemokines as well as cytokines generation^[39]. The effects facilitate mono cells and VSMCs to migrate into the internal membrane and macrophage foam cells formation, producing an early morphologic alteration of atherogenesis^[39-43]. Disorder of endothelium function such as damaged endothelium dependent and NO-derived relaxation is identified in cell and animal studies of DM^[21,44-47].

INSULIN RESISTANCE

Insulin resistance is another vital pathogenesis that exerts a major effect on the diabetic CVD complication. Insulin exerts effects by two pathways including PI3-K and mitogen-activated protein kinase (MAPK). Insulin signal producing by PI3-K has effects of anti-proliferative and anti-coagulant, the effects activated by MAPK have a proatherogenic function. On base of insulin resistance, although the first pathway is damaged, the second pathway maintains intact. Therefore, the decrease endothelial dependent vasodilatation as well as increase mitosis effects is a key result^[48,49].

Insulin resistance also critically takes part in vascular dysfunction in patients with T2DM^[50]. In fact, the reduction of PI3-K/Akt pathway causes eNOS depression, decreases NO generation^[51]. Combining with decreasing NO synthesis, intracellular oxidization of stored FFAs produces ROS contributing to vascular inflammation, AGEs synthesis, inhibited PGI2 synthase activity, and PKC activation^[51,52].

Rised ROS concentrations closely related with insulin resistance remove NO generation, generate peroxynitrite accompanying with a more decrease of NO biologic availability. Decreased cell concentrations of NO activate pro-inflammatory pathways promoted by increasing cytokine generation. In fact, TNF-α and IL-1 facilitate NFκB activity and adhesion molecules expression. TNF-α also induces C reactive protein expression which lowers the regulation of eNOS and elevates adhesion molecules and endothelin-1 (ET-1) generation^[50,53].

Adipokines associated with vasculopathy are leptin, adipocyte fatty acid-binding protein, interleukins, lipocalin-2 and pigment epithelium-derived factor, which could produce disorders of vessel function through increasing proliferation and migration of smooth muscle cells (SMCs), eNOS depression, and NFκB signaling activation accompanied with adhesion molecule expression and atherogenesis^[54].

PROTHROMBOTIC STATE

Damaged fibrinolysis, as a result of enhancing generation of PAI-1 and excessive activity of platelet result from of

glycoprotein IIb/IIIa superfluous expression and excessive production of thromboxane A2 in DM. Furthermore, DM rises concentrations of VII, VIII factor as well as thrombin-antithrombin compounds^[48,55-57]. Haemostasis chaos elevates the risk of cerebral thrombosis.

Abnormality of platelet function as well as elevation of both glycoprotein Ib and IIb/IIIa expression in DM augment interaction between platelet-von Willebrand factor (vWF) and platelet-fibrin^[58]. The internal-cellular platelet glucose level responses the external-cellular circumstance, is relative to increasing superoxide anion (O²⁻) generation as well as PKC activity, reduced platelet mediated NO^[59]. High blood glucose more alters hematoblastic functions through damaging calcium balance, thus changes platelet activation and aggregation such as platelet construct and mediators release^[60]. In DM, plasm coagulate factors VII, thrombin and impairment-dependent coagulate elements such as tissue factor (TF) elevated, and endogenic anti-coagulate factors like thrombomodulin as well as protein C reduced^[61-63]. In addition, the generation of plasminogen activator inhibitor-1 (PAI-1), a fibrinolysis inhibitor elevated^[64-66]. Therefore, a inclination of hematoblastic activating and aggregating accompanied with coagulate propensity is associated with a danger of thrombus formation complicated plaque burst.

Diabetic CVD largely results from an abnormality of elements participated in activation of coagulate factors and hematoblast^[67]. Insulin resistance, high blood glucose involve in the nosogenesis of the prothrombotic status^[68]. Insulin resistance rises PAI-1 and fibrinogen, decreases levels of tissue plasminogen activator. Hyperinsulinemia elevates TF expression in monocytes of T2DM contributing to increase TF procoagulant activity and thrombin production^[69]. Low level of inflammation also leads TF expression in vessel endothelial cells of DM patients, and results in atherothrombosis^[68,69].

Platelet hyperreactivity is one of major relations in elements leading to DM prothrombotic status^[70]. A lot of mechanisms lead to platelet dysfunction affect the adhesive, activated and aggregative stages of hematoblast induced thrombus formation. High blood glucose changes hematoblast calcium ion homeostasis contributing to a abnormality of cellular constructure, elevates the production of pro-aggregant elements^[58]. Furthermore, the increase production of glycoproteins Ib and IIb/IIIa in DM subjects contributes to thrombosis through interacting with vWF and fibrin.

In DM, the elevation of glucose levels contributes to the activation of PKC, down-regulates the production of platelet derived NO, rises the formation of O²⁻^[59], and also triggers the disorder of calcium homeostasis in platelets^[60]. The abnormal calcium regulation may significantly lead to disordered activity, because the intraplatelet calcium mediates a shape change, secretion, aggregation and thromboxane formation of platelet. Their disorders may cause by decreasing endothelial generation of the antiaggregants NO and prostacyclin, increasing generation of fibrinogen, and increasing generation of platelet

activators like thrombin and vWF^[58]. In general, diabetic disorders elevate the activation of platelet and depress internal depressors of platelet activity.

DM increases a blood coagulability which makes it more likely that atherosclerotic plaque rupture or erosion will lead to the thrombotic occlusion of artery. T2DM has damaged fibrinolytic capacity owing to increasing levels of PAI-1 in atherogenetic damage and nonatheromatous artery^[71]. DM elevates the TF (a forceful procoagulant) expression and plasma coagulate elements like factor VII, reduces contents of internal anti-coagulate factors like antithrombin III and protein C^[62,63,72]. A number of these disorders are associated with the occurrence of hyperglycemia and proinsulin split products^[73]. Therefore, DM increases a tendency of coagulation accompanying with damaging fibrinolysis, facilitates the formation and persistence of thrombi.

ENDOTHELIAL DYSFUNCTION

The endothelium is an organ composing of a mono cell layer arraying the intimal surface of the vasculature, serving as a paclose between blood and tissues. The normal paracrine and autocrine functions of endothelial cells include the synthesis of a series of substances that moderate vascular relaxation, mediate local inflammation, depress leucocyte migration and affect platelet activation.

Endothelial dysfunction consists of many abnormalities, encompassing changed vasomotor activity, VSMC dysfunction, excess generation of inflammatory cytokines and chemokines, damaged platelet function and abnormal coagulation, which contribute to elevating vasoconstriction, inflammation and thrombosis^[74].

The endothelium in DM is more frail in producing atherosclerotic plaques compared with the endothelium of non DM. A series of mechanisms may lead to the elevated risk of generating atherosclerotic plaques in DM. The abnormal cluster of hyperglycemia, increased FFAs and insulin resistance in DM, targeting the endothelial cell, causing oxidative stress and endothelial dysfunction^[75]. The endothelial dysfunction leads to a defective endothelium dependent vasorelaxation and vasoconstriction, a migration of monocytes, a VSMCs transport into internal membrane as well as a generation of macrophage foam cells, which trigger an atherogenesis production. As far as ET-1, besides a vascular constrictive function, which has the function of proinflammation, mitogenesis and proliferation yet^[48,76].

Hyperglycemia, elevated FFAs as well as insulin resistance exert through a usual pathogenesis characterized by rising ROS generation (Especially O_2^-), subsequently result in damaging endothelial dependent NO induced relaxation. Elevated ET-1 generation and VSMCs proliferation also lead to endothelium dysfunction. Hypertriglyceridemia, consequent atherogenesis and platelet hyperactivity as well as diminished fibrinolysis and hypercoagulability are features of vessel circumstances in DM. In general, an initial and progressive atherogenesis,

endothelium dysfunction as well as elevated thrombus production are extremely susceptible to generating thrombotic occlusive events at brain circle for this type of DM^[20].

Mechanisms of the endothelial dysfunction encompass elevating polyol pathway flux, changed cell redox status, increasing generation of diacylglycerol, specific PKC isoforms activation, and exacerbated non-enzymatic production of AGEs. A lot of pathways promote the generation of oxidative and nitrosative mediated oxidants and free radicals like O_2^- and peroxynitrite, exerting a important effect in the mechanism of the DM-relative endothelial dysfunction. The cellular sources of ROS like O_2^- are diverse and encompass AGEs, NADH, NADPH oxidases, mitochondrial respiratory chain, xanthine oxidase, arachidonic acid cascade, and microsomal enzymes^[77-82]. The oxygen and nitrogen stress mediated by hyperglycemia results in DNA-lesion as well as succedent poly (ADP-ribose) polymerase (PARP) activation^[83]. The endothelial dysfunction progression was related to a concurrent NAD^+ and NADPH loss in vascular systems, PARP depression inversed their alterations. Endothelium dysfunction in DM is relied on a PARP-derived, inverse cell NADPH insufficient^[83,84].

A mono layer of ECs is seated in the internal membrane of all vasculum, which offers a metabolically active interactive spot between blood and vessels regulating blood influx, nutritious transport, coagulation and thrombosis, and leukocyte diapedesis^[85]. ECs synthesize a lot of key bioactive substances, such as ROS, prostaglandin, endothelin as well as angiotensin II, which modulates vascular effect and structure. Moreover, it diminishes platelet activation, inhibits inflammation by decreasing leukocyte adhesion to endothelium and migrating into the vascular wall, and reduces VSMCs proliferation and migration^[38,86,87]. In general, these characters prevent atherogenesis and protect the vascular vessel.

DM damages endothelium dependent vasodilation prior to the generation of atheroma^[88,89]. Many of fundamental mechanisms lead to the lower bioavailability of vasoactivators in DM. Hyperglycemia diminishes generation of NO by inhibiting the activation of eNOS synthase and elevating the generation of ROS, particularly O_2^- , in endothelial and VSMCs^[78].

Insulin resistance contributes to excessive release of FFAs from adipose tissue^[90], activating the signal enzyme PKC, depressing PI3-K, and increasing the generation of ROS-mechanisms^[27]. Generation of peroxynitrite decreases synthesizing the vessel dilatory and antiplatelet prostanoid prostacyclin^[91]. The increased levels of FFAs in DM trigger the production of oxidized low-density lipoproteins (Ox-LDL), including vital initiating events for atherosclerosis. Ox-LDL can impair ECs and increase adhesion molecules expression like P-selectin^[92] and chemotactic factors like monocyte chemoattractant protein-1, macrophage colony stimulating factor^[93,94] and thus lead to endothelial dysfunction in DM^[78,95].

ABNORMAL RELEASE OF ENDOTHELIAL VASOACTIVATORS

Hyperglycemia rises the cyclooxygenase-2 mRNA expression in DM. Endothelin could be especially associated with the pathophysiology of vasculopathy in DM, because endothelin triggers inflammatory reaction, results in VSMCs contraction and growth^[95]. The abnormalities of endothelium associated factors or vasoactivators generation consisting of vascular oxidative stress^[96], inflammatory factors, NO, prostanoids (prostacyclin), ET-1, angiotensin II (ANG-II), tissue-type plasminogen activator (t-PA), PAI-1, vWF, adhesion molecules such as vascular cell adhesion molecule (VCAM) leukocyte adhesion molecules, intercellular adhesion molecule (ICAM) as well as cytokines^[97]. These vascular activated factors contribute to elevating vascular tone, resulting in microvascular and macrovascular impairment and apoptosis of microvascular cells, consequently contributing to DM associated vascular complications^[97]. In lots of pathological conditions, the abnormal balance of these regulatory mediators causes the onset and process of vascular endothelial dysfunction^[98]. Vascular endothelial dysfunction elevates effects of leukocyte, smooth muscle proliferation, vascular constriction, damaged coagulating, vessel inflammation, thrombus generation, and atherogenesis, these mechanisms are the base of later DM complications like retinopathy, nephropathy, vasculopathy as well as neuropathy^[99]. ET-1 is a forceful vasoconstrictor generated by ECs. The generation and the level of ET-1 in plasma elevated in DM patients, and it is reported a positive correlation between plasma ET-1 concentrations and the micro-vessel lesion of DM. Therefore, ET-1 could exert a possible key effect on endothelial dysfunction by a disorder between ECs mediated vascular dilator and vascular constrictor factors on mechanisms of the vessel complication in DM^[100]. Besides its direct vasoconstrictor functions, elevated levels of ET-1 may lead to endothelial dysfunction *via* generating a series of vascular active substances consisting of ROS, NO and inflammatory factors^[101-103].

CGRP is a key mediator of ET-1 vasoconstriction. The elevation of CGRP expression leads an abnormal balance in the CGRP/ET-1 ratio, inducing abnormal vascular constriction to result in topical endothelial dysfunction as well as vessel impairment^[104,105]. VCAM-1 is one of key ECs receptors, mediating leukocyte adhesion to the vascular ECs. Current studied results have highly proposed that VCAM-1 might exert a key effect on mechanisms of atherosclerosis on account of VCAM-1 effects on leukocyte adhesion and transmigration is key as well as its expression is upregulated in the initial phases of neogenetic atheroma plaques^[106]. Moreover, VCAM-1 expression is upregulated by pro-inflammatory stimuli like TNF- α and IL-1 β , is least partially mediated by NF κ B^[107]. The expression of VCAM-1 is upregulated in vessel stress circumstances like insulin resistance and chronic high blood glucose yet. In the circumstance,

the elevation in the activity of VCAM-1 expression is found yet^[108,109]. Besides binding leukocytes, VCAM-1 engagement leads to leukocyte transendothelial migration (TEM) *via* inducing gap formation between cells in the endothelial monolayer, facilitating TEM^[110]. The gap formation is moderated by VCAM-1^[111,112]. VCAM-1 accumulation elevates the internal cellular free calcium level yet^[111]. Therefore, the expression of VCAM-1 rises the permeability of vascular endothelium as well as the TEM of leukocyte, leads to the impairment of vessel and abnormal endothelial function, as well as mediates atherogenesis production. The ICAM-1 expression in ECs is risen in atherogenesis and in the animal model of atherogenesis^[113,114]. In normal conditions, ICAM-1 is presented in low concentrations in ECs, however ICAM-1 is significantly elevated while stimulating by pro-inflammatory factors such as the pro-inflammatory cytokines TNF- α , IL-1 β as well as interferon- γ ^[114]. Soluble ICAM-1 may mediate leukocyte adhesion, migration. Promoting leukocyte to attach to the ECs surface isn't the only effect of ICAM-1. Effect of ICAM-1 mediates signal in ECs, rises the IL-8 generation, facilitates the ICAM-1 and c-fos expression. It is likely that pro-inflammatory IL-8 and c-fos activated by ICAM-1 facilitates a positive feedback loop, contributing to excessive ICAM-1 and VCAM-1 expression and thus initiating the persistent recruitment of leukocytes to regions of atherosclerosis, facilitating the progress of atherogenesis through producing pro-inflammatory stimuli through indirect or direct endothelial dysfunction^[115-117].

P-selectin is mediated by pro-inflammatory stimuli stored in internal cellular vesicles of ECs combined with the plasma membrane being responsible to many stimuli like ischemia and chronic high blood glucose yet^[118]. On some conditions, P-selectin translocation to the endothelial cell surface is modulated by a ROS-dependent mechanism. P-selectin accumulation rises cytosol free Ca²⁺, mediates changes of cell morphology, facilitating endothelial dysfunction to ultimately generate vessel impairment^[119-121].

VCAM-1, ICAM-1, P-selectin exert a key effect on vascular integrity and permeability through endothelial dysfunction^[122]. Increased concentrations of soluble EAM could be one of the common causes for the pathogenesis of between atherogenetic CVD and endothelial dysfunction^[122]. The adhesive molecule expression in ECs facilitates the adhesion and transportation of leukocytes into the sub-endothelial space, consequently contributing to abnormal endothelial function and sub-endothelial structure alteration^[123]. Elevated vessel permeability owing to structure changes can then decrease insulin transportation to insulin sensitive peripheral tissues, ultimately form insulin resistance. In addition, insulin resistance may directly contribute to endothelial dysfunction^[124]. The investigation in non-diabetic subjects have proposed that lightly damaged glucose tolerance in the normal glycemic range may facilitate the process of endothelial dysfunction through side effects of oxidative stress, generation of AGEs, and

increased concentrations of FFAs^[125].

Vascular endothelial dysfunction may be prior to the development of insulin resistance, which results from a decrease of insulin sensitivity, generates a vicious cycle^[126-128]. Our studied results provided the further evidences that endothelial dysfunction exerts a causal role in the pathogenesis of CVD in T2DM, and also highlights new insights into the possible clinical value of endothelial function in CVD of T2DM. The pathophysiologic mechanisms of CVD in T2DM could be relative to an abnormal expressive balance of ET-1, CGRP, VCAM-1, ICAM-1 and P-selectin, causing endothelial dysfunction *via* a series of chemical factors like ROS, NO and inflammatory factors. Alternatively, we speculated that emotion, cerebral splanchno-motor and neuroendocrine center could participate in the mechanisms of CVD in T2MD through changes of ET-1, CGRP, VCAM-1, ICAM-1 and P-selectin expression, but further researches need to be warranted^[129].

VASCULAR SMOOTH MUSCLE DYSFUNCTION

The changes in vascular homeostasis owing to the dysfunction of ECs and SMCs are the primary characters of diabetic vascular diseases, which are favor of a pro-inflammatory or thrombotic status, finally contributing to artery thrombus formation. The diabetic affect on vascular function isn't confined to ECs. The abnormal regulation of VSM function is accelerated through damaging the sympathetic nervous system function^[130]. DM rises PKC activity, NF κ B and oxygen free radicals generation in VSM, which is similar to the effects in ECs^[131]. Furthermore, DM elevates the migration of VSMCs into early atherosclerotic impairment, replicating and generating external cellular matrix key process in later impairment production^[132]. VSMCs apoptosis during atherogenetic impairment is rised yet, so that DM patients are apt to have fewer VSMCs in impairment, increasing the tendency of plaque rupture. In DM, the cytokine generation reduces VSM synthesis of collagen and elevates generation of matrix metalloproteinases, producing an elevated propensity for plaque destabilization and rupture^[133].

DM promotes the VSMCs atherogenic activity. Hyperglycemia stimulates PKC, receptor for AGEs and NF κ B in VSMCs, which is similar in ECs. Promotion of these systems increases generation of O²⁻, which leading to the oxidant gathering circumstances^[27]. VSMCs are indispensable in the progression of atherosclerosis. In case the formation of the macrophage abundant fatty streak, VSMCs in the middle layer of the arteries migrate into the early intimal impairment, replicate and generate a complicate extracellular matrix vital process in the development formed atherosclerotic plaque. VSMCs heighten the atheroma by way of the collagen source, which makes it less possibly to rupture and results in thrombosis. In fact, the impairments that have disrupted

and resulted in fatal thrombosis are inclined to have few VSMCs^[134]. Hyperglycemic lipid modifications of LDL may partially modulate the risen migration and the following apoptosis of VSMCs in atherosclerosis impairment. LDL that has suffered nonenzymatic glycation promotes VSMCs migration, while oxidized glycated LDL can promote apoptosis of VSMCs^[135]. Therefore, DM changes VSMCs function through facilitating atherosclerotic lesion formation, plaque instability^[68].

OXIDATIVE STRESS

The generation of ROS is severely controlled in normal cells, but excessive generation at the condition of metabolized disorder contributes to cell lesions. O²⁻ and NO are correspondingly nonvalent, however, while the both are combined they produce highly active peroxynitrites that impairs and diminishes protein and lipid. Moreover, O²⁻ and NO can impair the iron sulfur center of enzyme and other protein, releasing iron atom and thus depressing enzyme and protein activity. There are a number of key proteins that are highly sensitive to the type of inhibition such as complexes I-III in the electron transfer chain, aconitase in the trichloroacetic acid cycle as well as biotin synthase^[136,137].

The production of lipid, protein and nucleic acid compounds participates in lots of complicated chain reactions in ways of a series of biological substrates containing reactive methylene groups. Intermediate productions in these chain reactions can have very strongly oxidative effects, thus cell lesion can be comprehensive^[138,139]. Lipids locate in plasm, mitochondria and endoplasmic reticulum membranes are main attacked objects of ROS and peroxidation. Terminal productions of lipid peroxidation like lipid peroxides can be toxic to cells, require to be resolved by glutathione. In the same way, proteins and nucleic acids can be suffered by peroxidation and nitrosylation. The terminal products aren't commonly directly toxic to cells, the gather of inactive proteins can excessively increase the ability of cells to recycle them, DNA impairment is known to promote the pathogenesis of apoptosis.

Diabetic CVD complications are majorly owing to a prolonged exposure of hyperglycemia^[140]. The early trigger high glucose levels change vessel function is the disorder between NO bio-availability and gather of ROS, contributing to endothelial dysfunction. In fact, high blood glucose mediated production of O²⁻ inactivates NO to generate peroxynitrite (ONOO⁻), a forceful oxidant, it easily pass through phospholipid membranes, causes substrate nitration^[21]. Hyperglycemia mediated ROS generation promotes a series of cellular mechanisms such as polyol and hexosamine flux, AGEs, PKC activation and NF κ B induced vascular inflammation^[52,141]. One of the major resources of ROS in the condition of high blood glucose is represented by PKC and its downstream subjects. The circumstance of high blood glucose promotes a chronic increase of

diacylglycerol concentrations in ECs accompanying the following membrane translocation of conventional and nonconventional PKC isoforms. In case activated, PKC would be responsible for different structure and functions alterations in vascular systems such as changes in permeability, inflammation, angiogenesis, growth, external cell matrix expansion and apoptosis of cells^[142]. A key result of PKC activation is ROS production. Hyperglycemia mediated the PKC activation rises superoxide generation by NADPH oxidase in vascular ECs^[27]. PKC also contributes to elevated generation of ET-1, which is favor of vasoconstricting and platelet aggregating^[142].

In the vascular wall, the generation of PKC-dependent ROS takes part in the atherogenetic progression *via* promoting vessel inflammation yet^[52,143]. ROS contributes to upregulation and nuclear translocation of NFκB subunit p65, thus, the transcription of pro-inflammatory genes encodes for monocyte chemoattractant protein-1 (MCP-1), selectins, VCAM-1, and ICAM-1. The latter event promotes the adhesion of monocytes to vascular endothelial cells, rolls and exudes in the subendothelium accompanying the following production of foam cells. The production of IL-1 and TNF-α derived from a active macrophage keeps elevation of adhesion molecule through augmenting NFκB signal in the endothelial cell and also stimulates SMCs growth and proliferation^[144].

Endothelial dysfunction in DM is the subsequence of damaged NO availability and the risen synthesis of vascular constrictor and prostanoid^[144]. The up-regulation of PKC induced cyclooxygenase-2 (COX-2) is related to an elevation of thromboxane A2 as well as a downregulation of prostacyclin (PGI2) release^[145]. The data speculate that PKC is upstream signaling molecules which affect vessel balance at the condition of hyperglycemia^[145]. Production of AGEs contributes to cell disorders by triggering of a AGEs receptor (RAGE) activation^[146,147]. AGE-RAGE signal conversely promotes ROS-sensitive biochemical pathways like the hexosamine flux^[52]. At the circumstance of hyperglycemia, an elevated flux of fructose-6-phosphate promotes a series of reactions leading different glycosylated patterns being responsible for down-regulation of enzymes involved in vessel balance. Especially, OglcNAcylation at the Akt site of eNOS protein contributes to decreasing eNOS activities and ECs disorders^[52,148]. Furthermore, transcription factors glycosylation results in upregulation of inflammation (IGFα, TGFβ1) and prothrombus genes (PAI-1)^[148,149]. Hyperglycemia mediated ROS generation promotes the polyol pathway flux participated in vessel redox stress yet^[141,150]. Therefore, hyperactivation of the pathway is relative to elevated atherogenetic damages in a DM mouse^[151,152].

ABNORMALITY OF MIRS PARTICIPATE IN ANGIOGENESIS, VASCULAR REPAIR AND ENDOTHELIAL HOMEOSTASIS

MicroRNAs (miRs) are a currently found one type of small no coding RNAs known as important effects on

mechanisms of high blood glucose mediated vessel lesion^[153,154]. The small no coded RNAs mediate many aspects of DM vasculopathy through moderating gene expression at the posttranscriptional time. DM shows a obvious abnormality of miRs participated in angiogenesis, vascular repair and endothelial homeostasis^[155]. When ECs are exposed to prolonged hyperglycemia, miR-320 is largely expressed and triggers a series of angiogenic factors and their receptors such as vascular endothelial growth factor and insulin like growth factor-1 (IGF-1). Increased expression of the miR is relative to decreasing cellular proliferation and migration. When the miR decrease recoveries the characters and elevates IGF-1 expression, facilitating angiogenesis and vascular repair^[156].

High blood glucose also elevates the miR-221 expression, a mediator of angiogenesis for c-kit receptor is associated with migrating as well as homing of endothelial progenitor cells (EPCs)^[157]. miR-221 and 222 were identified to induced AGE mediated vessel lesion yet^[157]. In fact, the decrease of miR-222 expression in human ECs exposed to hyperglycemia and in a DM mouse results in AGE associated ECs disorders through targeted, cyclin depended kinase proteins participated in cellular cycle depression (P27KIP1 and P57KIP2)^[157]. A current research revealed that miR-503 severely participated in high blood glucose mediated endothelial dysfunction in a DM mouse, is elevated in muscles of ischemia limbs in DM patients^[158]. The pernicious function of miR-503 at the condition of DM has been identified by the interaction with CCNE and cdc25A, which are key mediators of cell cycle process influencing the migration and proliferation of ECs. It is interesting that miR-503 depression can actualize the normalization of post-ischemic novel vascularization and blood stream repairs in a DM mouse. The studied results offer a base to predict protective effects on regulating miR-503 expression against DM vasculopathy.

Assay of plasm miR demonstrated a largely decrease of miR-126 in a cohort of DM patients^[155]. Current studied results propose that down-regulation of miR-126 expression is in part responsible for damaging vascular recovery capacities in DM^[159,160]. The expression of miR-126 decreases in EPCs isolated from DM and transfection using anti-miR-126 diminished the proliferation and migration of EPCs^[159,160]. By comparison, the recovered expression of miR-126 facilitated EPCs associated a recovery capacity and depressed apoptosis. The miR-126 effect in EPCs is regulated by Spred-1, an inhibitor of Ras/ERK signal pathway, a key mediator of cellular cycles. In general, the findings provide further evidences that miRs promotes a series of complicated signaling network through triggering the genes expression participated in the differentiation, migration as well as survival of cell^[161].

In summary, many factors affect the diabetic CVD complication, which are summarized in the Figure 1 of diagrammatic sketch. Such factors will promote optimal understanding of the pathogenesis of the diabetic CVD complication and lead to the identification of the specific preventive therapy. Ultimately, the knowledge

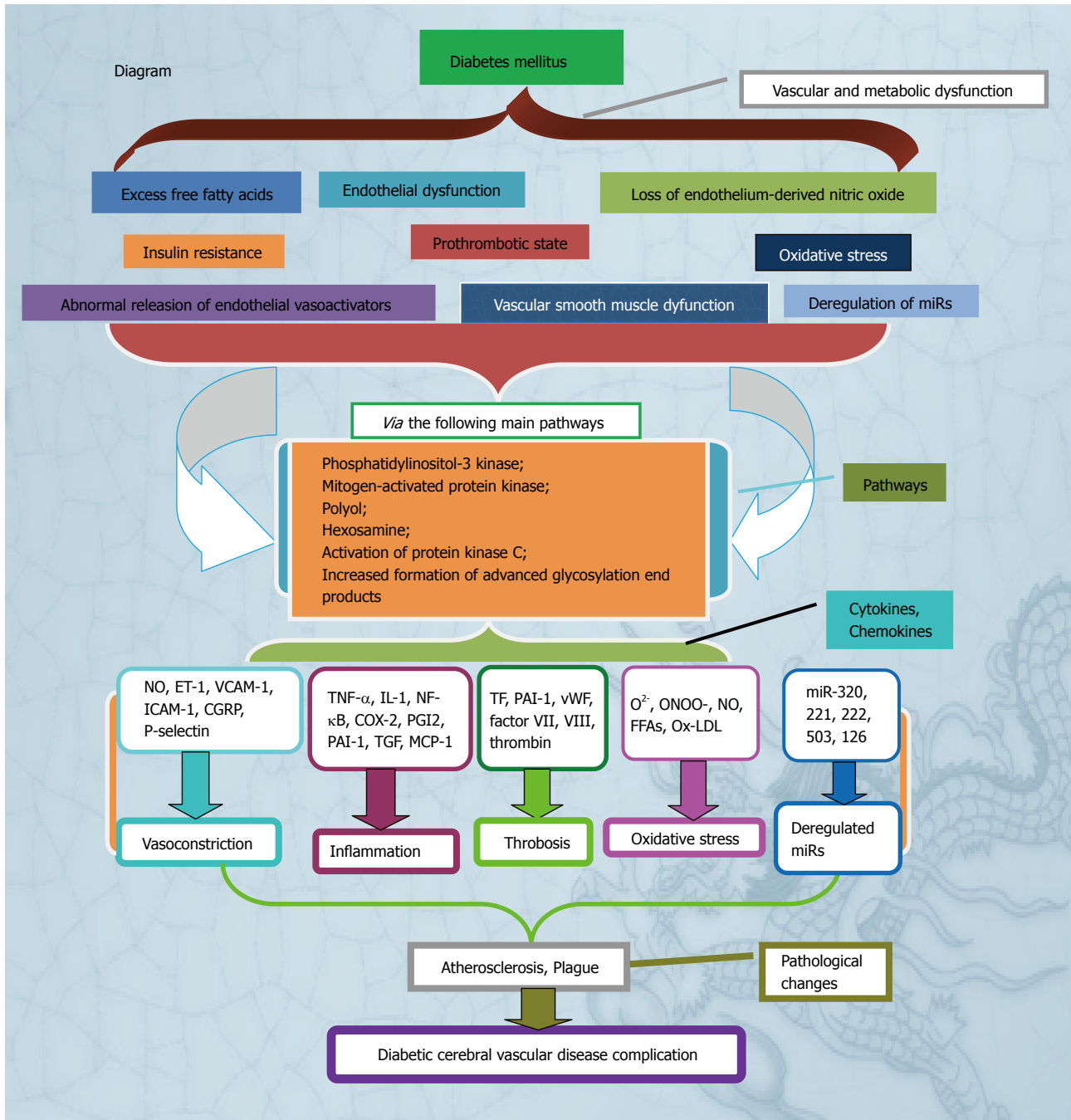


Figure 1 Diagram of pathogenesis of diabetic cerebral vascular disease complication. NO: Endothelium-derived nitric oxide; ET-1: Endothelin-1; VCAM: Vascular cell adhesion molecule; ICAM: Intercellular adhesion molecule; TNF: Tumor necrosis factor; IL: Interleukin; NF-κB: Nuclear factor-κB; COX-2: Cyclooxygenase-2; PGI2: Prostacyclin; PAI-1: Prothrombus genes; TGF-β: Transforming growth factor beta; MCP-1: Monocyte chemoattractant protein-1; TF: Tissue factor; Ox-LDL: Oxidized low-density lipoproteins; FFAs: Free fatty acids.

gained from these previous studies can be used to obtain the potential drug for preventing the diabetic CVD complication.

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Type 1 diabetes and polyglandular autoimmune syndrome: A review

Martin P Hansen, Nina Matheis, George J Kahaly

Martin P Hansen, Nina Matheis, George J Kahaly, Department of Medicine I, Johannes Gutenberg University Medical Center, 55131 Mainz, Germany

Author contributions: Hansen MP and Kahaly GJ conceived and designed the study; Hansen MP, Matheis N and Kahaly GJ acquired, analyzed and interpreted the data; Hansen MP and Kahaly GJ drafted the manuscript; Matheis N and Kahaly GJ revised the manuscript; all authors read and approved the final version of the manuscript.

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Correspondence to: George J Kahaly, MD, PhD, Department of Medicine I, Johannes Gutenberg University Medical Center, Langenbeckstreet 1, 55131 Mainz, Germany. kahaly@ukmainz.de
 Telephone: +49-6131-172290

Fax: +49-6131-173460

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Abstract

Type 1 diabetes (T1D) is an autoimmune disorder caused by inflammatory destruction of the pancreatic tissue. The etiopathogenesis and characteristics of the pathologic process of pancreatic destruction are well described. In addition, the putative susceptibility genes for T1D as a monoglandular disease and the relation to polyglandular autoimmune syndrome (PAS) have also been well

explored. The incidence of T1D has steadily increased in most parts of the world, especially in industrialized nations. T1D is frequently associated with autoimmune endocrine and non-endocrine diseases and patients with T1D are at a higher risk for developing several glandular autoimmune diseases. Familial clustering is observed, which suggests that there is a genetic predisposition. Various hypotheses pertaining to viral- and bacterial-induced pancreatic autoimmunity have been proposed, however a definitive delineation of the autoimmune pathomechanism is still lacking. In patients with PAS, pancreatic and endocrine autoantigens either colocalize on one antigen-presenting cell or are expressed on two/ various target cells sharing a common amino acid, which facilitates binding to and activation of T cells. The most prevalent PAS phenotype is the adult type 3 variant or PAS type III, which encompasses T1D and autoimmune thyroid disease. This review discusses the findings of recent studies showing noticeable differences in the genetic background and clinical phenotype of T1D either as an isolated autoimmune endocrinopathy or within the scope of polyglandular autoimmune syndrome.

Key words: Autoimmune thyroid disease; Polyglandular autoimmune syndrome; Addison's disease; Susceptibility genes; Type 1 diabetes

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Core tip: Type 1 diabetes (T1D) occurs in conjunction with several autoimmune endocrine and non-endocrine diseases. Recent studies have revealed noticeable differences in the genetic background and clinical phenotype of T1D either as an isolated autoimmune endocrinopathy or within the scope of polyglandular autoimmune syndrome. These findings are relevant for diagnostic and therapeutic procedures in daily practice as well as for the general understanding of endocrine autoimmunity.

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INTRODUCTION

Type 1 diabetes (T1D) is an endocrine disorder characterized by autoimmune destruction of insulin-producing pancreatic β -cells, which subsequently reduces insulin production and induces metabolic dysregulation^[1-4]. Although T1D onset was once thought to be restricted to children and adolescents, it can occur at any age, with the highest rate of incidence below the age of 30 years^[5-7]. Approximately 50 T1D susceptibility genes have been identified to date. These genes also carry a potential risk for various autoimmune diseases occurring simultaneously or within a narrow time interval and might explain to some extent why additional endocrine autoimmune diseases are comorbid in one third of all T1D patients^[8-12]. These associated autoimmune disorders are either glandular diseases [*e.g.*, Addison's disease or autoimmune thyroid disease (AITD)] that lead to polyglandular autoimmune syndrome (PAS) or non-glandular autoimmune diseases (*e.g.*, rheumatoid arthritis or celiac disease)^[13-15]. The variation in these comorbidities may hold the key to understanding the pathogenesis of autoimmune diseases, but also simultaneously complicates the diagnosis and treatment of T1D and is therefore of interest to both scientists and clinicians.

ISOLATED T1D

Approximately 5%-10% of all newly diagnosed patients with diabetes mellitus (nearly 400 million subjects worldwide) have T1D (20-40 million, accordingly)^[5,16]. This number may be even higher as 5%-15% of all adults with type 2 diabetes are positive for pancreatic islet autoantibodies^[17,18]. The age-adjusted incidence ranges from 0.1:100000/year (*e.g.*, China) to 40.9:100000/year (Finland), while the highest incidence rates are found in North American and European populations. Large studies confirmed a continuing rise of T1D incidence in Europe from 1989 through 2008 by approximately 3%-4% per year, which is higher than the average annual increase of 2.8%^[19]. There is a subtle gender bias, where males have the highest incidence between 10-14 years of age and females have the highest incidence between the ages of 5 and 9 years^[5,20,21]. The initial onset of T1D occurs primarily between the ages of 8 and 14 years, in close proximity to the start of puberty^[22].

Clinical spectrum and diagnosis

Clinical symptoms, caused by the high glucose levels from T1D, develop quickly and range from chronic fatigue, weight loss, polydipsia and polyuria to symptoms

of diabetic ketoacidosis (*e.g.*, nausea, acute abdomen or even coma). The diagnosis and differential diagnosis rely mainly on typical history and signs as well as measuring organ-specific autoantibodies directed against pancreatic islet cells, insulin, glutamate decarboxylase (GAD) and tyrosine phosphatase, which are positive in 95% of the cases at T1D onset. These pancreas autoantibodies may appear months or years before the clinical manifestation with various sensitivity, specificity and predictive relevance^[23,24]. Positive titers of islet cell autoantibodies (ICAs), glutamic acid decarboxylase autoantibodies (GADAs), insulinoma-associated protein 2 autoantibodies (IA2As), insulin autoantibodies (IAAs) and the recently discovered zinc transporter 8 autoantibodies (ZnT8As) are important serologic diagnostic parameters. An early presence of autoantibodies is associated with a greater risk for T1D. The first antibodies to appear in young children are IAAs with a peak under the age of five years; a valid titer can only be measured before initiation of insulin therapy^[25]. While titers of ICAs, ZnT8As and IAAs have been reported to decline after the onset of T1D, GADAs persist for years in the sera of diabetic patients independent of inflammatory β -cell destruction^[26]. Therefore, measurement of GADAs is preferred in adults with late onset diabetes mellitus. ZnT8As can be found in about a fourth of T1D patients seronegative for ICAs, GADAs, IA2As and IAAs, and in approximately one third of patients with autoimmune disorders associated with T1D (Table 1)^[27,28]. Considering the prevalence of organ-specific autoantibodies and their role in diagnosis of T1D, an autoimmune component in the disease manifestation seems undeniable.

Pathogenesis

Inflammatory infiltrates predominantly consisting of CD4⁺ and CD8⁺ lymphocytes and macrophages in the pancreatic tissue of patients with recent onset of T1D make an autoimmune etiology most likely^[3,29-31]. In addition to direct killing of β -cells by natural killer cells, with a subsequent expression and presentation of autoantigens and a loss of peripheral immunologic tolerance, recently detected β -cell regeneration in children with T1D and β -cell persistence in older patients highlight a more complex pathogenesis that includes the involvement of cytokines, regulatory T cells and hormones^[31-34]. Several studies have confirmed a higher cumulative risk for T1D in family members (Table 2). According to twin studies, the genetic predisposition and environmental effects might contribute 80% and 20%, respectively, to the clinical phenotype of T1D^[35,36]. Further studies focusing on serologic and genetic characteristics of these patients revealed a multitude of susceptibility genes, antigens, serologic markers and environmental risk factors.

Genetics

Familial clustering (λ_s) imparts a relative risk (RR) for siblings of T1D-affected patients compared to the general

Table 1 Characteristics of the relevant autoantibodies in type 1 diabetes^[125-134]

	Antigen	Sensitivity	Specificity	Percent at onset	Annotation
ICA	Islet cells	70%	99%	70%-90%	Single positivity similar predictive; in combination ≥ 3 increasing risk to approximately 90%; age independent
GADA	Glutamic acid decarboxylase (65 kDa)	65%-75%	99%	70%-80%	
IA2A	Tyrosine phosphatase-related islet antigen 2	50%-90%	99%	50%-70%	
IAA	Proinsulin/insulin	74%	99%	30%-50%	Inverse correlation with age; measurement prior to insulin therapy required
ZnT8	C terminal domain of the zinc transporter 8	65%-75%	99%	60%-80%	

IAA: Insulin autoantibody; IA2A: Tyrosine phosphatase-related islet antigen 2 autoantibodies; ICA: Islet cell autoantibodies; GADA: Glutamic acid decarboxylase autoantibodies; T1D: Type 1 diabetes; ZnT8: Zinc transporter 8.

Table 2 Involvement of family members of patients with type 1 diabetes^[5,27,135]

Affected family member	Presence of T1D
First degree relative (general)	5%-6%
Mother	2%
Father	7%
Monozygotic twin	30%-50%
Dizygotic twin	6%-10%

T1D: Type 1 diabetes.

population, amounting to $RR = 15^{[37]}$. Several affected sibling pair linkage studies showed the importance of genetic predisposition and the association of T1D with polymorphisms in the specific human leukocyte antigen (HLA) loci on chromosome 6p21.3^[38,39]. HLA class II loci are assumed to be responsible for 40%-50% genetic risk^[40,41]. HLA-DR3 or -DR4, which can be detected in approximately 95% of Caucasian Anglo-Saxon patients with T1D, partly reflect the distribution of the incidence among different countries and ethnicities in their genotype frequencies. Several studies graded the susceptibility of HLA class II genotypes^[42-45] as follows: the highest risk was found in DR3/4 heterozygotes, followed by DR4 homozygotes, DR3 homozygotes and DR4 heterozygotes combined with another DR allele^[27]. Furthermore, many non-HLA polymorphisms that appear to make a smaller contribution to the manifestation of T1D have been identified^[46]. Nevertheless, a concordance rate lower than 50% in monozygotic twins, a manifestation of T1D in 10% of the carriers of high-risk genes and a 15-fold difference in the disease incidence among European Caucasians indicates that genetics alone cannot explain disease onset^[8,47,48]. In contrast, an increase in patients with low-risk or protective HLA genotypes emphasizes the importance of environmental factors such as viral infections, nutrition and chemicals or epigenetics, respectively^[49-52].

ASSOCIATION OF T1D WITH OTHER ENDOCRINOPATHIES

Additional or associated autoimmune glandular and

non-glandular diseases in patients with T1D have been described and frequently involve organ-specific as well as systemic autoimmunity. The following autoimmune diseases are listed in the order of their frequency: autoimmune thyroid diseases (AITD, 15%-30%), autoimmune type A gastritis (15%), pernicious anemia (10%), celiac disease (4%-9%), vitiligo (1%-7%), rheumatoid arthritis (1.2%), systemic lupus erythematosus (1.15%), autoimmune adrenal failure or Addison's disease (0.5%) and multiple sclerosis (0.2%) (Table 3)^[53-60]. In addition to a common environment, many overlapping risk factors for T1D and other autoimmune diseases have been identified. While a role for HLA class I -recognizing CD8 T cells has been known to affect T1D and celiac disease, recent studies also showed a joint susceptibility for these diseases in HLA class II^[61]. HLA-DQ2 can be found in 90% of patients with celiac disease and in 55% of patients with T1D, while HLA-DQ8 is present in approximately 10% and 70%, respectively^[62]. In patients with HLA-DQ2-DQ8 heterozygosity, a transdimer (DQ2 α /8 β) binds a gliadin peptide and T1D-specific antigens, which implicates both gluten and the gut microbiome as additional factors or triggers for autoimmune diseases, respectively^[63-66]. Because the co-occurrence of non-glandular immunopathies such as autoimmune gastritis and pernicious anemia may lead to an atypical clinical presentation and additional discomfort, early and regular screening for serologic parameters (*e.g.*, parietal cell antibodies) and red blood cell count is recommended^[67].

The manifestation of additional glandular autoimmune diseases in association with T1D has recently become of particular interest for research on the common pathogenesis of general autoimmunity. PAS characterized by a combination of at least two autoimmune endocrinopathies can be classified into a juvenile form (PAS type I) and an adult form, which is then subdivided according to the specific constellation of autoimmune glandular diseases (PAS types II-IV)^[68-70].

T1D WITHIN THE SCOPE OF JUVENILE PAS TYPE I

PAS type I, also known as Whitaker's syndrome, autoimmune polyendocrinopathy-candidiasis-ectodermal-

Table 3 Prevalence of associated autoimmunity in patients with type 1 diabetes^[15,55,56,60,97,100,136-143]

Associated disease	Patients with type 1 diabetes		General population	
	Prevalence of organ-specific Abs	Overt disease	Prevalence of organ-specific Abs	Overt disease
Type 1 diabetes	ICA in 85%-90%	100%	ICA in 1%-3 %	0.1%-1.0%
Hashimoto's thyroiditis	TPO Abs in 15%-30%	10%-20%	TPO Abs in 2%-10%	0.5%-9.0%
Graves' disease	TSH-R Abs in 1%-18%	3%-6%	TSH-R Abs in 1%-2%	0.1%-2.0%
Addison's disease	21-OH Abs in 0.7%-2.0%	0.5%-0.8%	21-OH Abs in 0.6%	0.005%-0.140%
Autoimmune hypophysitis and/or hypopituitarism	Pituitary Abs in 3.6%	0.4%-0.9%	Pituitary Abs in 0.5%	0.24%-0.80%
Autoimmune type A gastritis and pernicious anemia	Gastric parietal cell Abs in 13%-25%	5%-10% (2%-6%)	Gastric parietal cell Abs in 2.5%-12.0%	2% (0.15%-1.00%)
Celiac disease	Transglutaminase Abs in 8%-12%	1%-9%	Transglutaminase Abs in 0.5%-1.0%	0.50%

Abs: Antibodies; ICA: Islet cell antibodies; TPO: Thyroperoxidase; TSH-R: Thyrotropin receptor antibodies; 21-OH: 21 Hydroxylase.

dystrophy or multiple endocrine deficiency autoimmune candidiasis syndrome, is a hereditary disorder with disease manifestation that occurs in a characteristic order at an early age. Mucocutaneous candidiasis is typically the first of the three major components to occur, typically prior to five years of age. Before the age of ten years, hypoparathyroidism becomes apparent and precedes Addison's disease, which is usually the last disorder to appear (in many cases before the age of 15 years). By definition, at least two of these major components must be present for PAS type I. Additional disorders were described that occurred prior to the fifth decade^[71,72]. T1D was found in 12%-33% of all patients with PAS type I^[72,73]. Several studies have suggested that a young age of clinical onset correlates with the manifestation of multiple concomitant autoimmune diseases^[74,75]. As a monogenetic disease with autosomal recessive inheritance caused by mutations in the autoimmune regulatory gene on chromosome 21, the prevalence of PAS I varies highly between ethnicities ranging from 1:6500 in Iranian Jews to 1:10000000 in the Japanese population, with a female/male ratio of 0.8-2.4^[76-78].

Screening for the co-occurrence of T1D in patients with PAS I is less effective. This is because the positive predictive value of ICAs and GAD65 autoantibodies is only 27%, whereas 18%-28% of PAS type I patients without T1D have islet cell autoantibodies present^[73,79]. This peculiarity led to the hypothesis that the detected autoantibody epitopes differ from those in patients with isolated T1D and that a limited, subclinical autoimmune reaction within the pancreas may exist without causing an overt clinical manifestation^[73,80]. A novel β -cell antigen, initially identified as a 51 kDa protein, was found to be aromatic-L-amino-acid decarboxylase^[81-83]. Though no correlation of T1D manifestation in PAS and anti-aromatic-L-amino-acid decarboxylase autoantibodies has been found yet, its high prevalence in PAS type I-associated diseases (*e.g.*, vitiligo and autoimmune hepatitis) warrants further research on its role in disease pathogenesis of autoimmune disorders^[82]. Similar to isolated T1D, the combination of autoantibodies in polyendocrinopathies has been suggested to provide a higher predictive value than any isolated autoantibody.

T1D WITHIN THE SCOPE OF THE ADULT PAS (TYPES II-IV)

T1D is the most frequent disorder of the PAS and is often the first disease to appear. The exact immunopathogenesis has not been fully elucidated, but several studies provide evidence for common immunologic mechanisms induced by environmental factors in a background with genetic polymorphisms^[84-86]. The frequent finding of combined manifestations of autoimmune glandular diseases led to a sub-classification for adult PAS as follows^[68,87]: (1) PAS type II: Addison's disease in combination with at least one additional autoimmune endocrinopathy (*e.g.*, T1D); (2) PAS type III: autoimmune thyroid disease in combination with T1D but excluding Addison's disease; (3) PAS type IV: combination of at least two autoimmune endocrinopathies but excluding PAS types I-III.

Clinical spectrum and diagnosis of T1D within PAS types II-IV

In approximately 40%-50% of patients with Addison's disease, additional autoimmune glandular diseases occur and become overt as PAS type II. Of these, T1D is apparent in 12%-24%^[88-90]. Autoantibodies directed against the adrenal cortex are found in 0.7%-3.0% of T1D patients. Although T1D often develops before Addison's disease, GAD65 antibodies are detected in 5%-7% of patients with Addison's disease but without T1D, thus a thorough follow-up should be performed in islet cell antibody-positive patients. The concomitant presence of Addison's disease and T1D leads to frequent hypoglycemia due to decreased gluconeogenesis and increased insulin sensitivity. Thus, autoimmune-induced adrenal failure should be considered in patients with T1D suffering from unexplained recurrent hypoglycemia and fatigue, whereas insulin therapy combined with cortisol substitution warrants close monitoring during treatment of T1D patients with adrenal failure.

PAS type III is the most frequent subtype of polyglandular autoimmune diseases, containing 41% of the possible endocrine component combinations^[68]. The co-occurrence of autoimmune-induced hypothyroidism (generally caused by chronic lymphocytic Hashimoto's

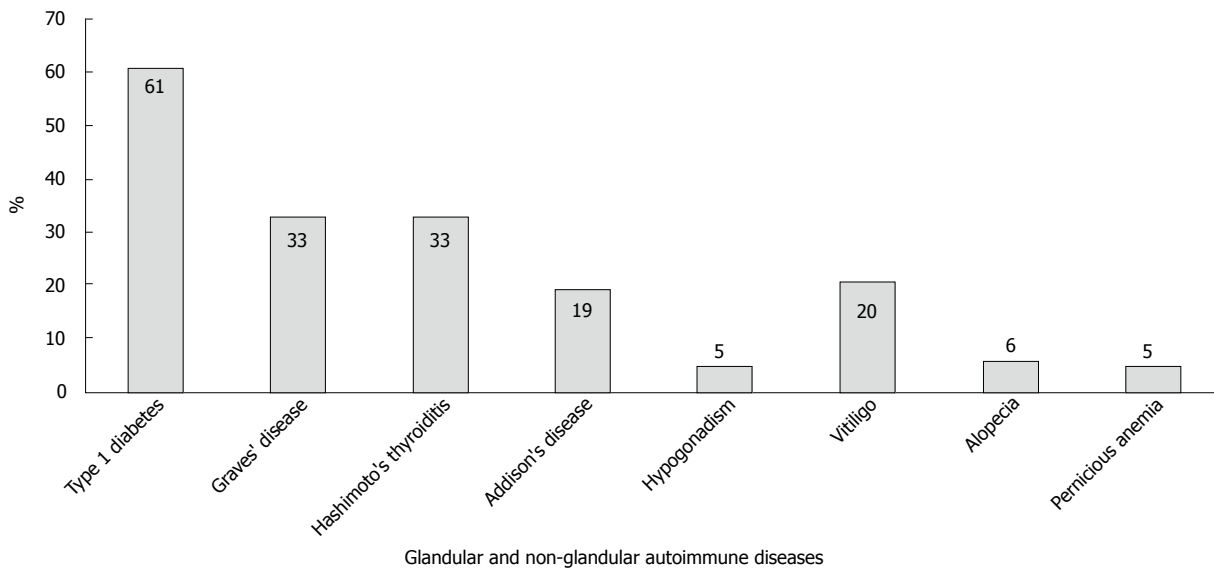


Figure 1 Endocrine and non-endocrine autoimmune diseases in patients with polyglandular autoimmune syndrome. The prevalence of glandular (dark grey) and non-glandular (light grey) autoimmune diseases in the 151 patients with adult polyglandular autoimmune syndrome (PAS) followed at the Johannes Gutenberg University Medical Center.

thyroiditis) and T1D is often accompanied by hypoglycemia due to increased insulin sensitivity. Hypothyroidism leads to a reduction in glucose resorption in the duodenum and glucose release from the liver. Because patients exhibit a decreased appetite and intake of calories, the risk for hypoglycemia is significantly enhanced^[91-93]. During the hypothyroid phase, the insulin dosage should be carefully evaluated and a reduction by approximately 20%-25% for 3-4 wk is recommended. After substitution with levothyroxine, the baseline insulin dosage may be administered again, after the patient becomes biochemically euthyroid. In hypothyroid children, chronic hypoglycemia and decreased food intake frequently lead to growth disorders. Either anti-thyroid peroxidase and/or antithyroglobulin autoantibodies are present in 19%-24% of T1D patients, whereas hypothyroidism (subclinical with normal free thyroid hormone levels but pathologically increased baseline serum thyroid-stimulating hormone) is observed in 10%-20% of patients^[12,94-96]. In comparison, subclinical and overt hyperthyroidism occur less frequently (3% and 6%, respectively)^[97]. Overt hyperthyroidism is accompanied in 50% of the cases by glucose intolerance and in 3% of the cases by overt diabetes. The impaired glucose tolerance is due to decreased insulin sensitivity and decreased hepatic storage of glycogen, whereas both secretion of glucagon and intestinal glucose absorption are enhanced. Thus, hyperthyroidism increases glucose resorption and hepatic glucose release leading to hyperglycemia. In T1D patients, this leads to insulin resistance and an increased release of fatty acids causing ketoacidosis^[53,91,98]. T1D usually manifests at a very young age. Moreover, in 60% of PAS type III patients, Graves' hyperthyroidism may occur prior to T1D, as has been reported in Japanese populations, usually within a time period of less than ten years^[99]. Onset of T1D in patients with Graves' disease and Hashimoto's thyroiditis occurred

at a mean age of 34 years in 0.78% and 1.17% of cases, respectively^[100]. ZnT8As, and especially GADAs, are observed more frequently in PAS type III than in isolated T1D, while IA2As may indicate a slow onset of T1D^[99]. In addition, in patients with T1D and PAS type III, gastric parietal cell and adrenocortical autoantibodies have been observed in 16.8% and 5.1% of cases, respectively^[96].

PAS type IV is a very heterogeneous and less well-defined group of polyglandular autoimmune diseases. It is frequently incorrectly published that this syndrome is defined as the combination of a monoglandular autoimmune disease (*e.g.*, T1D) with a non-glandular autoimmune disease (*e.g.*, autoimmune gastritis or celiac disease). In PAS type IV, pituitary antibodies have been detected in 3.6% of T1D patients, and clinically overt pituitary failure was noted in 0.9%^[101]. Aside from PAS type I, the combinations of T1D with autoimmune hypopituitarism or hypergonadotrophic hypogonadism as rare forms of PAS type IV have an estimated prevalence of < 1% and are rarely described in the literature^[102,103].

Our own findings

In a screening of 471 consecutive T1D patients that were followed at the endocrine outpatient clinic at the Johannes Gutenberg University Medical Center, multiple glandular involvement and PAS type III were found in 27% ($n = 127$) and 10%, respectively^[104]. Subsequent prospective screening of 15000 consecutive patients with monoglandular autoimmune disease (*e.g.*, T1D) revealed a high prevalence (1%) of patients with the adult PAS types II-IV, with a female bias of 75%. Figure 1 shows the various spectrums of autoimmune diseases registered in our PAS cohort. Significant male and female biases were noted for T1D and Hashimoto's thyroiditis, respectively. T1D manifested early (mean: 27.5 years), whereas other component diseases appeared later, ranging from an age

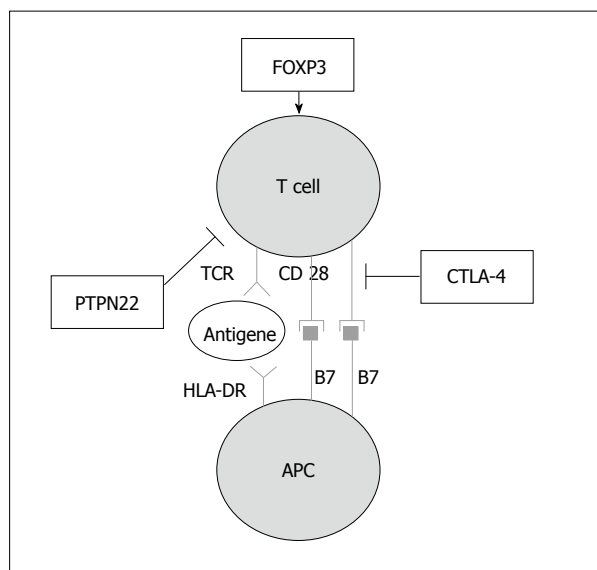


Figure 2 Immunologic synapse. This schematic depicts T cell activation and how it is influenced by expression of common susceptibility genes. Shared susceptibility genes for autoimmune thyroid disease and type 1 diabetes are involved in the immunological synapse. HLA-DR molecules present autoantigens to T cells, CTLA-4 expression suppresses T cell activation, PTPN22 expression negatively influences the T cell receptor (TCR) signaling pathway and FOXP3 expression regulates the differentiation of regulatory T cells (modified according to ref.[124]). APC: Antigen presenting cell; CTLA-4: Cytotoxic T lymphocyte antigen 4; HLA: Human leukocyte antigen; PTPN22: Protein tyrosine phosphatase non-receptor type 22; FOXP3: Forkhead box protein P3.

of 36.5-40.5 years. T1D was also the first component disease of adult PAS in half of the patients (48.3%), whereas Graves' disease (19.2%), Hashimoto's thyroiditis (17.2%), Addison's disease (14.6%) and vitiligo (12.6%) were less likely to be the first component disease. The predominant frequency of the coexistence of T1D and AITD was confirmed in our large collective. The time interval between manifestations of the first and second endocrinopathies varied considerably, with the longest time intervals between T1D and AITD, and a short time interval between Addison's disease and AITD^[105].

GENETICS OF THE ADULT PAS TYPES II - IV

Unlike the 1:1 gender ratio of isolated T1D and PAS type I, there is a clear female bias of 3:1 in adult PAS, with a prevalence of 1:20000^[13,104,106]. The incidence of adult PAS is approximately 1:100000/year and has a peak in the third or fourth decade of life. For a majority of the glandular autoimmune disorders, common susceptibility genes have been identified, including polymorphisms in protein tyrosine phosphatase non-receptor type 22, cytotoxic T lymphocyte antigen 4 (CTLA-4), MHC class I polypeptide-related sequence A, and HLA (Table 4, Figure 2). Thus, the association of endocrine autoimmune diseases is primarily due to a common genetic predisposition. The HLA class II haplotypes

Table 4 Odds ratio of susceptibility genes for autoimmune endocrinopathies^[117,144-160]

	T1D	HT	GD	AD
HLA-DR3	3.5	3.7	2-4	5
MICA	1.6	2.5	2	7
PTPN22	1.8	1.6	1.6	1.5
CTLA-4	1.5	5	1.5	1.8

AD: Addison's disease; CTLA-4: Cytotoxic T lymphocyte antigen 4; GD: Graves' disease; HLA: Human leukocyte antigen; HT: Hashimoto's thyroiditis; MICA: MHC class I polypeptide-related sequence A; PTPN22: Protein tyrosine phosphatase non-receptor type 22; T1D: Type 1 diabetes.

DRB1*03-DQA1*0501-DQB1*0201 and DRB1*04-DQA1-0301-DQB1*0302 have been reported to be associated with isolated T1D as well as with T1D within the scope of adult PAS^[10,107]. This joint susceptibility for both T1D and AITD has been demonstrated in both Caucasians and in Asians^[108-112]. CTLA-4 A/G49 single nucleotide polymorphisms (SNP) confer susceptibility to PAS type III^[113,114]. In particular, the CTLA-4 SNP rs3087243 (+ 6230 G > A) variant seems to predispose patients to a combined manifestation of T1D and Graves' disease^[115]. The 1858 C→T substitution in the protein tyrosine phosphatase non-receptor type 22 gene is associated with AITD, isolated T1D and PAS type III and the G1,123C SNP is associated with T1D and AITD in Asians^[116-119]. Additionally, a SNP in the forkhead box P3 (*FOXP3*) gene on the arm of the X chromosome has been associated with increased susceptibility to PAS type III in Caucasians^[113]. A mutation in *FOXP3* has also been shown to be the susceptibility gene in the extremely rare immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome^[120]. Typically, T1D is associated with severe enteropathy, hypothyroidism and autoimmune skin diseases such as psoriasis, neurodermitis and psoriasis vulgaris^[121]. There is a large variability in the organs affected by the additional autoimmune diseases in the severe IPEX syndrome and many patients die in infancy. Because *FOXP3* plays an important role in the function of regulatory T cells, a recent study suggested a similar CD25-correlated pathogenesis in isolated T1D and T1D within the context of the IPEX syndrome (Table 5)^[122,123].

CONCLUSION

In isolation as a monoglandular disease, or within the larger context of PAS, the manifestation of T1D justifies an extensive serologic and functional screening for additional autoimmune glandular and gastrointestinal diseases both in patients with T1D of recent onset as well as every two years during patient follow-up (Figure 3). In particular, in families with clustering of T1D patients or in families of patients with PAS, the risk for associated autoimmune diseases and endocrine or autoimmune involvement of the first-degree relatives is significantly

Table 5 Polyglandular autoimmune syndromes^[13,68,78,161-166]

	PAS Type I	PAS Type II-IV	IPEX
Onset	Childhood	Adulthood	Infancy
Incidence	< 1:100000/yr	1-2:100000/yr	Extremely rare
Male/Female ratio	3:04	1:03	Male >> Female
Genetics	Monogenetic (AIRE)	Polygenetic	X-linked (FOXP3)
Autoantibodies	Anti-interferon- α/ω antibodies 100%, additional Abs	Organ-specific Abs depending on the autoimmune components	ANA (42%) SSA (25%) TG Abs (25%)
Prevalence of T1D	2%-33%	40%-60%	80%
Additional autoimmune endocrine components	Hypoparathyroidism (80%-85%) Addison's disease (60%-70%) Hypogonadism (12%) Autoimmune thyroid disease (10%)	Autoimmune thyroid disease (70%-75%) Addison's disease (40%-50%) Hypoparathyroidism (0%-5%) Hypogonadism (0%-3%) Hypopituitarism (0%-2%)	Autoimmune thyroid disease (25%)
Concomitant non-endocrine diseases	Mucocutaneous candidiasis (70%-80%); autoimmune hepatitis; autoimmune gastritis; alopecia areata; vitiligo; keratoconjunctivitis	Autoimmune gastritis; pernicious anemia; neurodermitis; alopecia areata; myasthenia gravis; systemic lupus erythematosus; rheumatoid arthritis; autoimmune hepatitis	Malabsorption; autoimmune skin diseases; multiple sclerosis

Abs: Antibodies; AIRE: Autoimmune regulatory gene; ANA: Anti-nuclear antibodies; FOXP3: Forkhead box protein P3; IPEX: Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; PAS: Polyglandular autoimmune syndrome; TG: Transglutaminase; T1D: Type 1 diabetes.

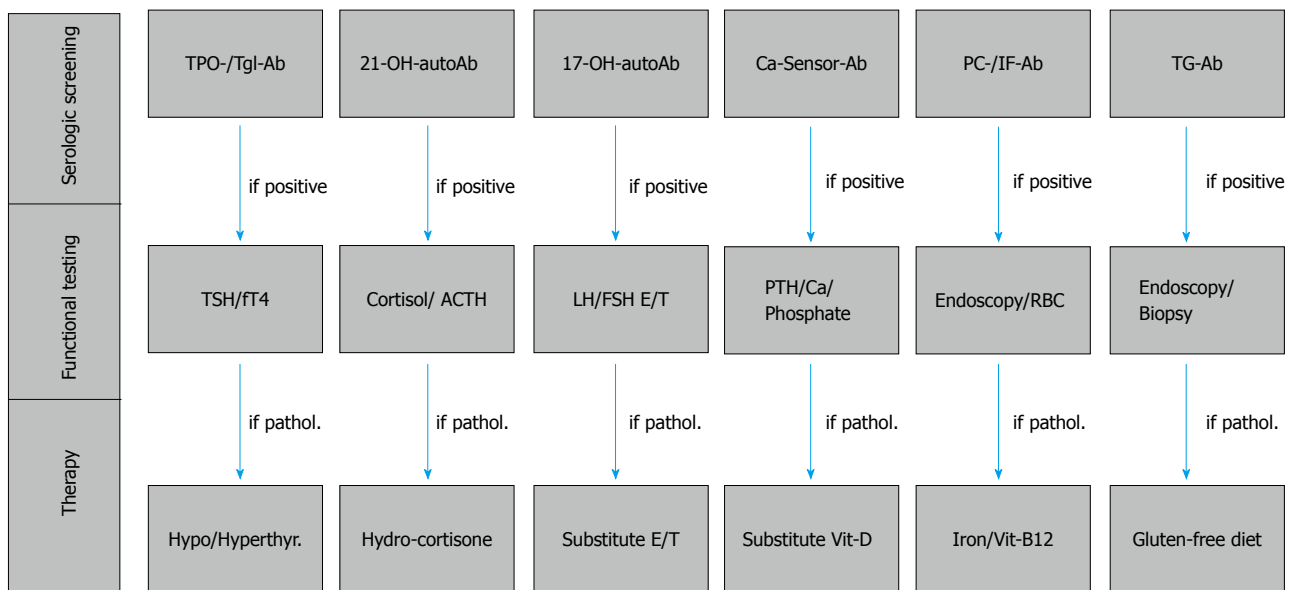


Figure 3 Serologic and functional screening in patients with type 1 diabetes. The serologic and functional screening for associated autoimmune diseases in patients with type 1 diabetes (T1D) performed at the onset of T1D and during follow-up appointments every two years. After diagnosis of thyroid dysfunction, adrenal failure, primary hypogonadism, hypoparathyroidism, type A autoimmune gastritis with or without pernicious anemia and celiac disease, substitution proceeds with levothyroxine, hydrocortisone, estradiol or testosterone, vitamin D, iron tablets and vitamin B12 intramuscularly, with a strict gluten-free diet. In contrast, hyperthyroidism due to the autoimmune Graves' disease will be managed first with the administration of anti-thyroid drugs (e.g., methimazole). Ab: Antibody; ACTH: Adrenocorticotropic hormone; Ca: Calcium; Ca-Sensor: Calcium-sensing receptor; E: Estradiol; FSH: Follicle-stimulating hormone; FT4: Free thyroxine; Hypo: Hypothyroidism; Hyperthy: Hyperthyroidism; IF: Intrinsic factor; LH: Luteinizing hormone; PC: Parietal cell; PTH: Parathyroid hormone; RBC: Red blood cell count; T: Total testosterone; TG: Transglutaminase/deaminated anti-gliadin; TgI: Thyroglobulin; TPO: Thyroid peroxidase; TSH: Thyrotropin; Vit: Vitamin; 17-OH: 17-hydroxylase; 21-OH: 21-hydroxylase.

high. Within a few years, approximately one third of T1D patients will develop thyroid autoantibodies and thyroid dysfunction leading to PAS type III. Furthermore, in subjects with either monoglandular T1D or the relatively rare autoimmune adrenal failure, organ-specific autoantibody screening and functional testing will help identify both patients at risk for developing PAS, as

well as subclinical PAS that may already be present. Clinicians should pay particular attention to autoimmune endocrinopathies, (e.g., Addison's disease or AITD), which are associated with T1D and strongly impact the patients' treatment with insulin. Thus, adrenal 21-hydroxylase autoantibodies should be assayed in all patients with T1D and GAD antibodies should be examined in all patients

with Addison's disease for early identification of subjects with a preclinical manifestation of a PAS. In conclusion, management of T1D within the context of PAS requires professional oversight and intervention provided in specialized centers for autoimmune endocrine and metabolic disorders.

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Diabetes and cardiac autonomic neuropathy: Clinical manifestations, cardiovascular consequences, diagnosis and treatment

Akif Serhat Balcioglu, Haldun Müderrisoğlu

Akif Serhat Balcioglu, Medical and Research Center of Alanya, Department of Cardiology, Başkent University, 07400 Alanya, Antalya, Turkey

Haldun Müderrisoğlu, Faculty of Medicine, Department of Cardiology, Başkent University, 06490 Ankara, Turkey

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Correspondence to: Akif Serhat Balcioglu, MD, Medical and Research Center of Alanya, Department of Cardiology, Başkent University, Saray Mahallesi, Yunus Emre Caddesi, No: 1, 07400 Alanya, Antalya, Turkey. serhatbalcioglu@gmail.com

Telephone: +90-242-5102525

Fax: +90-242-5115563

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dilatation. It causes a wide range of cardiac disorders, including resting tachycardia, arrhythmias, intraoperative cardiovascular instability, asymptomatic myocardial ischemia and infarction and increased rate of mortality after myocardial infarction. Etiological factors associated with autonomic neuropathy include insufficient glycemic control, a longer period since the onset of diabetes, increased age, female sex and greater body mass index. The most commonly used methods for the diagnosis of CAN are based upon the assessment of heart rate variability (the physiological variation in the time interval between heartbeats), as it is one of the first findings in both clinically asymptomatic and symptomatic patients. Clinical symptoms associated with CAN generally occur late in the disease process and include early fatigue and exhaustion during exercise, orthostatic hypotension, dizziness, presyncope and syncope. Treatment is based on early diagnosis, life style changes, optimization of glycemic control and management of cardiovascular risk factors. Medical therapies, including aldose reductase inhibitors, angiotensin-converting enzyme inhibitors, prostoglandin analogs and alpha-lipoic acid, have been found to be effective in randomized controlled trials. The following article includes the epidemiology, clinical findings and cardiovascular consequences, diagnosis, and approaches to prevention and treatment of CAN.

Key words: Diabetes mellitus; Autonomic neuropathy; Heart rate variability; Cardiac; Cardiovascular reflex tests

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Abstract

Cardiac autonomic neuropathy (CAN) is a frequent chronic complication of diabetes mellitus with potentially life-threatening outcomes. CAN is caused by the impairment of the autonomic nerve fibers regulating heart rate, cardiac output, myocardial contractility, cardiac electrophysiology and blood vessel constriction and

Core tip: Although very frequent, cardiac autonomic neuropathy (CAN) is one of the most commonly overlooked complication of diabetes. Higher incidence of cardiovascular events is encountered with CAN due to its relation with silent myocardial ischemia, arrhythmias, intraoperative cardiovascular instability,

orthostatic hypotension and cardiomyopathy. Diabetic patients should be screened for CAN due to the possibility of reversal of cardiovascular denervation in the early stages of the disease. Cardiovascular reflex tests and Holter-derived time- and frequency-domain measurements are frequently used for the diagnosis. Therapeutic approaches are promising and may hinder or reverse the progression of the disease when initiated during the early stages.

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INTRODUCTION

Cardiac autonomic neuropathy (CAN), a type of generalized symmetric polyneuropathy, is the most examined and clinically significant diabetic autonomic neuropathy^[1]. The autonomic nervous system has 2 major components: the parasympathetic and the sympathetic nervous systems. These may operate independently of each other or interact cooperatively to control heart rate, cardiac output, myocardial contractility, cardiac electrophysiology, and the constriction and dilatation of blood vessels^[2]. CAN is caused by damage to the autonomic nerve fibers that innervate the heart and blood vessels and leads to abnormalities in cardiovascular dynamics^[2]. The earliest finding of CAN, even at the subclinical stage, is a decrease in heart rate variability (HRV)^[3].

EPIDEMIOLOGY

Diabetes is estimated to affect approximately 350 million people globally^[4]. Diabetic neuropathies, including CAN, are frequent chronic complications of type 1 and 2 diabetes that influence quality of life and have potentially fatal outcomes^[2]. Prevalence rates between 1.6% to 90% have been reported, varying according to the diagnostic methods used, population studied and disease stage^[1]. The Diabetes Control and Complications Trial (DCCT) showed abnormal HRV values of 1.65%, 6.2% and 12.2% in patients with diabetes for a duration of less than 5 years, 5 to 9 years and more than 9 years, respectively^[5]. A study including 1171 patients with both type of diabetes mellitus reported impaired HRV tests in 25.3% of type 1 and 34.3% of type 2 patients^[6]. The different methodology between various studies makes epidemiological comparison difficult. Risk factors of decreased HRV in patients with type 2 diabetes include age, duration of diabetes, obesity and smoking^[2]. In type 1 diabetes, risk factors of CAN include higher levels of HbA1c, hypertension, distal symmetrical polyneuropathy, retinopathy and hyperglycemic exposure^[7,8].

PATHOGENESIS

Diabetic CAN is eventually caused by complex interactions among a number of pathogenic pathways. Hyperglycemia is the leading cause of the initiation of this pathogenic process^[9,10]. The pathogenesis of diabetic CAN is multifactorial, including increased mitochondrial production of free radicals due to hyperglycemia-induced oxidative stress. Neuronal activity, mitochondrial function, membrane permeability and endothelial function are impacted by advanced glycosylation end product formation, polyol aldose reductase signaling and poly(ADP ribose) polymerase activation and the alteration of the Na⁺/K⁺-ATPase pump function. Neuronal apoptotic processes are precipitated by endoplasmic reticulum stress induced by hyperglycemia, along with impaired nerve perfusion, dyslipidemia, alterations in redox status, low-grade inflammation and disturbance in calcium balance^[11]. The literature has described these mechanisms and their interactions but is beyond the scope of this article^[9-11].

CLINICAL MANIFESTATIONS

Diabetes can lead to dysfunction in the autonomic nervous system, causing various cardiovascular disorders, including resting tachycardia, postural hypotension, higher intra-/perioperative cardiovascular instability, more frequent asymptomatic myocardial ischemia and infarction, and greater mortality after myocardial infarction^[2]. Clinical symptoms associated with CAN generally occur at later stages and include postural hypotension, dizziness, lightheadedness, presyncope, syncope, and early fatigue and exhaustion during exercise^[1]. However, subclinical autonomic dysfunction, revealed as deterioration in HRV, can occur in the 1st year following diagnosis in patients with type 2 diabetes and within 2 years following diagnosis in type 1^[12]. Low *et al*^[13] reported a higher rate of autonomic symptoms in type 1 than in type 2 diabetes. A greater number of autonomic symptoms have been associated with an increased risk of CAN as measured by HRV^[14].

Because neuropathy first affects the longest nerve fibers, the first manifestation of diabetic CAN tends to be related with vagus nerve damage, which is responsible for nearly 75% of parasympathetic activity^[9]. This damage causes resting tachycardia as the sympathetic tone becomes dominant^[15]. Tachycardia eventually diminishes in a few years due to progressive sympathetic nerve fiber damage. However, increased heart rate persists in these patients^[16]. The progressive damage of the autonomic balance is indicated by additional symptoms, including intolerance to exercise, orthostatic hypotension and a further HRV reduction^[17]. Cardiac pain perception often deteriorates with the involvement of sensory nerve fibers, making patients prone to silent ischemia and myocardial infarction^[18,19].

Etiological factors associated with autonomic neuropathy include poor glycemic control, longer diabetes duration, increased age, female sex and greater body

mass index^[20]. Mortality rates of 25% to 50% within 5 to 10 years of diagnosis have been found in patients with symptomatic autonomic dysfunction^[21,22]. Among diabetic patients, the 5-year mortality rate is 3 times higher in those with autonomic involvement than in those without^[23].

CARDIOVASCULAR CONSEQUENCES

Impaired heart rate variability

HRV is a physiological variation in the interval between heartbeats and is regulated by the interaction of the sympathetic and parasympathetic tone^[24]. The functional response to the instantaneous metabolic needs of the body is regulated by this beat-to-beat variation. As high variability reflects the cardiac ability to adapt and implies good health, damage or disturbances to this control system results in lower HRV values. Even in a normal heart rate, the first finding of CAN is a decrease in HRV, which is apparent at the subclinical stage and can be detected through deep respiration^[25].

Resting tachycardia

Due to the dominant sympathetic tone, resting heart rates of 90 to 100 beats per minute with occasional increases to as many as 130 beats per minute are frequent findings in CAN with vagal impairment^[16,26]. Highest resting heart rates have been shown in patients with lone parasympathetic impairment^[16,26]. As the disease progresses and involves both parasympathetic and sympathetic nerves, the heart rate tends to return to the normal range but remains higher than in healthy individuals^[16,26]. Tang *et al*^[27] showed that resting heart rate is independently associated with CAN and has a high predictive value in predicting CAN in the general population^[27]. A steady heart rate less responsive to exercise, stress or sleep is suggestive of almost total cardiac denervation, which indicates severe CAN^[20].

Exercise intolerance

Exercise tolerance is worsened by CAN through the blunting of the increases in heart rate, blood pressure and cardiac output response to exertion^[20]. The development of hypotension or hypertension following strenuous exercise is more likely in individuals with CAN, particularly in the onset of a new exercise program^[1]. Therefore, patients with diabetes probably to have CAN should be checked for cardiac stress before beginning to exercise^[1]. Due to poor thermoregulation, such patients should avoid exercising in environments that are too hot or cold and hydrate adequately^[1].

Intraoperative and perioperative cardiovascular instability

The perioperative risk of cardiovascular morbidity and mortality are 2 to 3 times higher in diabetic individuals^[28]. Since the normal autonomic response of vasoconstriction and the increase in heart rate cannot appropriately compensate for the vasodilatation and negative chronotropic

effects of anesthesia, diabetic patients with CAN are subject to more pronounced decreases in blood pressure and heart rate during induction of anesthesia and, to a lesser extent, after intubation and extubation^[20,28]. In addition, more severe intraoperative hypothermia in patients with CAN resulting in decelerated metabolism of anesthetic drugs may cause deepening of anesthesia and/or delayed recovery^[29]. Accordingly, screening for CAN is recommended during the preoperative evaluation of diabetic patients for anesthetic management planning.

Non-dipping blood pressure profile and orthostatic hypotension

Blood pressure has diurnal variations. Decreases in nighttime and increases in daytime blood pressure show its circadian rhythm^[30]. Declines in parasympathetic tone occur at night and nocturnal unopposed sympathetic activity leads to deterioration of the circadian rhythm of blood pressure and results in the lack of or a less than 10% reduction in nocturnal blood pressure in patients with CAN^[31]. Such “non-dipper” CAN subjects experience more frequent left ventricular hypertrophy and are predisposed to cardiovascular events^[32]. Another blood pressure regulation abnormality related to CAN is orthostatic hypotension, which is defined as a decrease in systolic blood pressure by a minimum of 20 mmHg (at least 30 mmHg in patients with hypertension) or diastolic blood pressure of 10 mmHg in response to postural shifts from the supine to the standing^[2,20]. In diabetic individuals, orthostatic hypotension develops as a consequence of denervation of the efferent sympathetic vasomotor nerves, especially in the splanchnic vascular bed^[33]. Additionally, pathogenesis of orthostatic hypotension includes lower cutaneous, splanchnic and total vascular resistance^[10]. Impaired chronotropic and/or blood pressure response to exercise may accompany this condition^[10]. Orthostatic hypotension can cause many symptoms that reduce quality of life or lead to serious injury due to falling, such as lightheadedness, dizziness, faintness, visual blurring, presyncope and syncope while standing^[20]. However, an important number of patients are asymptomatic despite significant decreases in blood pressure^[2]. Orthostatic hypotension can be provoked by several drugs, which may be used concurrently for treatment of diabetes or its complications, including, vasodilators, diuretics, phenothiazines, insulin (through endothelium-dependent vasodilation) and tricyclic antidepressants for symptomatic relief of pain associated with diabetic neuropathy^[9].

Silent myocardial ischemia

Coronary artery disease has long been considered as a major complication of diabetes mellitus^[34]. Numerous studies have reported more extensive atherosclerotic disease, particularly that of the coronary arteries, in diabetic individuals^[35,36]. Silent myocardial ischemia is defined as the objective documentation of myocardial ischemia without angina or its equivalents^[37]. Several

reports have shown the predictive role of silent ischemia during exercise testing^[38] or ambulatory electrocardiography (ECG) monitoring^[39] on poor clinical outcomes and survival. A threefold increase in cardiac deaths was witnessed over a 2-year follow-up in individuals with silent ischemia during ambulatory ECG monitoring^[39]. Painless presentation of myocardial infarction in patients with CAN may include diaphoresis, dyspnea, fatigue, lightheadedness, palpitations, acute confusion, indigestion, nausea, and vomiting^[20]. Several explanations are possible for the variety of symptom patterns in individuals with diabetes, such as various pain perception thresholds, sensorial denervation secondary to autonomic neuropathy and psychological disavowal^[34]. Therefore, despite increasing ischemia, individuals with CAN and coronary artery disease may resume exercise as the longer threshold or subthreshold ischemia is not sufficient to induce pain, thus endangering the patient^[40]. Likewise, the Framingham Heart Study reported higher incidences of painless myocardial infarction in patients with diabetes than without (39% *vs* 22%)^[41,42]. Similarly, the National Registry of Myocardial Infarction 2 (NORMI-2) survey found that one-third of patients presented without angina^[43]. In the NORMI-2, diabetes was present in 32% of subjects without chest pain and 25.4% with angina^[43]. The Detection of Ischemia in Asymptomatic Diabetic Study, including 1123 patients with type 2 diabetes, reported that CAN is able to strongly predict silent ischemia and succeeding adverse cardiovascular events^[44]. Vinik *et al*^[10], in their meta-analysis of 12 studies, reported the association between CAN and the existence of silent ischemia detected by exercise stress tests with prevalence rate ratios of 0.85 to 15.53^[10]. Therefore, patients with CAN require more rigorous evaluation of the presence of coronary artery disease. The presence and extent of macrovascular coronary artery disease in such patients can be noninvasively tested by resting and stress thallium myocardial scintigraphy^[11]. Moreover, cardiovascular autonomic function testing should be included in the coronary artery risk assessment of all diabetic patients.

Myocardial infarction and increased risk of mortality

Myocardial infarction tends to be more extensive and severe in patients with diabetes^[42,45]. The timely diagnosis of myocardial ischemia or infarction is often delayed due to diminished angina perception and therefore the period before first medical contact is prolonged^[46]. Long-term survival rates following acute myocardial infarction are lower in patients with diabetes^[20]. In diabetic patients, 5-year survival rates of 38% have been reported following the first major coronary event, lowering to just 25% for those with subsequent events, while the rates are 75% and 50%, respectively, in non-diabetic patients^[45,47]. HRV has been demonstrated to be a good predictor of post-myocardial infarction mortality^[48-50]. For this reason, cardiovascular autonomic function testing is advisable for all diabetic patients after a myocardial infarction to identify the candidates who have a high risk of death^[51].

Sudden death

CAN is associated with a higher risk of malignant arrhythmias and sudden death^[9]. Previous studies have found 5-year mortality rates between 16% and 50% in patients with both CAN and either type of diabetes, often attributed to sudden cardiac death^[14,23]. Severe asymptomatic ischemia inducing fatal arrhythmias has been reported as the leading potential cause^[22]. Additionally, life-threatening arrhythmias and sudden death may be predisposed by QT prolongation^[52]. The European Diabetes Insulin-Dependent Diabetes Mellitus (EURODIAB IDDM) Complications Study established the association between impaired HRV and corrected QT prolongation^[53]. In addition, unopposed increases in sympathetic activity and resultant norepinephrine signaling and metabolism^[9], along with increased mitochondrial oxidative stress^[54] and calcium-dependent apoptosis^[55], is thought to contribute to myocardial injury^[54,56] and clarify the higher risk of sudden cardiac events and deaths. The EURODIAB IDDM Prospective Cohort Study, including 2787 patients with type 1 diabetes, reported CAN to be the strongest predictor of mortality over the 7-year follow-up period, even greater than traditional cardiovascular risk factors^[57]. A meta-analysis including 15 studies and 2900 diabetic patients showed CAN patients to have a pooled relative risk of mortality of 3.45 (95%CI: 2.66-4.47) and an increase in line with higher numbers of cardiovascular autonomic function abnormalities^[58]. Similar results were confirmed in 2 other studies of patients with type 1 and type 2 diabetes, strengthening the role in predicting mortality of abnormalities in both HRV and the QT index independent of conventional risk factors^[59,60]. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial^[61] also confirmed the association between CAN and mortality in type 2 diabetic patients. In this trial, Pop-Busui *et al*^[62] showed that mortality was between 1.55 and 2.14 times more likely in patients with baseline CAN than those without^[62]. Three large studies (the ACCORD trial^[61], the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation trial^[63], and the Veterans Affairs Diabetes Trial^[64]) investigated the role of more intensive treatment and the frequency of cardiovascular events on individuals with long-term type 2 diabetes. In these trials, tight glycemic control was not shown to reduce cardiovascular adverse events^[2] and the ACCORD trial was terminated early due to the increased mortality risk in the intensive therapy group^[65]. Hypoglycemia is known to reduce the threshold for malignant ventricular arrhythmias that can result in sudden death^[9,66]. In addition, hypoglycemic periods were reported to lead to impaired autonomic cardiovascular function even in healthy volunteers^[67]. Hence, the cause of a lack of a decrease, or even an increase, in cardiovascular events in the intensive therapy arm may be linked to deterioration of cardiac autonomic function due to episodes of hypoglycemia.

Cardiomyopathy

Diabetic cardiomyopathy is defined as structural and

functional myocardial abnormalities without coronary artery disease, hypertension or valvular heart disease^[68]. It is characterized by diastolic dysfunction^[69]. The responsible mechanisms are left ventricular hypertrophy (increased left ventricular mass and concentric remodeling^[70]), myocardial lipotoxicity, increased oxidative stress, cell death, interstitial and perivascular fibrosis, impaired contractile reserve, changes in myocardial substrate and energy metabolism, altered substrate utilization and mitochondrial dysfunction^[69]. In patients with CAN, the initial augmentation in cardiac sympathetic activity stimulates the renin-angiotensin-aldosterone system and increases heart rate, stroke volume and peripheral vascular resistance^[71]. In addition, the combination of sympathetic hyperactivity and regional myocardial sympathetic denervation cause reduced coronary blood flow and diastolic dysfunction, which may lead to impairment of systolic function^[2].

Stroke

Previous studies have revealed the relationship between CAN and cerebrovascular events. Ko *et al*^[72] reported that the presence of CAN, assessed by HRV testing, was significantly associated with the ischemic stroke in a study of 1458 patients with type 2 diabetes with a 7-year follow-up^[72]. Another study of 133 subjects with type 2 diabetes showed that stroke could be predicted by parasympathetic and sympathetic autonomic function abnormalities^[73].

DIAGNOSTIC METHODS OF CARDIAC AUTONOMIC NEUROPATHY

Variability in heart rate and blood pressure values can provide data regarding both parasympathetic and sympathetic autonomic function and is useful in clinical settings.

Heart rate variability

The most commonly used methods for the diagnosis of CAN are based on HRV assessment. HRV testing is noninvasive and objective in the evaluation of cardiac autonomic function and can be performed by recording electrocardiograms during deep breathing, standing, and Valsalva maneuvers^[74]. HRV analysis enables the independent measurement of the sympathetic and parasympathetic components of the autonomic nervous system and can be assessed with a number of simple clinical tests^[75] or easier digital 24-h electrocardiographic recordings^[24]. In the 1970s, Ewing *et al*^[75] discovered 5 simple tests of short-term R-R alterations to identify CAN in patients with diabetes: (1) heart rate response to respiration, which measures beat to beat sinus arrhythmia (R-R variation) during paced deep expiration and inspiration (E:I ratio); (2) heart rate response to standing, known as the 30:15 ratio, which is the ratio of the longest R-R interval (between beats 20 to 40) to the shortest R-R interval (between beats 5 to 25); (3) the

Valsalva maneuver, measuring the heart rate response during and after a provoked increase in intra-thoracic and intra-abdominal pressures; (4) blood pressure response to orthostasis, which evaluates baroreflex mediated blood pressure fluctuations after changes in posture; and (5) blood pressure response to isometric exercise, as defined by the diastolic blood pressure increase due to continuous muscle contraction using a handgrip dynamometer^[75]. Tests 1 and 2 reflect parasympathetic function, and 4 and 5, sympathetic function^[76,77]. Although the Valsalva ratio primarily represents parasympathetic activity, the resultant autonomic changes are complicated and include both the sympathetic and parasympathetic components^[78]. An American Diabetes Association statement describes these validated cardiac autonomic reflex tests (CART) in detail and recommends their use in the diagnosis of CAN (Table 1)^[1]. HRV with deep breathing is the most commonly used autonomic function test and has a specificity of approximately 80%^[79,80].

The assessment of HRV has become easier and more detailed due to new, digital, high frequency, 24-h multi-channel electrocardiographic recorders and the use of statistical indexes in the time and frequency domains.

Time domain methods

Time domain methods determine the heart rate at any point in time or the intervals between consecutive normal complexes^[24]. Each QRS complex is detected and the normal-to-normal (NN) intervals (that is all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined through a continuous ECG record^[24]. Simple time-domain variables that can be analyzed include mean NN interval, mean heart rate, the difference between the longest and the shortest NN interval and the variation between night and day heart rate^[24]. More complex time-domain parameter calculations can be performed from a series of instantaneous heart rates or cycle intervals, particularly those recorded during 24-h periods^[24]. The complex parameters can be divided into 2 classes: those derived from direct measurements of the NN intervals and those derived from the differences between NN intervals. The parameters calculated by direct measurements include: standard deviation of the NN interval (SDNN), reflecting all cyclic components responsible for variability in the recording period; standard deviation of the average NN interval (SDANN) calculated over periods (usually 5 min), estimating heart rate changes from cycles longer than 5 min; and the mean of the 5-min standard deviation of the NN interval (SDNN index) calculated over 24 h, measuring variability from cycles of less than 5 min. Secondly, the parameters calculated by the differences between NN intervals include the square root of the mean squared differences of successive NN intervals (RMSSD), the number of interval differences of successive NN intervals of more than 50 msec (NN50), and the division of NN50 by the total number of NN intervals (pNN50)^[24]. Although influenced by several

Table 1 Cardiovascular autonomic reflex tests^[1]

Test	Technique	Normal response and values
Beat-to-beat HRV	With the patient at rest and supine, heart rate is monitored by ECG while the patient breathes in and out at 6 breaths per minute, paced by a metronome or similar device	A difference in heart rate of > 15 beats per minute is normal and < 10 beats per minute is abnormal. The lowest normal value for the expiration-to inspiration ratio of the R-R interval decreases with age: age 20-24 yr, 1.17; 25-29, 1.15; 30-34, 1.13; 35-39, 1.12; 40-44, 1.10; 45-49, 1.08; 50-54, 1.07; 55-59, 1.06; 60-64, 1.04; 65-69, 1.03; and 70-75, 1.02. Normally, a tachycardia is followed by reflex bradycardia. The 30:15 ratio should be > 1.03
Heart rate response to standing	During continuous ECG monitoring, the R-R interval is measured at beats 15 and 30 after standing	Healthy subjects develop tachycardia and peripheral vasoconstriction during strain and an overshoot bradycardia and rise in blood pressure with release. The normal ratio of longest R-R to shortest R-R is > 1.2
Heart rate response to the Valsalva maneuver	The subject forcibly exhales into the mouthpiece of a manometer to 40 mmHg for 15 s during ECG monitoring	Normal response is a fall of < 10 mmHg, borderline fall is a fall of 10-29 mmHg and abnormal fall is a decrease of > 30 mmHg with symptoms
Systolic blood pressure response to standing	Systolic blood pressure is measured in the supine subject. The patient stands and the systolic blood pressure is measured after 2 min	The normal response for diastolic blood pressure is a rise of > 16 mmHg in the other arm
Diastolic blood pressure response to isometric exercise	The subject squeezes a handgrip dynamometer to establish a maximum. Grip is then squeezed at 30% maximum for 5 min	

HRV: Heart rate variability; ECG: Electrocardiogram.

arrhythmias and requiring normal sinus rhythm and atrioventricular nodal function, these short-term variation measurements estimate high frequency variations in heart rate and are therefore highly correlated^[24]. SDNN represents both the sympathetic and parasympathetic modulation of HRV, and RMSSD and pNN50 the parasympathetic system^[24].

Frequency domain methods

CAN may also be evaluated using spectral analysis of HRV, which divides the R-R signal into sine and cosine waves to estimate the amount of variability as a function of frequency^[24]. Three main spectral components are distinguished in a spectrum calculated from short-term recordings of 2 to 5 min: very-low-frequency (≤ 0.04 Hz) of fluctuations in vasomotor tone related to thermoregulation, low-frequency (0.04-0.15 Hz) associated with the baroreceptor reflex, and high-frequency (0.15-0.4 Hz) related to respiratory activity^[24]. The sympathetic system is thought to modulate the 2 low-frequency components and the parasympathetic system the high-frequency component^[20]. Accordingly, while decreases in very-low- and low-frequency peaks indicate sympathetic dysfunction, a decrease in the high-frequency peak is a sign of parasympathetic dysfunction^[1]. A decrease in the ratio of low-frequency-to-high-frequency demonstrates sympathetic imbalance^[1]. Various mathematical methods can be used to analyze the spectral components of HRV. Most common is the Fourier transform because of its simplicity and high processing speed^[24]. A noise-free signal is necessary in order to correctly perform the spectral analysis. Because artefacts and extra beats must be removed, this correction leads to data loss and is associated with an underestimation in each case. Additionally, specific reference values must be obtained as each HRV-analysing device has different technical properties for spectral measurements.

It is not yet clear which of these 2 methods is preferable: time-domain methods including the standardized CART of Ewing or frequency-domain methods. However, many time- and frequency-domain variables obtained over the 24-h period are highly correlated with each other^[24]. On the other hand, studies comparing Holter-based analysis and CART found a high (83%) correlation between the both techniques^[81]. The advantages of Holter-based techniques include simpler, less stressful and faster implementation during daily routine use, independence of patient cooperation and greater sensitivity allowing for the identification of disorders in the early stages. However, CART can be applied quickly (less than 15 min) using stand-alone operator friendly devices during routine physical examination.

Heart rate turbulence

Another Holter-based technique for evaluating CAN is the heart rate turbulence (HRT)^[82]. HRT refers to sinus rhythm cycle length fluctuations following isolated premature ventricular beats. After an initial acceleration, the sinus rate decelerates after a premature ventricular beat. There are 2 components of HRT; turbulence onset and turbulence slope. A transient vagal inhibition triggers the mentioned initial acceleration in heart rate as a response to the missed baroreflex afferent input due to hemodynamically ineffective ventricular contraction. The successive deceleration in heart rate is caused by a sympathetically mediated overshoot of arterial pressure through vagal recruitment^[82]. HRT evaluation can be used in the risk assessment after acute myocardial infarction and in the monitoring of disease progression in heart failure and CAN^[82]. We previously demonstrated that among HRV and HRT indexes, turbulence slope has the greatest correlation with CAN severity^[83]. A turbulence slope of below 3.32 msec/R-R is 97% sensitive and 71% specific for the diagnosis of CAN as detected by the

CART in patients with type 2 diabetes^[83].

Other diagnostic tools

Other methods currently used in research settings are scintigraphic evaluation of sympathetic innervation of the heart, which can reveal cardiac sympathetic nerve population changes and early anatomical regional deficits of sympathetic denervation^[84-86]; microneurography, which records electrical activity released by peroneal, tibial or radial sympathetic nerves and identifies sympathetic dysfunction^[87]; neurovascular flow, using noninvasive laser Doppler measures of peripheral sympathetic reactions to nociception^[88]; and baroreflex sensitivity, which evaluates the capability to reflexively increase vagal activity in response to a sudden increase in blood pressure^[89]. As many of these tests assess the influence on sympathetic component, they do not provide information about early stage CAN. In a recent study, it was shown that altered cardiac autonomic balance can be detected through exercise stress testing in diabetic subjects even with minimal evidence of CAN^[90].

Criteria for diagnosis and staging

CART are the gold standard clinical tests for cardiovascular autonomic neuropathy^[91]. Following the 8th international symposium on diabetic neuropathy in 2010, criteria for diagnosis and staging of CAN are defined in the CAN Subcommittee of the Toronto Consensus Panel statement^[92]. Accordingly, only 1 abnormal CART result is sufficient to diagnose possible or early CAN; among the 7 autonomic function analysis (5 CART, time-domain and frequency-domain HRV tests), 2 or 3 abnormal tests indicate definite or confirmed CAN; and severe/advanced CAN can be indicated by concurrent orthostatic hypotension^[91].

SCREENING FOR CAN

By the time clinical signs occur, CAN has often reached to a late stage, making management more difficult. Therefore, patients should be screened for CAN at the time of diagnosis of type 2 diabetes and within 5 years of diagnosis of type 1 diabetes (except presence of symptoms suggesting autonomic neuropathy earlier)^[1]. In addition, screening may be of benefit before undergoing an operation or beginning a new intense exercise program^[93,94]. Screening should include a clinical history and an evaluation for evidence of autonomic dysfunction. Main HRV tests (E:I ratio, heart rate response to Valsalva maneuver, and heart rate response to standing) should also be performed. As an alternative to CART, easier screening methods has been attempted to develop. For instance, sudomotor function tests that assess the cholinergic innervation of sweat glands have been found to be useful for early screening of CAN^[95]. Ge *et al*^[96] offered a new risk score system not requiring specific tests for screening CAN, using clinical parameters including age, body mass index, hypertension and resting heart rate. The risk score can be between 0 and 15, and

a score of 6 can detect CAN in 72.87% of previously undiagnosed individuals^[96]. Screening should be repeated annually in the presence of negative results^[1].

THERAPEUTIC APPROACHES

Early determination of CAN is significant for the success of therapeutic strategies as cardiovascular denervation seems to be reversible at onset^[97]. In less affected patients, lifestyle changes including graded supervised exercise associated with weight loss improve HRV^[97].

Optimizing glycemic control

Blood glucose optimization is the essential treatment for CAN. The Framingham Heart Study showed the significant association between reduced HRV and increased fasting plasma glucose level^[98]. This finding is present in diabetics as well as individuals with impaired glucose tolerance^[99]. Additionally, the DCCT reported that intensive insulin therapy reduced the incidence of CAN in comparison to conventional insulin therapy after approximately 5 years (14% *vs* 7%; $P < 0.004$) in type 1 diabetics^[100]. The Epidemiology of Diabetes Intervention and Complication Study (EDIC) is a longitudinal cohort follow-up study for the DCCT^[101]. Pop-Busui *et al*^[102] demonstrated that during EDIC follow-up, CAN progressed in both the conventional and intensive insulin therapy groups, while its incidence and prevalence remained lower in the intensive therapy group despite similar glycemic control^[102]. Accordingly, the early initiation of intensive glucose control in type 1 diabetics can help to minimize the development of CAN^[102]. On the other hand, the benefit of glycemic control in type 2 diabetics is less certain^[1,2]. The Veterans Affairs Cooperative Study reported a similar prevalence of autonomic neuropathy in type 2 diabetics after 2 years of intense glucose control in comparison with conventional glycemic control^[103]. Similarly, in the Anglo-Danish-Dutch Study of Intensive Treatment in People With Screen Detected Diabetes in Primary Care Danish arm, CAN was frequently found 6 years following diagnosis of type 2 diabetes and this prevalence was not significantly affected by intensive multifactorial treatment in comparison with routine care^[104]. Conversely, in the Steno-2 Trial patients with type 2 diabetes were given intensive multifactorial treatment (*e.g.*, targeting hyperglycemia, hypertension and dyslipidemia, including acetylsalicylic acid for secondary prevention) and targeted strict glycemic control as well as other cardiovascular risk factor modification, which reduced the incidence of autonomic dysfunction by approximately 60%^[105]. Briefly, the intensive blood glucose, HbA1c, blood pressure and lipid levels control using pharmacological therapy with lifestyle changes are recommended for all diabetic patients^[1].

Other therapies

Functional disorders of the autonomic nervous system can be treated with a variety of medications. In a trial

including 73 type 2 diabetic subjects, a four-month period of treatment with alpha-lipoic acid, which reduces oxygen free radicals, improved HRV detected by standardized CART^[1,106]. While the use of aldose reductase inhibitors (epalrestat, fidarestat and AS-3201), which reduce nerve sorbitol, had a positive influence on HRV in patients with mild abnormalities, they were ineffective in advanced CAN patients^[1,107]. Total HRV has been shown to be increased and parasympathetic/sympathetic balance improved by angiotensin-converting enzyme (ACE) inhibition in patients with mild autonomic neuropathy through increases in nerve blood flow^[1]. Prostaglandin analogs have been shown to be effective through the same mechanism^[1]. Cardiospecific beta-blockers are considered to have positive effects on autonomic dysfunction. For example, the addition of metoprolol to ramipril therapy in patients with type 1 diabetes resulted in recovery of HRV parameters^[108]. Furthermore, bisoprolol improved HRV in heart failure^[109]. In a study including individuals with long-term diabetes and diabetic neuropathy, the combination of ACE inhibition and angiotensin-receptor blockade improved autonomic neuropathy^[110]. In addition, Ozdemir *et al.*^[111] showed that losartan therapy significantly improved HRV in patients with ischemic cardiomyopathy already receiving ACE inhibitors and beta-blockers. Similarly, sympathovagal imbalance in heart failure patients was improved following the administration of spironolactone along with enalapril, furosemide, and digoxin^[112]. Such evidence reveals that combination therapies appear to provide better results than monotherapies.

Orthostatic hypotension

Because orthostatic hypotension is a relatively late complication of CAN, the treatment is challenging due to advanced disease. Nonpharmacological treatments include: increased water consumption; the use of lower-extremity stockings; avoidance of sudden postural changes to standing up; avoidance of medicines such as vasodilators, diuretics, phenothiazines and tricyclic antidepressants that provoke hypotension; eating frequent, small meals to prevent postprandial hypotension; and avoidance of exercises and maneuvers that increase intra-abdominal and intra-thoracic pressure resulting in venous return decrease^[20]. Some physical preventive maneuvers, such as crossing of the legs and squatting may counter decreases in blood pressure^[9]. While pharmacological treatments, such as midodrine, clonidine, octreotide, fludrocortisone acetate, erythropoietin, nonselective beta-blockers and pyridostigmine bromide appear promising, all have mild to severe side effects, including hypertension^[9].

CONCLUSION

Although very common and serious, CAN is a frequently overlooked complication of diabetes. Related with intraoperative and perioperative cardiovascular instability, abnormal blood pressure profile, orthostatic hypotension,

silent myocardial ischemia, arrhythmias, diabetic cardiomyopathy, and stroke, CAN is associated with significant increases in morbidity and mortality. Patients may have subclinical CAN for several years before it becomes clinically apparent. Because the progression of cardiovascular denervation is partly reversible or can be slowed down in the early stages of the disease, recent guidelines strongly recommend screening for CAN in patients with diabetes. Assessment of CAN is possible through a variety of methods, such as CART, HRV and imaging modalities. Operator friendly devices and use of Holter-based analysis has simplified CAN testing. Treatment principles include early diagnosis, optimization of glycemic control, life style changes and management of cardiovascular risk factors. Medical therapy, including aldose reductase inhibitors, ACE inhibitors, prostaglandin analogs and alpha-lipoic acid, have been found to be effective in randomized control trials for the treatment of autonomic neuropathies. Orthostatic hypotension, which may lead to life-threatening injuries, is an undesired manifestation and indicates severe or advanced CAN.

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Ocular complications of diabetes mellitus

Nihat Sayin, Necip Kara, Gökhan Pekel

Nihat Sayin, Department of Ophthalmology, Kanuni Sultan Suleyman Education and Research Hospital, 34303 Istanbul, Turkey

Necip Kara, Department of Ophthalmology, Gaziantep University, 27000 Gaziantep, Turkey

Gökhan Pekel, Department of Ophthalmology, Pamukkale University, 20070 Denizli, Turkey

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Correspondence to: Nihat Sayin, MD, Department of Ophthalmology, Kanuni Sultan Suleyman Education and Research Hospital, Atakent Mahallesi, 4. Cadde. C 2-7 Blok. Kat: 3 Daire: 13. Küçükçekmece, 34303 Istanbul, Turkey. nihatsayin@yahoo.com

Telephone: +90-533-4383755
 Fax: +90-212-5714790

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becoming more common. Ocular complications associated with DM are progressive and rapidly becoming the world's most significant cause of morbidity and are preventable with early detection and timely treatment. This review provides an overview of five main ocular complications associated with DM, diabetic retinopathy and papillopathy, cataract, glaucoma, and ocular surface diseases.

Key words: Diabetes mellitus; Diabetic retinopathy; Ocular complication; Neovascular glaucoma; Cataract; Ocular diseases

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Core tip: Ocular complications associated with diabetes mellitus (DM) are progressive and rapidly becoming the world's most significant cause of morbidity and are preventable with early detection and timely treatment. This review provides an overview of five main ocular complications associated with DM, diabetic retinopathy and papillopathy, cataract, glaucoma, and ocular surface diseases.

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Abstract

Diabetes mellitus (DM) is a important health problem that induces ernestful complications and it causes significant morbidity owing to specific microvascular complications such as, retinopathy, nephropathy and neuropathy, and macrovascular complications such as, ischaemic heart disease, and peripheral vasculopathy. It can affect children, young people and adults and is

INTRODUCTION

Complications of diabetes mellitus (DM) are progressive and almost resulting by chronic exposure to high blood levels of glucose caused by impairments in insulin metabolism and biological macromolecules such as carbohydrates, lipids, proteins and nucleic acids^[1]. DM and its complications are rapidly becoming the world's most significant cause of morbidity and mortality^[2,3]. The DM pandemic has expanded speedily in the developed

and developing countries. It is expected that DM will reach epidemic proportions within the near future^[4]. DM affects more than 240 million people worldwide, and this number is expected to reach roughly 370 million by 2030^[5,6]. DM can lead to several ocular complications such as diabetic retinopathy, diabetic papillopathy, glaucoma, cataract, and ocular surface diseases^[7]. Diabetes related ocular complications are general public health problem, so we purpose of putting emphasis on the frequencies, pathogenesis, and management of these ocular complications.

DIABETIC RETINOPATHY

Diabetic retinopathy (DR), a microangiopathy affecting all of the small retinal vessels, such as arterioles, capillaries and venules, is characterized by increased vascular permeability, ocular haemorrhages, lipid exudate, by vascular closure mediated by the development of new vessels on the retina and the posterior vitreous surface^[8]. DR, the most common microvascular complication of DM, is predicted to be the principal reason of new blindness among working population^[9,10]. DR is the major reason of blindness in adults 20-74 years of age in the United States of America^[11]. In patients with type 1 and type 2 diabetics with disease duration of over twenty years, the prevalences of DR are 95% and 60%, respectively^[12]. Roughly 25% of type 1 diabetic patients have been reported to be influenced with DR, with the frequency increasing to about 80% after 15 years of anguish^[13]. The type 2 DM is responsible for a higher percentage of patients with visual loss^[13]. The incidence of DR is related primarily to duration and control of diabetes and is related to hyperglycemia, hypertension, hyperlipidemia, pregnancy, nephropathy, and anemia^[14-16]. According to reports published by Wisconsin epidemiologic study of diabetic retinopathy (WESDR)^[17], the general 10-year incidence of DR was 74%. Moreover in 64% of people with baseline DR developed more severe DR and 17% of those advanced to occur proliferative DR^[18].

Pathogenesis

There is a very strong relationship between chronic hyperglycemia and the development of DR^[19,20]. Hyperglycemia triggers a sequence of events causing vascular endothelial dysfunction. Many interdependent metabolic pathways have been put forward as important connections between hyperglycemia and DR. These implicated metabolic pathways include increased polyol^[21] and protein kinase C (PKC) pathway^[22] activity, upregulation of growth factors of which vascular endothelial growth factor (VEGF)^[22], generation of advanced glycation endproducts (AGEs)^[23,24], chronic oxidative damage^[25], increased activation of the renin angiotensin system (RAS)^[26], chronic inflammation and abnormal clumping of leukocytes (leukostasis)^[26].

When excessive amounts of glucose increase the polyol way is activated to reduce glucose into sorbitol.

The aldose reductase enzyme and nicotinamide adenine dinucleotide phosphate are involved in this biochemical reaction. Sorbitol is further metabolized to fructose by sorbitol dehydrogenase. Since sorbitol movement is severely restricted by cellular membrane, excessive accumulation of sorbitol in the cell occurs^[27,28]. The increased sorbitol has potential osmotic damage in retinal cells^[29] (Figure 1).

Chronic hyperglycemia increases quantity of diacylglycerol (DAG), which is leading to activate protein kinase C^[30]. This activation leads to increase vascular permeability and upregulation of VEGF in the retinal structure. However, this abnormal pathway may lead to increase the activation of leukostasis^[31-33] and significant changes in extracellular matrix (ECM) protein synthesis (Figure 2). Eventually, DAG and PKC pathway adversely affect inflammation, neovascularization, and retinal haemodynamics, which redounds to progression of DR^[26].

VEGF is a crucial mediator in microvascular complications of DM. Normally, numerous retinal cells such as, retinal pigment epithelial (RPE) cells, Mueller cells, and pericytes, produce VEGF^[31-33]. When a hypoxia occurs VEGF is secreted much more than normal production by hypoxic retinal tissues^[31]. Clinical studies have reported that there is a strong correlation between DR and intraocular VEGF concentrations. Intravitreal and intracameral VEGF levels were prominently increased in patients with proliferative diabetic retinopathy (PDR)^[34]. Additionally, VEGF has a crucial role in the pathogenesis of diabetic macular edema (DME) by increasing vascular permeability^[35,36].

AGEs have been implicated in several diabetic complications, such as DR, and DME. Under chronic hyperglycemic circumstances, proteins are nonenzymatically glycated and the excessive amount of AGEs alter structures and functions of ECM, basement membranes, and vessel wall.

Oxidative stress is also a serious condition that may result in microvascular complications^[37,38]. Severe production of reactive oxygen radicals may increase the oxidative stress and reduce antioxidant capacity^[39].

RAAS is the endocrine system that takes an essential role to regulate vascular blood pressure, electrolyte, and fluid balance and shows an aberration in patients with DM^[40], although the accurate process of RAAS leads to DR is not well clarified.

Inflammation is a prominent part of the pathogenesis of DR^[41,42]. In response to hyperglycemic stress, AGE formation, and hypertension, a sequence of inflammatory mediators are increased in DM. Retinal subclinical inflammation contributes to elevated intraocular perfusion pressure by means of endothelial nitric oxide synthase (eNOS), the development of neovascularization (NV) due to hypoxia and VEGF. Although there are no strong association between systemic inflammation and development of DR^[43,44], leukostasis is a likely to be a significant local factor in DR pathogenesis, causing capillary occlusion.

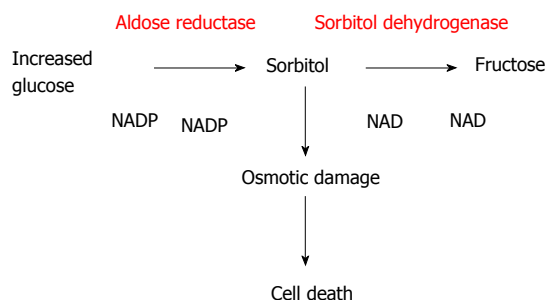


Figure 1 The polyol pathway.

The classification of DR

Previously, DR was classified into three forms, such as, background, pre-proliferative, and proliferative DR. The current classification is based on the location, extent, and degree of various clinically significant features, such as microaneurysms, intraretinal hemorrhages, venous abnormalities such as beading, intraretinal microvascular abnormalities (IRMA), and NV. Recently, DR is classified as either nonproliferative or proliferative.

Nonproliferative diabetic retinopathy: (1) Mild non-proliferative diabetic retinopathy (NPDR): There are a few microaneurysms; (2) Moderate NPDR: In this form, there are less than 20 microaneurysms. Hard yellow exudates, cotton wool spots, and venous beading are present also in only one quadrant; (3) Severe NPDR: It is identified as any of following clinic features; Microaneurysms in all 4 quadrants; Venous beading in 2 or more quadrants; IRMA in 1 or more quadrant; and (4) Very severe NPDR: This form includes 2 or more of the criteria for severe NPDR.

PDR: As a response to ischemia, NV grows at the optic nerve (NVD) and elsewhere in the retina except the optic disc (NVE). In general, NV grows at the border zone of perfused and non-perfused retina. These new vessels are permeable, and the leakage of plasma contents probably causes a structural change in the adjacent vitreous. Also, NV may cause preretinal and subhyaloid vitreous hemorrhages and can become membrane formations on the posterior hyaloid surface.

Diabetic macular edema

Macular edema is defined as retinal thickening or the existence of hard exudates at 2 disk diameter of the macula. Diabetic macular edema (DME) is the most common cause of moderate or severe visual loss in diabetic patients. DME occurs apart from the stage of DR, so it should be evaluated independently. In diabetic eyes, central macular thickness does not correlate directly with visual acuity, but there is a vigorous link between the unity of the photoreceptor inner/outer segment junction and visual acuity^[45].

Clinically significant macular edema

The Early Treatment Diabetic Retinopathy Study

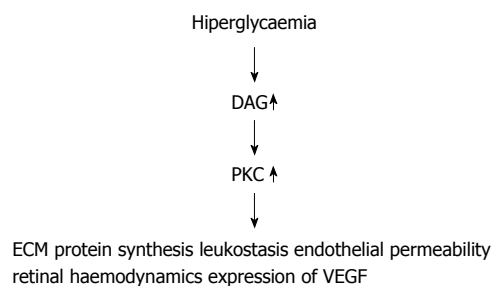


Figure 2 The protein kinase C pathway. DAG: Diacylglycerol; PKC: Protein kinase C; VEGF: Vascular endothelial growth factor; ECM: Extracellular matrix.

(ETDRS) described the clinically significant macular edema (CSME) as the following conditions: (1) Retinal thickening within 500 microns of the center of the fovea; (2) Hard yellow exudate within 500 microns of the center of the fovea with adjacent retinal thickening; and (3) Retinal thickening 1 disc area or larger, any part of which is within 1 disc diameter of the center of the fovea.

The ETDRS indicated that the presence of CMSE guide ophthalmologist for the focal laser treatment.

DME classification based on optical coherence tomography

Optical coherence tomography (OCT) shows four different types of DME: Sponge like retinal swelling, cystoid macular edema (CME), macular edema with serous retinal detachment (SRD) and tractional macular edema (TDME)^[46-48].

Sponge like retinal swelling: There is an increased diffuse retinal thickness with reduced intraretinal reflectivity. This type of retinal swelling has a better visual outcome than the CME, SRD and TRD types after laser treatment^[49].

CME: In this type, there is diffuse or focal retinal thickening with intraretinal cystic spaces.

SRD: There is an accumulation of subretinal fluid below reflective elevation. It is possible to confirm the presence of SRD only by OCT.

TDME: TDME is identified by a hyperreflective membrane on OCT with loss of foveal depression and macular edema.

First examination and follow-up

The WESDR study represented that, for type 1 diabetic patients, the frequency of NPDR at less than 5 years was 17% and the frequency of PDR was nearly 0%^[50]. These frequencies were nearly 99%, and 50% after 20 years later, respectively. So, the first eye exam should be performed almost 4 years after diagnosis with annual follow-up exams.

The same study indicated that, for type 2 diabetic patients, the frequency of NPDR at 5 years was nearly 30% and the frequency of PDR was nearly 2%^[51]. These frequencies were nearly 80%, and 15% after 15

years later, respectively. So, the first eye exam should be examined at diagnosis with annual follow-up exams.

Mild NPDR can be followed with dilated fundus exams every 12 mo. If DME that is not CSME is present, follow-up every 3 mo is advised. If CSME is present, treatment is advised promptly. Severe NPDR should be followed up every 2 mo. If very severe NPDR is present, patients should be followed more closely. After treatment of PDR, they should be observed every 3 mo not to overlook complications, such as TRD and CSME.

Current therapy

The treatment of DR includes increased metabolic control, laser treatment, intravitreal medication, and surgery.

Metabolic control

Poor metabolic control is a good marker for development and progression of DR. So, related risk factor such as, hyperglycemia, hypertension, and hyperlipidemia should be controlled. It reduces the risk of retinopathy occurrence and progression^[52].

Glysemic control

The trial research group^[53] showed that, for type 1 diabetic patients, a 10% reduction in the hemoglobin A1c (HbA1c) was associated with a 43% and 45% diminution in improvement of DR in the rigorous and traditional treatment group, respectively^[53]. The another trial group^[54] found that, for type 2 diabetic patients, tighter blood glucose control had been found to correlate most closely with a lower rate of DR^[54]. However, very strict control of blood glucose may lead to cause worsening of DR due to up regulation of insulin-like growth factor-1 (IGF-1)^[52,55,56].

Control of blood pressure

Hypertension is more common in type 2 diabetic patients rather than patients with type 1 DM. Approximately 40%-60% of patients with hypertension are over the age range of 45 to 75^[57]. Although the relationship between hypertension and progression of retinopathy is not certain, good blood pressure control pulls down the risk of DR. An another study^[58] reported that strict control of blood pressure reduces the risk of diabetic ocular complications^[58].

Control of serum lipids

There is a positive correlations between the severity of DR and plasma lipid levels, particularly LDL-HDL cholesterol ratio^[59]. Hard yellow exudates, which are lipid rich, have been found to correlate with plasma protein levels. Dietary and medicine therapy may reduce hard exudates^[60,61]. Systemic lipid-lowering drugs such as, fenofibrate reduced the need for focal laser treatment of CSME in type 2 diabetic patients^[62].

Laser treatment

Laser treatment has been considered the evidence-based treatment for DME and PDR for a long time.

Randomized studies have demonstrated the efficacy of laser photocoagulation to prevent vision loss from DME^[63,64]. In eyes observed with CSME, prompt photocoagulation is highly recommended. Treatment is performed at areas of focal leaking microaneurysms by using focal laser photocoagulation or at areas of diffuse leakage by using grid laser photocoagulation. Laser spot size should not be greater than 100 μm for focal laser treatment. Grid laser treatment is characterized by mild RPE whitening spots as far as 2 optic disks diameters from the center of the fovea^[65]. Combination treatment is applied in most patients, which involves focal and grid laser treatment.

Patients are reevaluated for retreatment at 3 mo intervals. For each retreatment, clinicians repeat the fluorescein angiogram to determine sites of persistent dye leakage. If patients have focal leakage with a circinate lipid ring, it may not be necessary to repeat angiogram before the treatment because the leaking focal lesions are in the lipid ring.

Panretinal laser photocoagulation (PRP) treatment became a standard of care for DR when the results of the Diabetic Retinopathy Study (DRS) were published^[66,67]. DRS showed that PRP enormously reduced the risk of severe vision loss from 16% to 6.4% in patient with PDR. The goal of PRP is not to improve visual acuity. It is applied to regress of the NVD or NVE and to prevent the blinding complications of DRP. Generally, laser treatment should be performed over a period of 4-6 wk by applying 1.500-2.000 burns, with a size of 500 μm , spacing spots 0.5 burn widths from each other with a 0.1-0.2 s duration^[65].

Intravitreal medication

The results of several investigations showed that these different intravitreal agents are effective not only in the prevention of visual loss, but also allowed a regain of visual acuity. The two main categories of intravitreal drugs recently used in the management of DME and PDR are steroids and anti-VEGF agents.

The use of intravitreal steroids are preferred to manage the DME. They have antiinflammatory and antiangiogenic effects that stabilize of the inner blood-retina barrier. Intraocular steroid injections have beneficial effects in PDR, by inhibiting production of the VEGF^[68,69]. Many various studies reported the benefits of injections of triamcinolone acetonide (IVTA) to reduce DME and increase visual acuity^[70-74].

The effects of intravitreal steroids are temporary and last for about 3 mo. In this cases, intravitreal steroids may be repeated. But complications such as elevated intraocular pressure and infection may occur. However, IVTA is more likely to be associated with cataract progression. Combination of IVTA and laser treatment has more beneficial effects in pseudophakic eyes than laser alone^[74].

Recently, a novel, biodegradable, slow-release dexamethasone implant (DEX implant, Ozurdex) was

developed to gradually release 0.7 mg of preservative-free dexamethasone in the vitreous cavity after a small incision^[75]. DEX implant have the advantage of a lower incidence of cataract and glaucoma than IVTA^[76]. The maximum effects of the DEX implant occur at 3 mo and gradually diminish from month 4 to 6^[77].

Anti-VEGF agents (pegaptanib, bavituzumab, ranibizumab, aflibercept) have been investigated as a treatment for DME and for PDR. Also, anti-VEGF injections might be useful adjuncts to facilitate effective fibrovascular membrane dissection in eyes with active vascularity components^[78]. TRD occur or progress within 1-4 wk of anti-VEGF injection, so, in general, these cases should be scheduled in a timely manner after the injection^[79].

Nowadays, clinicians have the option of four anti VEGF agents: Pegaptanib (Macugen), Bevacizumab (Avastin), Ranibizumab (Lucentis), Aflibercept (Eylea).

Pegaptanib is a selective VEGF antagonist that binds to the VEGF165 isoform. Intravitreal pegaptanib is currently an approved treatment in neovascular choroidal membrane, but several trials addressed the efficacy and safety of intravitreal pegaptanib injections in the treatment of PDR and DME^[80-82].

Bevacizumab^[83] is a full-size humanized antibody that binds to all VEGF-A isoform. Intravitreal bevacizumab is currently used beneficially in the off-label treatment of DR. There have been many studies with intravitreal bevacizumab injections and DME. The results of these retrospective or prospective trials showed an improvement in visual acuity and OCT outcomes. However, bevacizumab injections were also associated with short-term efficacy and a high recurrence rate^[83-88].

Ranibizumab is a high affinity anti-VEGF Fab specifically designed for ophthalmic use. It binds to all isoforms of VEGF-A and related degradation products and neutralizes their biological activity. Several studies confirmed its efficacy in treating DME^[89-94].

Aflibercept^[95] is an intravitreally administered fusion protein that is designed to bind both the VEGF-A and the placental growth factor with higher affinity in comparison to other anti- VEGF agents^[95]. Aflibercept has a longer duration of action in the eye after intraocular injection. This new agent has been recently investigated in the treatment of DME^[96,97].

Surgery

Pars plana vitrectomy (PPV) is considered an option for patients not responding to combined anti-VEGF- laser and/or steroid-laser therapy in DME^[98]. PPV, including posterior hyaloid, internal limiting membrane (ILM) and epiretinal membrane (ERM) removal, might achieve DME resolution. However, the removal of the vitreous gel might improve inner retina oxygenation and thus promote the resolution of DME^[98-101].

PPV was introduced in the early 1970 as a promising treatment for the severe late complications of PDR, including vitreous hemorrhage, TRD, and fibrovascular

proliferation^[102]. The proper timing for PPV in PDR was under discussion for a long time. The Diabetic Retinopathy Vitrectomy Study (DRVS) considered the early PPV effects compared to deferral PPV in patients with severe vitreous hemorrhage (VH)^[103]. The DRVS showed that at 2-year follow up, early PPV for nonclearing VH primarily increased the chance for retaining vision $\geq 20/40$. Today, PPV can be performed as early as it is needed by the patients. The aim of PPV in PDR includes removal of opacity from the vitreous space, and the removal of tractional membrane from the retinal surface. Anti-VEGF injections might be useful adjuncts to ease effective fibrovascular membrane dissection in eyes with active vascularity components^[78].

Finally, enzymatic vitrectomy performed by the intravitreal injection of autologous plasmin enzyme might be effective and could be considered as an alternative for diabetic patients before performing other treatments, such as intravitreal injections of anti-VEGF or steroids, surgical vitrectomy or laser. Several investigations on enzymatic vitreolysis, such as microplasmin, showed that many agents might achieve vitreous dissolution, PVD, or VH clearance^[104,105].

Indications for PPV in PDR: Severe nonclearing vitreous hemorrhage; Nonclearing vitreous hemorrhage; Premacular subhyaloid hemorrhage; TRD involving the fovea; Tractional and rhegmatogenous retinal detachment; Macular edema due to vitreomacular traction; Nontractional macular edema that is refractory to pharmacotherapy and laser therapy.

DIABETIC PAPILLOPATHY

Definition and incidence

Diabetic papillopathy (DP) is an uncommon ocular manifestation of DM identified by unilateral or bilateral disk swelling associated with minimal or no optic nerve dysfunction^[106-108]. DP, which is self-limited disease, was reported in 1971 in T1DM patients for the first time^[109]. So, it is very difficult to predict the exact incidence of DP. The prevalence of DP in both types of DM is about 0.5%, regardless of glycemic control and seriousness of DRP^[106-108]. The percentage of patients with DP presenting a NPDRP is higher than in the PDRP.

Pathogenesis

The pathophysiology is not fully understood and several theories have been suggested. There are no links between DP and either DRP or metabolic control. Some researchers suggest that DP is a subtype of non-arteritic anterior ischemic optic neuropathy (NAION), but there are some differential features between NAION and DP, for instance, DP is an asymptomatic optic disc edema, whereas NAION is an acute optic disc infarction^[110,111]. However, the most plausible mechanism responsible for DP is a limited impairment to the peripapillary vascular network, and superficial capillary network endothelial

cells^[111,112].

Clinical evaluation

The other causes of disk swelling, and PDRP with NV on the disc have been ruled out to verify the diagnosis of DP^[113]. DP, which occurs generally in patients with uncontrolled diabetes, has following features: painless visual loss, macular edema, disk hyperfluorescence on fluorescein angiography, and significant visual improvement after the treatment^[106].

However, several diseases can imitate DP, such as infection, inflammation, metastatic infiltration, hypertension, and papilledema^[106,108,114]. Pseudopapilloedema, that is seen in patients with disc drusen^[113], can be confused with DP.

In order to reach differential diagnosis, investigations are required, such as fluorescein angiography, orbital magnetic resonance imaging, blood tests including serum angiotensin-converting enzyme, anti nuclear antibody, vitamin B12, folate, erythrocyte sedimentation rate, C reactive protein, and fluorescent treponemal antibody test.

Current therapy

So far, definitive treatment has not been found to change its native progression, as in most cases the disc edema resolves within a few months with no visual impairment. Intravitreal anti-VEGF injection increased visual acuity and decreased disk edema in patients with DP^[114-117]. At the same time, it is unknown that how anti-VEGF agents affect to the patients with DP. Another study showed that periocular corticosteroids stabilize the blood-ocular barrier at the disc and the macula and causes resolution of the disc and macular edema^[118]. Some degree of optic atrophy is seldom present after treatment. Tight control of blood pressure optimises the visual outcome.

GLAUCOMA

Association of DM and glaucoma has been investigated much in the literature. DM is the major etiologic factor for neovascular glaucoma (NVG)^[119]. However, the association of DM with other types of glaucoma such as open angle glaucoma (OAG) and angle closure glaucoma (ACG) is controversial. Since glaucoma is a type of optic neuropathy and DM alone could cause optic neuropathy, a complex relation may occur between DM and glaucomatous optic neuropathy. On the other hand, central corneal thickness (CCT) is found to be thicker in patients with DM that could cause higher intraocular pressure (IOP) readings^[120]. Since the mechanisms of glaucoma subtypes are different from each other; it would be more logical to investigate the association of glaucoma subtypes individually with DM.

OAG and DM

OAG is one of the most common causes of vision loss worldwide. In several studies, DM was reported as a risk

factor for OAG, along with other risk factors such as elevated IOP, older age, family history of glaucoma and black race^[121-123]. It was found that as the duration of type 2 DM increases, risk of having OAG also increases^[123]. On the other hand, an association of having a history of DM and risk of OAG was not found in several studies^[124,125]. It is possible that diabetic patients are more likely to have an ocular examination than the general population and are thus more likely to be diagnosed with OAG^[122]. Small vascular abnormalities including optic nerve vessels and oxidative damage are some of the possible mechanisms by which DM might increase risk of OAG^[122]. In the aspect of treatment, OAG patients with DM undergoing trabeculectomy do not have the same long-term IOP control and surgical survival rate when compared with patients without DM^[126]. Medical treatment, laser trabeculoplasty, and surgery (filtering surgery, aqueous drainage devices, *etc.*) are the treatment options.

ACG and DM

The association between DM and ACG is not very clear. But several studies showed that DM might be considered as a risk factor for ACG^[127,128]. Saw and colleagues^[127] reported that diabetic patients have shallower anterior chambers than individuals without DM, irrespective of age, gender, and socioeconomic factors. Senthil *et al*^[128] found that DM is associated with ACG, possibly because of the thicker lenses of diabetic patients. Weinreb *et al*^[129] reported that pseudophakic pupillary block with ACG might occur in patients with DM. Also, treatment of DR with argon laser panretinal photocoagulation could cause ACG soon after the laser^[130]. Medical treatment (topical, oral, and intravenous agents) and laser iridotomy are the treatment options.

NVG and DM

NVG is a severe and intractable glaucoma type. DR is one of the most common etiologic factors for NVG. NVG might occur in cases with no retinal or optic disc neovascularization, but it is more likely seen in PDR^[131]. The association of iris and angle NV with DM mostly increase with the duration of the disease and blood sugar control^[132]. Although iris and angle NVs are common in DM, they do not always progress to NVG; but NVs always develop prior to IOP increase^[132]. This is due to a fibrovascular membrane that occurs on the anterior surface of the iris and iridocorneal angle. This membrane then causes anterior synechiae, angle closure, and rise of IOP^[131,132].

NVG may develop in diabetic patients after cataract surgery, laser posterior capsulotomy and pars plana vitrectomy^[132]. NVG following these operations probably results from a combination of surgical inflammation and disruption of a barrier preventing diffusion of angiogenesis factors to the anterior segment^[132]. Prompt diagnosis and treatment are very important to prevent blindness due to NVG. Panretinal photocoagulation is the

key treatment method for prevention of NVG in DRP^[131]. Panretinal photocoagulation laser therapy in the early stages may be efficacious in inhibiting and even reversing new vessel proliferation in the anterior segment of the eye. Medical treatment, cyclophotocoagulation, cryotherapy, and surgery (trabeculectomy with antimetabolites and valve implantation) are the other therapeutic options.

Other glaucoma types and DM

Pseudoexfoliation (Psx) has been supposed to be a generalized or systemic disorder of the extracellular matrix^[133]. Psx increases the risk of glaucoma development^[133]. It was reported that there is not a significant relationship between DM and psx^[134]. Also, HbA1c levels do not vary among patients with DM based on psx status^[134]. Ellis *et al*^[135] found that DM is not associated with ocular hypertension. On the other hand, it was revealed that DM is significantly associated with bilateral eye involvement in normotension glaucoma, maybe due to several impaired neurovascular autoregulation processes related to DM^[136].

Glaucomatous optic neuropathy and DM

Retinal ganglion cell death is the major cause of blindness in glaucoma. DM may increase susceptibility of retinal ganglion cells to apoptosis when there is a co-morbidity with elevated IOP in glaucoma^[137]. DM disrupts vascular tissues, compromises neuro-glial functions, and thus may take a role in the pathogenesis of optic neuropathy related with glaucoma^[138]. In the literature, it was shown that DM may accelerate apoptosis of retinal inner neurons, alter metabolism of astrocytes and Müller cells, and impair microglial function^[138]. All of these factors contribute to visual acuity, contrast sensitivity and color vision loss in comorbidity of DM and glaucoma^[138].

Miscellaneous issues related to glaucoma and DM

DM is associated with increased corneal stiffness, and corneal hysteresis which have been shown to have an effect on glaucoma risk^[125,139]. IOP may increase in patients with DM due to aqueous outflow resistance in trabecular meshwork, because of glycation and crosslinking of meshwork glycoproteins^[140].

Since DM is frequently found with other systemic disorders, such as hypertension, this comorbid condition may also affect glaucoma risk. Shoshani *et al*^[141] reported that DM may interfere with normal vascular regulation and contribute to glaucoma progression. Moïse *et al*^[142] suggested that blindness due to glaucoma may be prevented by using a regular Mediterranean diet and maintaining regular intake of vegetables in patients with DM.

CATARACT

Definition and incidence

Cataract, the commonest cause of curable blindness

worldwide, is the opacification of the crystalline lens^[143,144]. Diabetic cataract is considered a complication of DM, which can affect individuals at younger ages^[145]. Cataract formation in diabetics seems to be related to the hyperglycemia or to hastened senile lens opacity. A snowflake like cataract is occurred commonly in patients with insulin-dependent diabetes and more prone to progress than others.

Diabetic patients are 2-5 times more at risk for cataract formation and are more likely to get it at an earlier age^[146,147]. Although cataract frequency varies based on ethnic populations and geographic locations (ranges from 35% to 48%), it is higher in diabetics when compared to non-diabetics^[148-152]. In a study by Raman *et al*^[153], it has been indicated that the mixed cataract was more common than mono type cataract (42% *vs* 19%, respectively). A combination of cortical, nuclear, and posterior subcapsular cataract was the most common form of the mixed types (20%), followed by the combined posterior subcapsular cataract and cortical (16%). Among the monotype cataracts, rate of cortical cataract was the highest (15%), followed by nuclear cataract (5%) and posterior subcapsular cataract (1%)^[153]. On the other hand, cataract frequency varies from 1% to 27% in patients with type 1 diabetes^[154].

Pathogenesis

Several different pathogenetic mechanisms that may precipitate formation of diabetic cataracts have been proposed: increased osmotic stress caused by activation of the polyol pathway^[155], non-enzymatic glycation of lens proteins^[156-159], and increased oxidative stress^[160-164].

The polyol pathway

In cases of high blood glucose levels in diabetic patients, the crystalline lens is exposed to a hyperosmotic aqueous humour and its glucose concentration progressively increases. During hyperglycemic conditions excess glucose to sorbitol. Sorbitol is further metabolized to fructose. In diabetic patients, the excessive accumulation of sorbitol in the crystalline lens produces a high osmotic gradient that leads to a fluid infusion to equilibrate the osmotic gradient. The accumulation of sorbitol in lens cell causes a collapse and liquefaction of lens fibers, which eventually results in the cataract formation^[165,166]. Moreover, increased osmotic stress in the crystalline lens produced by excess accumulation of sorbitol initiates apoptotic process in epithelial cells which contributes to the cataractogenesis^[155,167,168].

Non-enzymatic glycation

Advanced glycation occurs during normal aging but to a greater degree in diabetic patients in which it contributes the formation of lens opacity^[156]. Advanced glycation produced by a nonenzymatically reaction between the piece of the excess glucose and proteins, which may leads to production of superoxide radicals and AGE formation^[169]. Excessive accumulation of AGEs in the crystalline lens of diabetic patients plays an essential role in cataractogenesis^[157-161].

Increased oxidative stress

It is well known that chronic hyperglycemia may increase the oxidant load^[162] and facilitate the onset of senile cataract^[163]. In diabetic eyes, antioxidant capacity is reduced free radical load is increased, which increases the susceptibility of crystalline lens to oxidative damage. The decrease in antioxidant capacity is facilitated by advanced glycation and defects of antioxidant enzyme activity^[164].

Clinical evaluation

DM can cause anterior segment changes as well as posterior segment; therefore, a comprehensive ophthalmologic examination including visual acuity measurement, evaluation of relative afferent pupil defect, slit-lamb biomicroscopy, gonioscopy, intraocular pressure measurement, and dilated fundus examination are mandatory. In selected cases, ancillary tests such as fundus angiography and OCT may also be useful.

The level of cataract should correspond to patient's visual complaints including decreased visual acuity, decreased contrast sensitivity, and glare. If the biomicroscopic examination shows mild cataract but the patient reports severe visual dysfunction, other ocular diabetic complications such as DR should be investigated. Recently, there has been a shift in emphasis towards early cataract removal in diabetics to enable adequate identification for examination of posterior segment, and facilitate panretinal photocoagulation and treatment of underlying macular edema^[170]. Pre-existing PDR and macular edema may exacerbate after cataract surgery^[171] which contributes to the poor visual outcomes^[172]. Therefore if posterior segment is visualized, diabetic patients with pre-existing retinopathy should be preoperatively treated.

Current therapy

First of all, good blood glucose control is main goal to prevention of diabetic cataract. It has however been suggested that cataractogenesis can be prevented through nutrition and supplementation, including high content of nutritional antioxidants^[173], lower dietary carbohydrate^[174] and linolenic acid intake^[175], and aldose reductase inhibitors^[144,176].

Currently, the main treatment for the diabetic cataract is surgery. Phacoemulsification results in better visual results, less intraocular inflammation and less capsular opacification as compared to extracapsular surgery^[177]. Femtosecond assisted cataract surgery may be a better option for diabetics; however, there has been no comparative study comparing the results of femtosecond assisted to conventional cataract surgery in diabetics. It is advisable to perform a large capsulorrhexis with a large diameter IOLs, thus allowing better visualization of the posterior segment for examination and further treatment of DR.

After cataract surgery, using topical anti-inflammatory drugs such as steroids and nonsteroidal anti-inflammatory drops may be useful to control inflammation and macular edema. Despite an uneventfully performed cataract

surgery, DR and macular edema can become exacerbated after surgery, hence patients should be followed closely with fundus examinations and ancillary tests.

OCULAR SURFACE DISEASES

Ocular surface diseases, such as dry eye is frequently present in diabetic patients. Ocular surface diseases related with DM are developed in many mechanisms including abnormal ocular surface sensitivity^[178,179], decreased tear production^[179-181], and delayed corneal re-epithelialization^[181].

DRY EYE SYNDROME

Definition and incidence

Dry eye is a condition which is a complex disease of tear film and anterior surface of the cornea. The resulting changes in the ocular surface may lead to ocular discomfort, and visual disturbance. Tear osmolarity, and ocular surface inflammation^[182] are also increased in diabetic patients causing dry eye disease. Burning, foreign body sensation, photophobia, blurred vision^[183], and blurred vision are present in patients with dry eye. Both dry eye disease and DM increase the risk of corneal infections and scarring, in advanced disease, corneal perforation and irreversible tissue damages^[184] may occur. Patients with dry eye have serious corneal complications such as, superficial punctuate keratitis, neurotrophic keratopathy, and persistent epithelial defect^[185]. Dry eye syndrome (DES) is more like to occur in the industrial country. Studies showed that approximately 1.68 million men and 3.2 million women^[186] aged 50 and older are affected with DES in the United States^[187]. DES, one of the most common diagnosis for diabetic patients^[188], is a condition in which abnormal tear film and an changed anterior surface of the cornea is present. Studies show at least 50% of DM patients have either symptomatic or asymptomatic DES. 92 patients with diabetes types I and II have been evaluated by Seifart^[189]. The patients were aged from 7 to 69 years old as well as normal healthy controls comparable in number, age and sex. The study demonstrated that 52.8 of all diabetic patients complained about eye dry symptoms, whereas 9.3% of the healthy controls complained about dry eye symptoms.

Pathogenesis

DM can lead to DES through a variety of mechanisms^[190-192], but the association between DM and DES is unclear^[193]. The most possible mechanism responsible for dry eye in DM is extensive hyperglycemia bring about corneal neuropathy. Corneal neuropathy leads to tear film instability and lower tear break up time (TBUT) values due to conjunctival goblet cell loss. Mucin, which covers the villus surface of the corneal epithelium and reduce evaporative tear loss^[181] is produced by conjunctival goblet cells.

The other suggested mechanisms for disruption of

corneal integrity include AGE accumulation^[194,195] and polyol pathway^[196,197] bi-product accumulation within the corneal layers. It is believed that DM affects tear production and quality by compromising the functional integrity of the lacrimal gland. Corneal sensitivity is also reduced in DM, which affects the stimulation of basal tear production. Both lacrimal gland integrity^[180] and corneal sensitivity are shown to be affected by diabetic neuropathy^[180,198]. These proposed mechanisms imply that DM affects both tear production and corneal integrity, suggesting disruption to one or both may cause and lead to the exacerbation of DES.

Clinical evaluation

During routine eye examination clinicians should be aware of dry eye in diabetic patients^[199]. Dry eye index scores can be used for uncovering the presence of dry eye and for evaluating the response to therapeutic treatment. Several questionnaires are available, with the most common being the Ocular Surface Disease Index (OSDI)^[200]. However, there is still no standardized dry eye disease questionnaire that is universally accepted.

The most common test for determining tear film quality in use today is the TBUT which shows the tear film stability. The TBUT value is the time from the last complete blink to the appearance of dry spot. The Schirmer test is used for measuring the aqueous tear manufacture. Normally, the Schirmer filter paper gets wet 10 mm for 5 min. A result yielding less than 5 mm shows aqueous tear deficiency. Fluorescein is useful in assessing dry eye where its application can detect the epithelial defects due to dry eye disease.

Risk factors for DES include duration of DM and higher HbA1c levels^[188,201]. So, strict blood glucose control and close follow-up reduce the risk of DES^[188].

Current therapy

DES may cause loss of vision, scarring, perforation, and corneal infection. If patients with dry eye are treated in time, there will be no complications of DES^[185]. The patients should be treated with tear supplements called “artificial tears” which contains surfactants, different viscosity agents, and electrolytes^[202].

Dry eye disease is the outcome of many factors resulting in inflammation of the cornea and conjunctiva. Artificial tears can reduce blurred vision, and the symptoms of dry eye, temporarily. These agents do not contain the cytokines and growth factors which are comprised in normal tears and do not have direct anti-inflammatory effect^[203,204]. Anti-inflammatory drugs are widely used for the treatment of DES. The most widely used anti-inflammatory agents are topical corticosteroids, NSAID, and cyclosporine A^[203-205].

Corticosteroids can reduce the symptoms and signs of dry eye^[206] to control inflammatory process. On the other hand, after long-term use, steroids produce severe side effects such as bacterial, viral, and fungal infection, elevated IOP, and cataract formation. NSAIDs are increasingly used as dry eye treatment instead of steroids because of their

non-severe side effects. Topical cyclosporine A are used to increase tear production^[207] and the number of goblet cells decreased by chronic inflammation due to dry eye disease^[207].

DIABETIC KERATOPATHY

Definition and incidence

DM can trigger acceleration of ocular surface abnormalities which have been termed diabetic keratopathy^[208]. In contrast to healthy persons, patients with diabetes have corneal epithelial erosions that may recur and be associated with unresponsiveness to conventional treatment regimens^[209-211]. This clinical condition is known as diabetic keratopathy^[212-214]. Diabetic keratopathy includes various symptomatic corneal conditions, such as, punctate keratopathy and persistent corneal epithelial defect^[208].

Diabetic keratopathy is a common complication of patients with evidence of DR. A study reported that several symptomatic corneal epithelial lesions have been occurred in diabetic patients at the rate of 47% to 64%^[208]. In another study, authors showed that the incidence of diabetic keratopathy in diabetic patients with DR was 2 times greater than that of patients without DR^[215]. Several studies reported that the incidence of diabetic keratopathy increased following pars plana vitrectomy^[216,217], penetrating keratoplasty^[218], laser iridectomy^[219], and refractive surgery^[220] in diabetic patients.

Pathogenesis

Several pathophysiological abnormalities have been shown in diabetic keratopathy, including, an abnormally thickened and discontinuous basement membrane, abnormal adhesion between the stroma and basement membrane^[219-223], increased epithelial fragility^[206], decreased epithelial healing rates, increased sorbitol concentrations^[224], decreased oxygen consumption and uptake^[225], increase in the polyol metabolism^[196], decreased or alter epithelial hemidesmosomes, and increased glycosyltransferase activity^[214,226].

Recently, studies have demonstrated^[194,195,227] that there is a relationship between AGE and development of diabetic keratopathy. Increased AGE in the laminin of the corneal epithelial basement membrane causes abnormal weak attachment between the basal cells and basement membrane of the cornea in diabetics^[194]. Also, the loss of the corneal sensation and neural stimulus have been regarded as the reason of the development of diabetic keratopathy^[228]. Axonal degeneration of corneal unmyelinated nerves occurs under chronic hyperglycemic conditions.

Clinical evaluation

Diabetic keratopathy is a condition that can result in blindness and should be closely monitored. Early diagnosis and treatment of diabetic keratopathy, particularly, before corneal complications occur, is very crucial. If

the diagnosis is late, patients will become resistance to the routine treatment of corneal defects. Nonhealing corneal epithelial erosion may also occur after pars plana vitrectomy for advanced PDR^[208,211]. If corneal epithelium is removed manually for clarity by surgeons, this conditions may accelerate dramatically. So, when diabetic patients are examined after vitrectomy their corneas should be examined carefully.

Current therapy

Keratopathy is generally treated with artificial tears, and antibiotics. Additionally, bandage contact lens, and tarsorrhaphy can be used for re-epithelialization. In selected cases new treatments modalities will be used such as, topical administration of naltrexone, nicergoline^[229], aldose reductase inhibitor^[194,214,230], and some growth hormones^[231] to accelerate re-epithelialization. All of these drugs were associated with a high corneal epithelial wound healing rate.

Recently, new topical drugs such as substance P and IGF-1 were tested on diabetic animals to accelerate re-epithelialization. Successful outcomes were obtained with these new drugs^[231]. Corneal epithelial barrier function was improved by topical aldose reductase inhibitors, but superficial punctate keratopathy could not be prevented by these topical drugs. Aminoguanidine had beneficial effects in corneal epithelial defects, by improving attachment between the epithelial cells and basement membrane of the cornea^[185,194]. The *in vivo* beneficial effect of aminoguanidine were unknown^[194]. In additional to these new drugs, amniotic membrane transplantation is used to treat persistent corneal epithelial defects^[232].

CONCLUSION

DM and its ocular complications remain a major cause of blindness despite increased understanding of these ocular conditions and identification of successful treatments. All of diabetic ocular complications can be prevented by early diagnosis and therapy. Therefore, periodic eye examinations are required for the reduction of diabetes-related vision loss. Good blood glucose control and other systemic risk factors such as hypertension, and hyperlipidemia are main goal to prevention of ocular complications of DM.

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β -cell dysfunction: Its critical role in prevention and management of type 2 diabetes

Yoshifumi Saisho

Yoshifumi Saisho, Department of Internal Medicine, Keio University School of Medicine, Tokyo 160-8582, Japan

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Correspondence to: Yoshifumi Saisho, MD, PhD, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. ysaisho@z5.keio.jp

Telephone: +81-3-33531211

Fax: +81-3-33592745

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of glycemic control and treatment failure; thus, it is important to preserve or recover β -cell functional mass in the management of T2DM. Since β -cell regenerative capacity appears somewhat limited in humans, reducing β -cell workload appears to be the most effective way to preserve β -cell functional mass to date, underpinning the importance of lifestyle modification and weight loss for the treatment and prevention of T2DM. This review summarizes the current knowledge on β -cell functional mass in T2DM and discusses the treatment strategy for T2DM.

Key words: β -cell; Insulin secretion; Type 2 diabetes; Prevention; Treatment

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Core tip: Recent studies have revealed that a deficit of β -cell functional mass is an essential component of the pathophysiology of type 2 diabetes (T2DM). β -cell dysfunction is present at the diagnosis of T2DM and progressively worsens with disease duration. β -cell dysfunction is associated with worsening of glycemic control and treatment failure; thus, it is important to preserve or recover β -cell functional mass in the management of T2DM. This review summarizes the current knowledge on β -cell functional mass in T2DM and discusses the treatment strategy for T2DM.

Abstract

Type 2 diabetes (T2DM) is characterized by insulin resistance and β -cell dysfunction. Although, in contrast to type 1 diabetes, insulin resistance is assumed to be a major pathophysiological feature of T2DM, T2DM never develops unless β -cells fail to compensate insulin resistance. Recent studies have revealed that a deficit of β -cell functional mass is an essential component of the pathophysiology of T2DM, implying that β -cell deficit is a common feature of both type 1 and type 2 diabetes. β -cell dysfunction is present at the diagnosis of T2DM and progressively worsens with disease duration. β -cell dysfunction is associated with worsening

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INTRODUCTION

The number of patients with diabetes is continuously increasing all over the world. Worldwide, there were

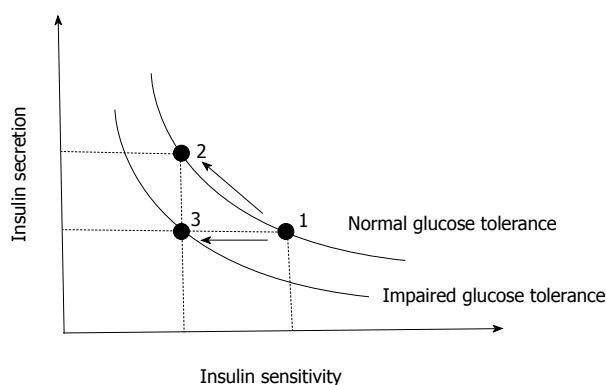


Figure 1 Insulin secretion-insulin sensitivity relationship. In a physiological condition, when insulin sensitivity decreases, insulin secretion increases to maintain normoglycemia (1→2), showing a hyperbolic curve. When insulin secretion fails to compensate, the hyperbolic curve shifts to the left and abnormal glucose tolerance develops (1→3).

382 million patients with diabetes in 2013, which will rise to 592 million in 2035^[1]. Diabetes is associated not only with diabetic microangiopathy such as retinopathy, nephropathy and neuropathy, but also with a 2- to 4-fold increase in risk of cardiovascular disease^[2,3]. Among the people with diabetes, more than 90% have type 2 diabetes (T2DM). Therefore, optimal treatment and prevention strategies for T2DM are urgently needed.

T2DM is characterized by insulin resistance and β -cell dysfunction. Recent evidence suggests an important role of β -cell function in the development and management of T2DM. In this review, the current knowledge regarding β -cell dysfunction in T2DM is summarized and its critical role in the prevention and treatment of T2DM is discussed.

DEFICITS OF β -CELL FUNCTION AND β -CELL MASS IN T2DM

Disposition index: A true assessment of β -cell function

T2DM is characterized by insulin resistance and β -cell dysfunction^[4,5]. However, since the development of an insulin radioimmunoassay, it was found that in people with T2DM, plasma insulin concentration is rather higher than that in those with normal glucose tolerance (NGT), indicating that insulin resistance rather than insulin deficiency is central in the pathogenesis of T2DM. Therefore, in contrast to type 1 diabetes, obesity, hyperinsulinemia and insulin resistance are often emphasized as characteristics of T2DM, and β -cell function in T2DM is often less emphasized or even ignored.

However, the higher plasma insulin concentration in patients with T2DM is often confounded by a higher plasma glucose level, which itself stimulates insulin secretion. Moreover, insulin sensitivity also affects insulin secretion. In normal physiological conditions, normoglycemia is maintained under a balance between insulin sensitivity and insulin secretion, and when insulin

sensitivity decreases, insulin secretion increases to maintain normoglycemia. Thus, insulin secretion should always be assessed in relation to insulin sensitivity. Bergman and Cobelli have found that this relationship between insulin secretion and insulin sensitivity is expressed as a hyperbolic curve, and as a result the product of insulin sensitivity and insulin secretion is constant as long as normoglycemia is maintained^[6,7] (Figure 1). The product of insulin sensitivity and insulin secretion, called the disposition index, refers to insulin secretion adjusted by insulin sensitivity and reflects true β -cell function *in vivo*.

Once insulin secretion is not able to sufficiently increase to compensate the decrease in insulin sensitivity, the insulin sensitivity-insulin secretion relationship is shifted to the left and abnormal glucose tolerance develops (Figure 1). In this case, the disposition index is decreased, indicating that abnormal glucose tolerance develops only when β -cells are no longer able to compensate decreased insulin sensitivity.

β -cell function in T2DM

When β -cell function is assessed using the disposition index, a number of studies have consistently shown that β -cell function is diminished in people with T2DM^[4,8,9]. Using the disposition index, DeFronzo *et al*^[9] have shown that β -cell function is decreased by -80% in patients with impaired glucose tolerance (IGT) and is even less in patients with T2DM (Figure 2). Importantly, β -cell function starts to decline with higher plasma glucose levels, even within the range of normal plasma glucose levels^[9], which suggests that β -cell function is already impaired prior to the development of IGT.

β -cell mass in T2DM

If β -cell function is impaired in patients with T2DM, what about the β -cell mass? β -cells are located in the islets of Langerhans, which are scattered within the exocrine pancreas. Each islet contains ~1000 β -cells together with other endocrine cells such as alpha cells, delta cells, pancreatic polypeptide (PP) cells and epsilon cells, and a total of ~1 million islets exist in the pancreas. β -cell mass refers to the total mass of β -cells and is approximately 1 g in humans.

Due to the anatomical characteristics of β -cells scattered throughout the whole pancreas, it is difficult to visualize β -cells *in vivo*, and direct measurement of β -cell mass *in vivo* in humans remains to be established^[10,11]. Thus, to date, the measurement of β -cell mass inevitably relies on histological analysis of the pancreas obtained surgically or at autopsy.

Since insulin resistance and hyperinsulinemia are often emphasized in people with T2DM, β -cell mass in people with T2DM is also often assumed to be increased or at least not decreased. However, based on histological analysis, Butler *et al*^[12] have reported that β -cell mass is decreased by -40% and -65% in lean and obese people with T2DM, respectively, compared with non-diabetic controls matched for age and BMI. Other groups have

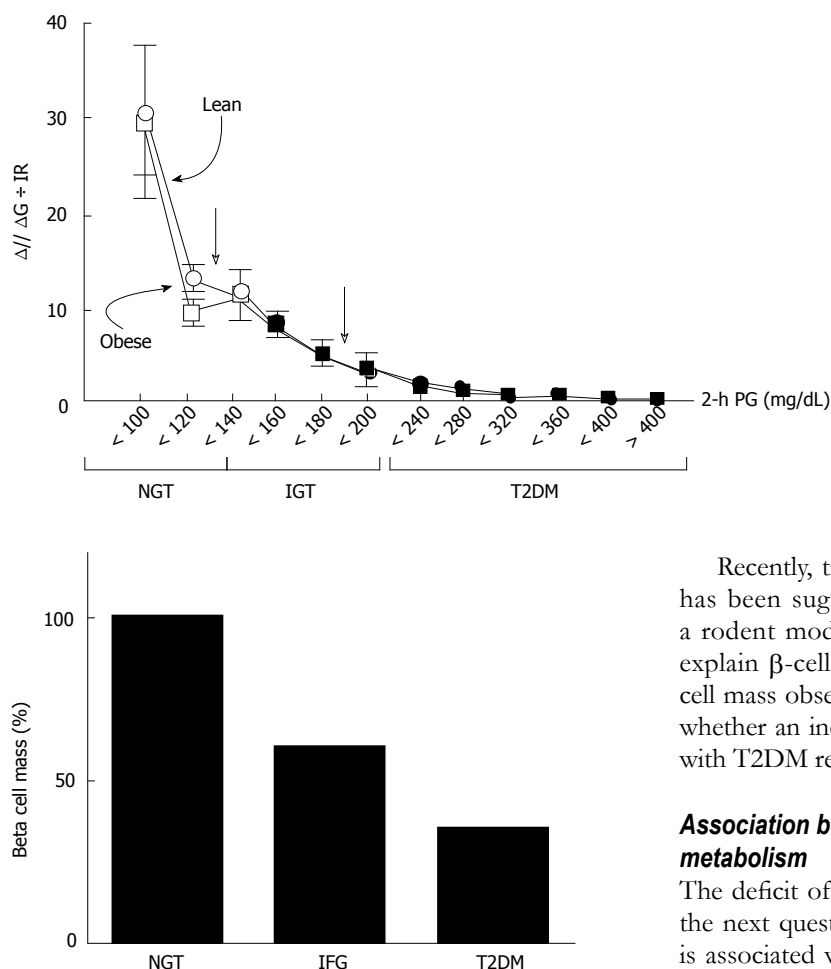


Figure 3 β -cell mass in patients with normal glucose tolerance, impaired fasting glycemia and type 2 diabetes. Adapted and modified from the study by Butler *et al*^[12]. NGT: Normal glucose tolerance; IFG: Impaired fasting glycemia; T2DM: Type 2 diabetes.

also reported a significant (-30%-40%) decrease in β -cell mass in patients with T2DM^[13-15]. These findings suggest that deficit of β -cell mass is a common pathophysiological feature of type 1 and T2DM (Figure 3), while the cause and degree of the deficit are different between type 1 and T2DM.

Mechanisms of β -cell deficit in T2DM

β -cell mass is regulated by the balance of newly formed β -cells and β -cell loss^[16-18]. Butler *et al*^[12] have shown that β -cell apoptosis is increased in patients with T2DM, whereas neither β -cell replication nor neogenesis is decreased, suggesting that increased β -cell loss is the main cause of reduced β -cell mass in T2DM. Various mechanisms that induce β -cell apoptosis have been proposed such as hyperglycemia (glucotoxicity)^[19], fatty acids (lipotoxicity)^[20], amyloid or islet amyloid polypeptide (IAPP, also called amylin)^[21-24], oxidative stress^[25], inflammatory cytokines^[26], mitochondrial dysfunction^[27], endoplasmic reticulum (ER) stress^[28,29] and dysfunction of autophagy^[30]. A recent study suggested that several mechanisms are simultaneously associated with β -cell failure in humans with T2DM^[31].

Figure 2 Insulin secretion/insulin resistance (disposition) index (I/G + IR) during 75g-oral glucose tolerance test in individuals with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes as a function of the 2 h plasma glucose concentration in lean and obese subjects. I/G: Insulinogenic index (Insulin 0-30 min/Glucose 0-30 min); IR: Homeostasis model assessment of insulin resistance [HOMA-IR; fasting insulin (mU/L) \times glucose (mmol/L)/22.5]. Adapted from ref.[9]. NGT: Normal glucose tolerance; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes; PG: Plasma glucose.

Recently, transdifferentiation of β -cells to α cells has been suggested as a mechanism of β -cell loss in a rodent model of diabetes^[32]. This mechanism could explain β -cell loss and the reciprocal increase in α cell mass observed in humans with T2DM^[15,31], although whether an increase in α cell mass occurs in humans with T2DM remains controversial^[33,34].

Association between β -cell mass and glucose metabolism

The deficit of β -cell mass in patients with T2DM raises the next question of whether the change in β -cell mass is associated with the severity of glucose intolerance. It has been reported that there is a reciprocal relationship between β -cell mass and fasting plasma glucose level^[35], suggesting that glucose intolerance develops when the β -cell mass decreases by -50% of the normal level. A similar relationship has been observed in rodents^[36], pigs^[37] and monkeys^[38]. An increased risk of the development of IGT or diabetes after hemipancnectomy has been reported in dogs and humans^[39-43]. It has also been reported that β -cell mass was decreased by -20%-40% in patients with IGT and impaired fasting glycemia (IFG)^[12,44]. We have also reported that there was a significant negative correlation between β -cell mass and glycated hemoglobin (HbA1c) level in non-diabetic individuals^[45], suggesting that β -cell mass is related to glucose intolerance even prior to the development of T2DM. A significant correlation between β -cell mass and HbA1c was also observed in patients with T2DM^[31].

Association between β -cell mass and β -cell function

The relationship between β -cell mass and β -cell function is more complicated. Whether β -cell dysfunction in T2DM is mainly due to a functional defect of each β -cell or due to a defect of β -cell mass has been extensively argued^[46]. A close correlation between β -cell function assessed by maximum acute insulin response (AIRmax) induced by arginine infusion under a hyperglycemic state and β -cell mass of transplanted islets has been reported^[47]. On the other hand, β -cell dysfunction was markedly improved after an overnight β -cell rest by somatostatin

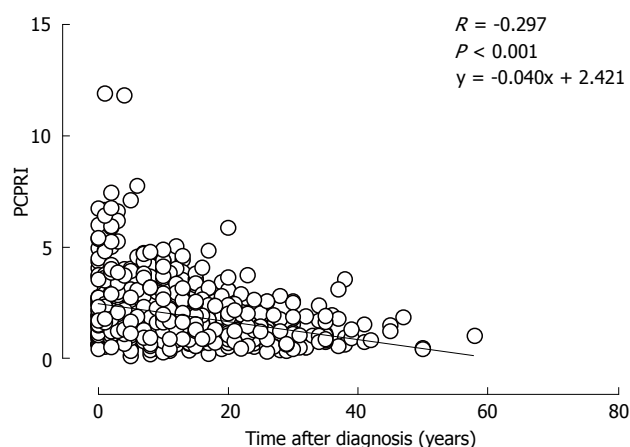


Figure 4 Relationship between postprandial C-peptide to glucose ratio (postprandial C-peptide index) and time after diagnosis in patients with Type 2 diabetes. Reproduced with permission from ref.[51]. PCPRI: Postprandial C-peptide index.

infusion^[48]. Thus, it remains uncertain whether β -cell function *in vivo* sufficiently reflects β -cell mass in patients with T2DM.

Meier *et al*^[49] assessed the relationship between β -cell mass and β -cell function in patients who had undergone pancreatic surgery and found that there was a significant positive correlation between β -cell mass and β -cell function, especially postprandial C-peptide level, suggesting that C-peptide measurement in clinical settings reflects β -cell mass.

Taken together, these results indicate that β -cell function and β -cell mass seem to be correlated with each other, although on some occasions they can be dissociated, and both β -cell function and mass seem to decrease during the development of glucose intolerance. Since β -cell function and mass are difficult to separate, currently they are referred to as “ β -cell functional mass”, and it is now certain that β -cell functional mass decreases during the development of T2DM.

Progressive decline in β -cell functional mass in T2DM

A deficit of β -cell functional mass is not only present in patients with T2DM, but it also progressively declines with disease duration. In the UK Prospective Diabetes Study (UKPDS), β -cell function assessed by homeostasis model assessment (HOMA) in patients with T2DM was already decreased by -50% at the time of diagnosis and progressively declined by -5% annually^[50]. This also indicates that in patients with T2DM, β -cell function starts to decline -10 years prior to the onset of the disease. A gradual but significant decline in β -cell function assessed by C-peptide level has been confirmed in cross-sectional cohort studies of Japanese patients with T2DM^[51,52] (Figure 4). Intriguingly, a significant negative correlation between β -cell mass and duration of T2DM has also been reported^[13].

Limited β -cell regenerative capacity in humans

Since deficits of β -cell function and mass are now

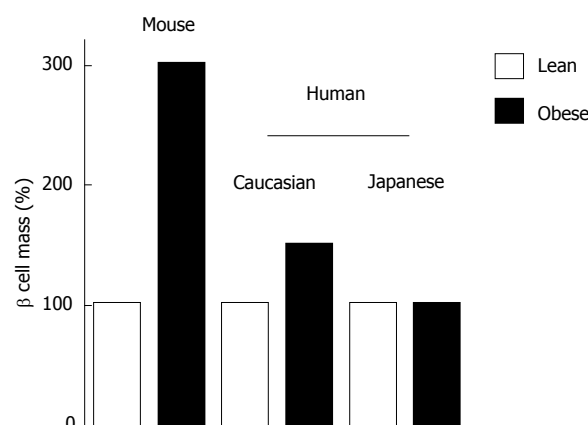


Figure 5 Change in β -cell mass with obesity. In mice, β -cell mass increases 3-fold with obesity. In humans, a 50% increase in β -cell mass has been reported in Caucasians, while no increase was reported in Japanese. Adopted and modified from ref.[54,59,85].

recognized as hallmarks of T2DM as well as type 1 diabetes, β -cell regeneration is considered to be an important therapeutic strategy for both types of diabetes.

Rodent studies show an adaptive change in β -cell mass in response to obesity or pregnancy^[53-58], suggesting the presence of endogenous β -cell regenerative capacity in the postnatal period. However, recent observation of the human pancreas suggests that endogenous β -cell regenerative capacity is limited in humans.

We have reported that β -cell mass in obese non-diabetic individuals is -1.2 g compared with -0.8 g in lean non-diabetic individuals, an -50% increase^[59], whereas β -cell mass increases 3- to 10-fold in response to obesity or insulin resistance in rodents^[53,54] (Figure 5). This striking difference in β -cell regenerative capacity between humans and rodents suggests that the results of rodent studies are not necessarily applicable to humans^[60,61].

In humans, β -cell mass increases from -37 mg to -1 g in the first five years of life, and during this period replicating β -cells are often observed^[62,63]. However, after that, replicating β -cells are rarely seen and β -cell mass reaches a plateau. The β -cell mass then remains constant during adulthood^[13,59,64], suggesting that β -cell turnover is limited in humans after the first five years of life. Estimation of β -cell life using either ¹⁴C measurement or cellular lipofuscin body content also suggests very slow turnover of β -cells in adult humans^[65,66]. Recent studies have suggested that there is an increase in β -cell neogenesis in humans with obesity, pregnancy and IGT^[67-70]; however, the extent of its contribution to β -cell mass remains unclear. Limited β -cell regenerative capacity has also been observed in monkeys^[71,72]. Even in rodents, β -cell regenerative capacity significantly decreases with aging^[54,73,74].

A hypothetical schema of the change in β -cell functional mass during the development of T2DM is shown in Figure 6. The magnitude of the increased demand for insulin due to insulin resistance caused by excess caloric intake and physical inactivity exceeds the magnitude of β -cell mass expansion, resulting in an increase in β -cell

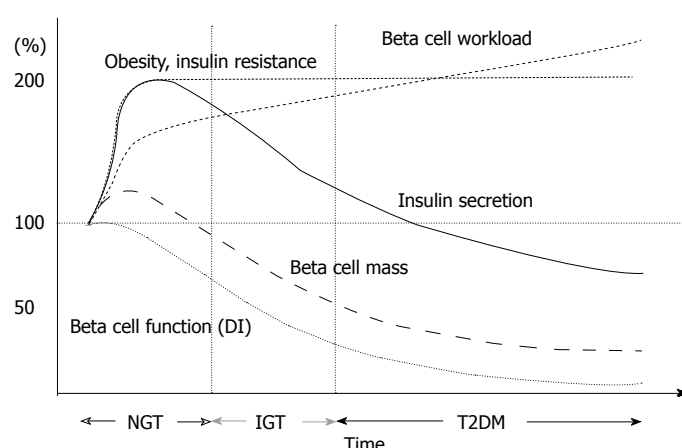


Figure 6 Hypothesis for change in β -cell function and mass during development of abnormal glucose tolerance. The magnitude of the increased demand for insulin due to insulin resistance caused by excess caloric intake and physical inactivity exceeds the magnitude of β -cell mass expansion, resulting in an increase in β -cell workload. In individuals who are susceptible to type 2 diabetes (T2DM), increased β -cell workload may lead to β -cell failure and the development of T2DM. Adopted and modified from ref.[109].

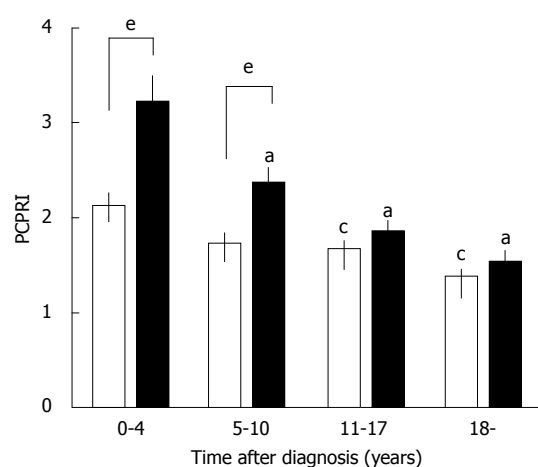


Figure 7 Postprandial C-peptide to glucose ratio (postprandial C-peptide index; PCPRI) in subjects according to obesity and time after diagnosis (0-4, 5-10, 11-17 and ≥ 18 years). There were significant differences in PCPRI between lean (open bars) and obese subjects (solid bars) in the first and second quartiles of time after diagnosis, but no significant difference was observed in the third and fourth quartiles. ^a $P < 0.05$ vs obese subjects ≤ 4 yr after diagnosis, ^b $P < 0.05$ vs lean subjects ≤ 4 yr after diagnosis, ^c $P < 0.05$ vs lean subjects. Reproduced with permission from ref.[51].

workload. In individuals who are susceptible to T2DM, increased β -cell workload may lead to β -cell failure and the development of T2DM. In addition, once hyperglycemia develops, it also causes β -cell dysfunction and apoptosis, which further exacerbate β -cell failure. Importantly, because insulin resistance persists, the β -cell workload continues to increase, with a reduction in β -cell mass. As a result, glucose metabolism progressively deteriorates in patients with T2DM. In our retrospective cohort, the progressive decline in β -cell function seemed to be exaggerated in the presence of obesity in Japanese patients with T2DM^[51] (Figure 7). Another Japanese cohort showed a decreasing trend in fasting insulin level despite an increasing trend in BMI at the first clinic/hospital visit of patients with T2DM during the past ten years^[75]. Recent studies have shown that even metabolically healthy obese individuals are at increased risk of future development of diabetes, cardiovascular events and all-cause mortality^[76-78]. Thus, weight loss itself may be important to preserve β -cell function and improve

clinical outcomes.

β -cell functional mass in Asian population

T2DM is characterized by obesity, but the degree of obesity differs between ethnic groups^[79,80]. In Caucasians, most patients with T2DM are obese, and the mean BMI of patients with T2DM is ~ 30 kg/m². In contrast, the mean BMI of Asian patients with T2DM is ~ 23 kg/m², suggesting that about half of patients with T2DM are not even overweight (*i.e.*, BMI ≥ 25 kg/m², the definition of obesity in Asian countries).

The difference in adiposity between Caucasians and Asians has been postulated to explain this ethnic difference. Visceral adiposity is more apparent in Asians compared to Caucasians with the same BMI^[81,82], indicating that Asians have a lower capacity for subcutaneous fat deposition and are more vulnerable to visceral fat accumulation compared with Caucasians. Nonetheless, a meta-analysis of studies examining the insulin sensitivity-insulin secretion relationship in individuals with NGT clearly showed that Asians have less insulin secretion with higher insulin sensitivity compared with Caucasians^[83]. Direct comparison of insulin sensitivity and insulin secretion between Japanese and Caucasians showed that most of the difference in insulin secretion between the two ethnicities can be explained by the difference in BMI between the two^[84]. Since the incidence of T2DM is comparable between the two ethnicities despite the different degree of obesity^[1], it is plausible that the lower degree of obesity in Asians could be attributable to the lower β -cell functional capacity in this population.

We have recently examined the change in β -cell mass in Japanese obese nondiabetic individuals (mean BMI 20.4 kg/m²) compared to age- and sex-matched lean individuals (mean BMI 28.5 kg/m²)^[85]. As a result, in contrast to the studies in Caucasians showing a significant increase in β -cell mass with obesity^[13,59], there was no significant increase in β -cell mass in Japanese obese individuals (Figure 5). Another Japanese study also confirmed our findings^[86]. These studies suggest that Asians have less β -cell regenerative capacity compared with Caucasians, which is probably derived from both genetic and environmental factors, and the lower β -cell

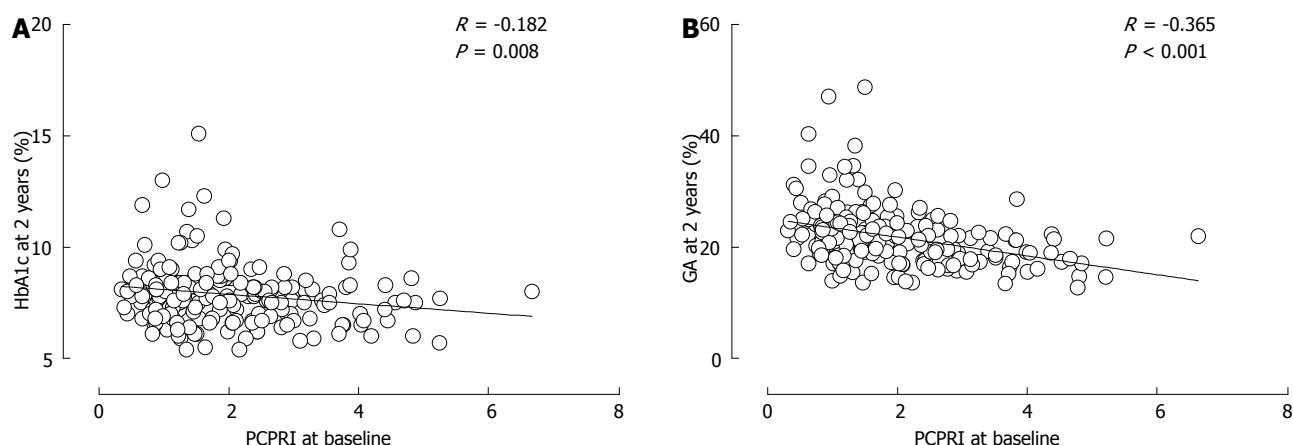


Figure 8 Correlation between baseline postprandial C-peptide index and HbA1c (A) and glycated albumin (B) after 2 years. Reproduced with permission from ref.[93]. PCPRI: Postprandial C-peptide index; GA: Glycated albumin.

functional capacity in Asians may contribute the different phenotype of T2DM between the two ethnicities. Because of the limited capacity of β -cell regeneration in Asians, excess β -cell workload could be induced in individuals with less obesity compared with Caucasians, which may lead to β -cell failure and the development of T2DM.

IMPLICATIONS FOR TREATMENT AND PREVENTION OF T2DM

β -cell function and glycemic control

If a deficit of β -cell functional mass is a hallmark of T2DM, what is the clinical consequence? In UKPDS and A Diabetes Outcome Progression Trial (ADOPT), treatment failure was associated with a progressive decline in β -cell function^[50,87,88]. In the Treatment Options for T2DM Adolescents and Youth (TODAY) study, similar results were observed in adolescents with T2DM, and in this study baseline β -cell function was associated with treatment efficacy^[89]. In our retrospective cohort analysis, we found that a lower baseline C-peptide level was associated with poorer glycemic control and the need for insulin therapy thereafter^[90-93] (Figure 8). In these studies, postprandial C-peptide index [*i.e.*, postprandial serum C-peptide (ng/mL)/plasma glucose (mg/dL) \times 100] was the best predictor of future insulin therapy among other C-peptide indices such as fasting C-peptide index and urinary C-peptide level. Since it was also significantly correlated with β -cell mass^[49], postprandial C-peptide index may be a useful marker of β -cell function in clinical settings.

Thus, poorer β -cell function is associated with poorer glycemic control and treatment failure, indicating the important role of β -cell function in the treatment of T2DM.

β -cell function and glycemic variability

Furthermore, β -cell function is associated with glycemic variability. In patients with T1DM, it has been reported that lower β -cell functional capacity is associated with

greater glycemic variability^[94-96].

We and others have reported that serum and urinary C-peptide levels are negatively correlated with glycated albumin (GA) to HbA1c ratio in patients with T2DM^[97-99] (Figure 9). Since albumin is more susceptible to glycation than is hemoglobin^[100,101], GA more sensitively reflects glycemic variability than does HbA1c^[97,102,103]. Thus, the inverse association between C-peptide level and GA to HbA1c ratio in patients with T2DM indicates that β -cell dysfunction is associated with greater glycemic variability in not only patients with type 1 diabetes but also those with T2DM.

Notably, we found that the relationship between postprandial C-peptide index and GA to HbA1c ratio in patients with T2DM was comparable to that in those with type 1 diabetes^[97] (Figure 9B). This suggests that the impact of β -cell dysfunction on glycemic variability is irrespective of the type of diabetes, again indicating the central role of β -cell function in the pathogenesis of diabetes.

Recently, it has been reported that greater glycemic variability as well as poorer glycemic control is associated with the development of micro- and macro-angiopathy^[104-107]. Thus, it should be stressed that greater glycemic variability and poorer glycemic control due to β -cell dysfunction may result in increased risk of diabetic complications.

Treatment strategy for T2DM

Since β -cell dysfunction is associated with poor glycemic control in patients with T2DM, preservation and recovery of β -cell functional mass is an important therapeutic strategy for T2DM. Moreover, the current issues in the treatment of T2DM summarized in Table 1 are, to put it simply, all associated with either excess or insufficiency of insulin supplementation^[108]. Thus, the recovery of physiological insulin secretion in patients with T2DM is also a key to resolving these issues.

To preserve or recover β -cell function, a reduction in excess β -cell workload appears to be the most effective strategy to date. In ADOPT, better glycemic control was

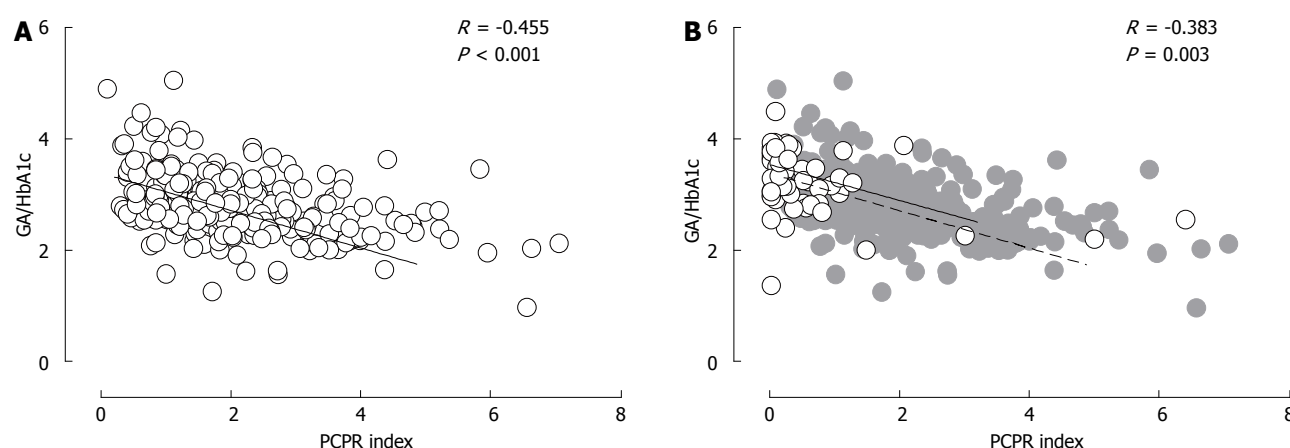


Figure 9 Correlation between postprandial C-peptide index and glycated albumin to HbA1c ratio in patients with type 2 diabetes (A) and type 1 diabetes (B). In Figure 9B, the data of patients with type 1 diabetes are superimposed on the data of those with type 2 diabetes (gray circles and dotted line). Reproduced with permission from ref.[97]. PCPR: Postprandial C-peptide index; GA: Glycated albumin.

Table 1 Current issues in treatment of type 2 diabetes

Issue	Cause
Hypoglycemia	Excess insulin
Weight gain	Excess insulin
Concern of increased risk of malignancy and/or atherosclerosis	Excess insulin, especially peripheral hyperinsulinemia
Postprandial hyperglycemia	Insufficient insulin in postprandial state, especially in portal vein

obtained with metformin or rosiglitazone monotherapy compared with glyburide in patients with T2DM^[87]. Thus, therapy should be focused on improving insulin sensitivity to reduce β -cell workload.

A proposed treatment strategy for T2DM is shown in Figure 10, as also described previously^[108,109]. It is emphasized that, to reduce β -cell workload, lifestyle modification and weight reduction remain the most important therapy at any stage of T2DM. Although lifestyle modification failed to reduce the incidence of cardiovascular disease in the Action for Health in Diabetes (Look AHEAD) trial^[110], it has been reported that lifestyle modification improved cardiovascular risk factors, reduced the need for and cost of medication, reduced the rate of sleep apnea and urinary incontinence, improved well-being and depression symptoms, and increased the rate of diabetes remission^[111-116]. In a cohort analysis of the ADDITION-Cambridge study, it has been reported that healthy behavioral changes after the diagnosis of T2DM were associated with a significant reduction in risk of cardiovascular events^[117], suggesting that early lifestyle intervention may be important to improve cardiovascular outcome.

Metformin is currently positioned as first-line therapy in most guidelines for the treatment of T2DM^[118,119]. Since metformin is effective in lean patients as well as obese patients with T2DM^[120,121], it should be used in both lean and obese individuals unless contraindicated.

Its efficacy in reducing HbA1c (by -1.5%), low risk of hypoglycemia, favorable effect on body weight and low cost also support metformin as a first-line drug.

Thiazolidinediones (TZDs) have also been shown to reduce β -cell workload and maintain glycemic control in the long term^[87,88]. Rosiglitazone has been shown to increase low-density lipoprotein (LDL) cholesterol and the risk of coronary heart disease in patients with T2DM^[122], and its use has been suspended or strictly restricted in Europe and United States^[123,124], although recently the US Food and Drug Administration has lifted most of its restrictions^[125]. On the other hand, pioglitazone has been shown to suppress the progression of atherosclerosis and reduce the risk of cardiovascular disease^[126-129]. However, TZDs often induce weight gain and edema due to fluid retention, and are contraindicated in patients with heart failure^[118]. Recent studies have also shown an increase in risk of bone fracture in women^[130] and risk of bladder cancer^[131-133] in patients treated with pioglitazone. The risk of bladder cancer may be dose dependent. In addition, since low-dose pioglitazone also reduces the risk of weight gain and edema, it may be preferable to use pioglitazone at lower doses, especially in women. Pioglitazone should also be used with caution in postmenopausal women with osteoporosis because of the increased fracture risk.

α -glucosidase inhibitors (AGIs) delay the absorption of carbohydrate from the small intestine, and thereby reduce postprandial hyperglycemia, resulting in reduced β -cell workload in a postprandial state. AGIs have also been reported to reduce the progression to T2DM in patients with IGT^[134,135]. Improving postprandial hyperglycemia by AGIs may also improve the cardiovascular outcome^[136-138]. Therefore, although the reduction in HbA1c by AGIs is relatively small (-0.5%), their use is also considered in patients with T2DM, especially those with postprandial hyperglycemia. The major side effect of AGIs is gastrointestinal disturbance such as flatulence, diarrhea and abdominal pain. In Japan, AGIs are the only medication indicated for patients with IGT.

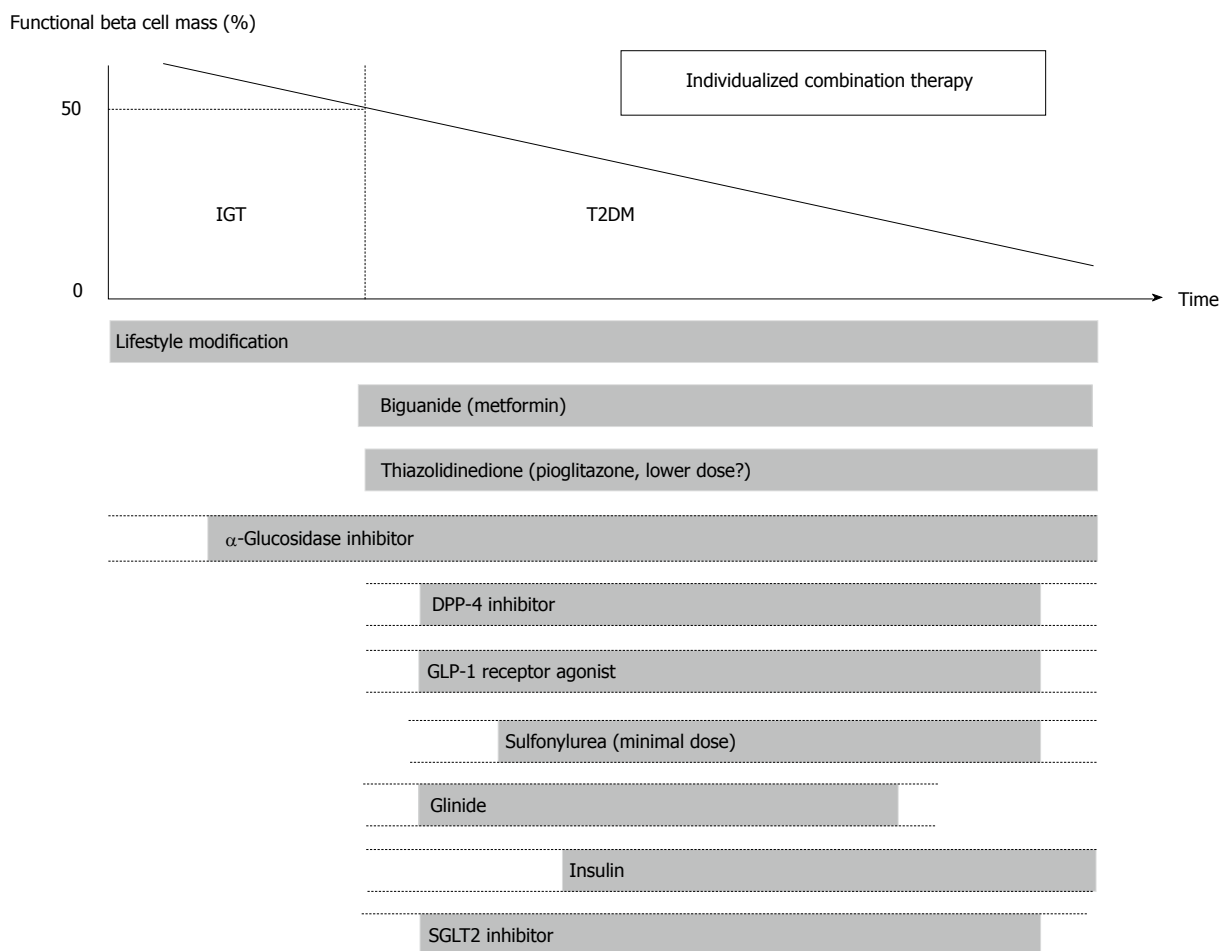


Figure 10 Proposed concept of treatment strategy for type 2 diabetes in relation to functional β -cell mass. α -glucosidase inhibitor is partly approved for use in patients with impaired glucose tolerance in Japan. Medications not approved in Japan are not included in the figure. Since currently no single therapy or agent can cure and even manage type 2 diabetes (T2DM), an effective combination of current medications in addition to lifestyle modification aiming at reduction in β -cell workload is important to preserve or recover β -cell function. Adopted and modified from ref.[108,109]. IGT: Impaired glucose tolerance.

Thus, AGIs are also considered for the treatment of T2DM at the early stage of the disease, if tolerated.

On the other hand, the use of insulin secretagogues, which increase β -cell workload, may be somewhat limited. Sulfonylureas (SUs), while remaining among the most highly prescribed drugs for the treatment of T2DM, increase the risk of hypoglycemia and weight gain, resulting in a high rate of treatment failure^[87]. These issues of SUs may be derived from their non-physiological augmentation of insulin secretion from β -cells.

Incretin drugs include dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 receptor agonists (GLP-1RAs). Both drug types reduce HbA1c mainly through an increase in insulin secretion, but also through suppression of glucagon secretion^[139]. GLP-1RAs also slow gastric emptying and reduce appetite, resulting in weight loss. The most important characteristic of incretin drugs is probably that the enhancement of insulin secretion occurs in a glucose-dependent manner. Thus, the action of incretin drugs as insulin secretagogues is more physiological than that of SUs, thereby resulting in a low risk of hypoglycemia and weight gain with incretin

therapy^[140-142]. Whether this physiological enhancement of insulin secretion results in long-term maintenance of glycemic control remains to be elucidated. Although an increase in β -cell mass with incretin therapy has been reported in rodent studies^[143,144], this effect has not been confirmed in humans^[145-147]. Since incretin therapy is usually well tolerated without serious adverse effects, the use of incretin drugs is rapidly increasing^[148].

Glinides, short-acting insulin secretagogues, enhance early-phase insulin secretion, thereby reducing postprandial hyperglycemia^[149]. Since a defect in early-phase insulin secretion is a hallmark of glucose intolerance^[150], the enhancement of early-phase insulin secretion without prolonged hyperinsulinemia by glinides is more physiological, unlike the action of SUs, and is assumed to increase β -cell workload as well as the risk of hypoglycemia to a lesser degree compared with SUs.

Thus, the use of insulin secretagogues may be limited because of an increase in β -cell workload as well as increased risk of hypoglycemia. Since incretin enhances insulin secretion in a more physiological manner and is also expected to improve β -cell function and/or mass, incretin drugs could be used at any stage of T2DM.

On the other hand, SUs may be used rather to enhance incretin action at only a minimal dose. To recover physiological insulin secretion, a combination of an incretin drug and a glinide may also be useful.

Insulin has been shown to improve β -cell function in patients with IGT and T2DM^[151-153]. Since initial intensive insulin therapy has been shown to preserve β -cell function thereafter^[152], insulin therapy should be considered as early as possible in patients with T2DM. Insulin therapy is also the most effective medication to reduce HbA1c^[118]. However, the increased risk of hypoglycemia, weight gain and non-physiological insulin delivery (*i.e.*, systemic *vs* portal), in addition to the fear of injections, limit its use. Insulin therapy to overpower insulin resistance without eliminating excess calories may worsen ectopic lipid overload^[154].

A sodium-glucose cotransporter 2 (SGLT2) inhibitor has recently been approved in several countries including United States, EU and Japan. SGLT2 inhibitors suppress reabsorption of glucose by SGLT2 in the proximal renal tubule and increase glucose excretion in urine (~ 60 – 80 g glucose/d)^[155]. As a result, SGLT2 inhibitors not only decrease HbA1c, but also reduce body weight and blood pressure and improve the lipid profile. The action of SGLT2 inhibitors is independent of insulin. Thus, the efficacy of SGLT2 inhibitors seems to be regardless of β -cell function. SGLT2 inhibitors show a low risk of hypoglycemia but increase the incidence of bacterial urinary tract infections and fungal genital infections especially in women. A higher risk of hypotension has also been reported^[156]. SGLT2 inhibitors may be suitable for obese patients with T2DM and metabolic syndrome; however, their longer term safety including cardiovascular and cancer risk and efficacy remain unknown^[156,157].

Nonetheless, since currently no single therapy or agent can cure or even manage T2DM, an effective combination of current medications in addition to lifestyle modification aiming at reduction of β -cell workload is important to preserve or recover β -cell function.

Finally, marked weight reduction by bariatric surgery such as gastric bypass or sleeve gastrectomy has been reported to markedly improve glycemic control and even achieve remission of T2DM in severely obese T2DM patients^[158,159]. This also suggests the importance of reducing β -cell workload, although change in incretin secretion has also been proposed as another mechanism by which glucose metabolism is improved after gastric bypass. On the other hand, it has been reported that gastric bypass markedly improved incretin's effect on insulin secretion, but not insulin secretion induced by intravenous glucose infusion^[160], suggesting limited recovery of β -cell function even with marked weight loss. Also, the remission of T2DM after bariatric surgery is associated with residual β -cell function^[161,162], indicating the importance of residual β -cell function to manage and/or cure T2DM.

Implications for prevention

The progressive decline in β -cell functional capacity

during the development of glucose intolerance also implies the important role of preservation or recovery of β -cell function to prevent T2DM.

Similarly to the treatment of T2DM, prevention strategies should focus on reducing β -cell workload or inducing β -cell rest. These include lifestyle modification and/or weight reduction, and use of metformin or TZD. Lifestyle modification, *i.e.*, nutritional therapy and increase in physical activity, and weight reduction improve insulin sensitivity and thereby reduce β -cell workload. A number of studies have shown the efficacy of lifestyle intervention to prevent the development of T2DM in patients with IGT^[163-166]. In the Diabetes Prevention Program (DPP), intensive lifestyle modification with more than 7% weight loss suppressed the progression to T2DM by $\sim 58\%$ in patients with IGT^[165]. In the same study, metformin therapy also reduced the progression to T2DM by $\sim 31\%$ ^[165]. TZDs have also been shown to effectively suppress the progression from IGT to T2DM^[167-169]. A significant reduction in the development of diabetes was also observed in patients with IGT treated with AGIs^[134,135]. In the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial, adding basal insulin was also shown to suppress progression from IGT to T2DM^[151], probably through inducing β -cell rest. On the other hand, in the Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) trial, nateglinide, a short-acting insulin secretagogue, failed to show a reduction in progression to T2DM in patients with IGT^[170], suggesting that therapeutic strategies to increase β -cell workload may not be effective to prevent deterioration of glucose metabolism.

Importance of empowerment of patients

Although several anti-diabetic agents have been shown to effectively prevent the onset of T2DM, the importance of lifestyle modification remains unchanged, since the rapid increase in incidence of T2DM is certainly associated with the change in diet (*i.e.*, westernization) and physical inactivity, resulting in increased incidence of obesity. In the NAVIGATOR trial, it has been reported that both baseline level and change in daily ambulatory activity were associated with a reduced risk of cardiovascular events in patients with IGT^[171]. A six-year lifestyle intervention program for Chinese people with IGT showed a significant reduction in the incidence of cardiovascular and all-cause mortality as well as diabetes during 23 years of follow-up^[172]. A combination of diet and exercise appears more beneficial than either alone in obese older adults^[173]. Lifestyle modification may improve cardiovascular outcomes even after the onset of T2DM^[177].

Nonetheless, it is difficult to continue lifestyle modification in most patients. Patients' motivation is one of the most important factors in successful patient-centered management of T2DM^[118]. Therefore, it is important to motivate and encourage them to improve their adherence to daily lifestyle modification. In this context,

understanding the natural history of the development of T2DM and the importance of reducing β -cell workload to prevent or manage the disease may help to motivate or encourage patients to adhere to daily lifestyle changes.

Furthermore, as a whole society, not only patients with IGT or T2DM, but the healthy, general population should also be educated to motivate or encourage them to pursue a healthy lifestyle to prevent diseases associated with obesity and physical inactivity, resulting in improvement of quality of life (QOL). Changing our understanding of T2DM and a “modern” lifestyle may be needed to overcome this pandemic burden of T2DM all over the world.

CONCLUSION

This review summarizes the current knowledge of β -cell function and β -cell mass in T2DM. Recent evidence has emerged that a deficit of β -cell function along with β -cell mass is a hallmark of T2DM. Therefore, it is now acknowledged that a deficit of β -cell functional mass is a common characteristic of both type 1 and type 2 diabetes, indicating a core pathogenesis of diabetes. Genome-wide association studies have currently detected over 60 genetic loci associated with T2DM, most of which are assumed to relate to the β -cell, also indicating the importance of β -cells in the pathogenesis of T2DM^[174-178]. It is important to stress that diabetes never develops unless β -cells fail to compensate insulin resistance. In addition, β -cell function is related to treatment failure and glycemic control, suggesting its critical role in the management of T2DM. These findings suggest that recovery of β -cell functional mass is an important therapeutic strategy to manage or even cure T2DM. Although, unfortunately, currently no treatment strategy or medication to recover β -cell functional mass has been established, current evidence suggests that reducing β -cell workload is most effective to preserve β -cell functional mass. Thus, therapy or prevention of T2DM should focus on this point, and, therefore, lifestyle modification and weight loss remain the most important therapeutic strategy. Use of medication without lifestyle modification may even result in adverse outcomes. From the point of view of prevention, we need to tackle this pandemic burden of T2DM as a whole society, and correct understanding of the pathogenesis of T2DM may help motivate people to maintain a healthy lifestyle.

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I apologize to the many authors of original research whose publications I could not cite owing to space restrictions.

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Roles of interstitial fluid pH in diabetes mellitus: Glycolysis and mitochondrial function

Yoshinori Marunaka

Yoshinori Marunaka, Department of Molecular Cell Physiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Yoshinori Marunaka, Department of Bio-Ionomics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Yoshinori Marunaka, Japan Institute for Food Education and Health, St. Agnes' University, Kyoto 602-8013, Japan

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Correspondence to: Yoshinori Marunaka, MD, PhD, Professor and Chairman, Department of Molecular Cell Physiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566,

Japan. marunaka@koto.kpu-m.ac.jp

Telephone: +81-75-2515310

Fax: +81-75-2510295

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factors regulating various cell function such as enzyme activity and protein-protein interaction *via* modification of its binding affinity. Therefore, to keep cell function normal, the pH of body fluids is maintained constant by various systems. Insulin resistance is one of the most important, serious factors making the body condition worse in diabetes mellitus. I have recently found that the pH of body (interstitial) fluids is lower in diabetes mellitus than that in non-diabetic control, and that the lowered pH is one of the causes producing insulin resistance. In this review article, I introduce importance of body (interstitial) fluid pH in regulation of body function, evidence on abnormal regulation of body fluid pH in diabetes mellitus, and relationship between the body fluid pH and insulin resistance. Further, this review proposes perspective therapies on the basis of regulation of body fluid pH including propolis (honeybee product) diet.

Key words: pH; Interstitial fluid; Insulin; Binding affinity to receptors; Propolis

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Core tip: This review article provides new findings on changes of body (interstitial) fluid pH in type 2 diabetes mellitus, the role of body (interstitial) fluid pH in occurrence of insulin resistance, and future possibility of treatment for type 2 diabetes mellitus from a viewpoint of improvement of body (interstitial) fluid pH.

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Abstract

The pH of body fluids is one the most important key

INTRODUCTION

Metabolic syndrome increases the risk developing type

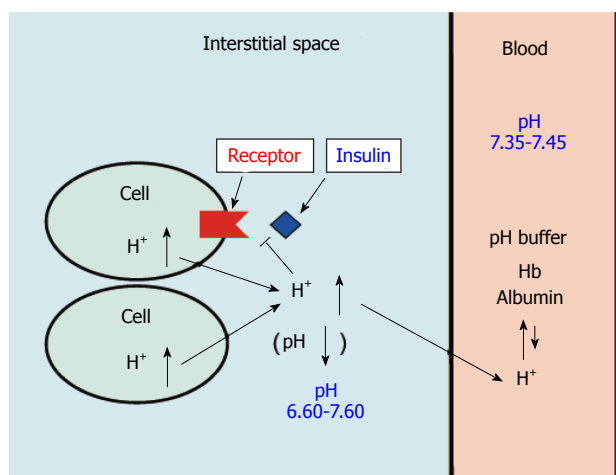


Figure 1 pH of interstitial fluid and blood, and binding affinity of insulin to its receptor. Interstitial fluids have little pH-buffering molecules, while blood has very strong, powerful pH buffering molecules such as hemoglobin and albumin. Thus, even under mild but not severe metabolic disorder conditions, blood pH is kept constant within a normal range (7.35-7.45), but interstitial fluid pH would be lower than a normal level.

2 diabetes mellitus, cardiovascular disease and cancer, *etc.*, meaning that it would be a pre-stage for diseases. Therefore, to prevent progression of metabolic syndrome is one of the most effective strategies for prevention of occurrence of type 2 diabetes mellitus. Insulin resistance is one of the most important, serious factors developing symptoms of type 2 diabetes mellitus. Patients with insulin resistance show hypertension, one of typical clinical symptoms for diagnosis of cardiovascular disorders^[1-4]. Further, patients with hyperinsulinemia, which is generally caused by insulin resistance, show hypertension mediated through various types of disorders such as renal failure, vascular dysfunction, and hyper-activation of sympathetic nerve^[5-10]. These findings suggest that preventing development of insulin resistance would be one of the most important key subjects keeping the healthy body function.

Pathogenesis of insulin resistance means insufficiency of insulin action on glucose uptake in skeletal muscle^[11], sustaining blood glucose at high levels after meals; this is known as one of typical symptoms of type 2 diabetes mellitus. The number of patients suffering from type 2 diabetes mellitus still continuously increases, and this becomes one of the most serious worldwide social problems^[12]. Thus, clarification of mechanisms causing insulin resistance is one of the most important key subjects on prevention and treatment for diabetes mellitus. Unfortunately, the mechanisms of occurrence of insulin resistance have not yet been fully clarified.

As described above, one of the most major symptoms of type 2 diabetes mellitus is insulin resistance causing hyperglycemia, which leads to progression of pancreatic β -cell dysfunction due to hyper-secretion of insulin. Continuous hyperglycemia due to poor uptake of glucose into cells such as skeletal muscles, adipocytes, hepatocytes, *etc.*, in general, irreversibly leads to macro- and micro-vascular complications, resulting in myocardial infarction,

stroke, blindness, renal dysfunction, and peripheral neuropathy. International Diabetes Federation (IDF) reports that in 2012, 370 million people are recognized as diabetes mellitus in the worldwide, and the number of people with diabetes mellitus is considered to increase up to 550 million by 2030^[12]. Various types of drugs have been developed for treatment of type 2 diabetes mellitus, however a tremendous number of people still suffer from type 2 diabetes mellitus. This means that although some newly developed drugs for treatment of type 2 diabetes mellitus are very efficient, the drugs are still not effective to fully treat patients suffering from type 2 diabetes mellitus.

Interstitial fluids provide circumstances where extracellular signaling molecules such as hormones and neurotransmitters regulate cell function. This means that alteration of interstitial fluid composition affects efficiency of signal transduction of extracellular signaling molecules in intracellular signal transduction. Specially, it is notable that pH of interstitial fluids is very variable, since interstitial fluids contain little pH buffering molecules (Figures 1 and 2). On the other hand, blood has powerful pH buffering such as hemoglobin, albumin, *etc.*, (Figures 1 and 2), keeping strictly pH of blood at a range between 7.35-7.45. These facts mean that even if pH of blood stays at normal levels, 7.35-7.45, pH of interstitial fluids would deviate from the normal range under metabolically pathophysiological conditions. As described above, hormones and neurotransmitters act on their receptors in the interstitial (extracellular) fluids (spaces) but not inside blood vessels (Figures 1 and 2). Further, it is notable that pH regulates activity of various types of enzymes and binding affinity of hormones and neurotransmitters to their receptors (Figure 1). This means that pH of interstitial fluids plays one of the most important key roles in regulation of cell function keeping homeostasis of the body function and condition, nevertheless unfortunately little information on pH of interstitial fluids is available.

As mentioned above, activity of most enzymes and binding affinity of hormones and neurotransmitters to their receptors directly depend on pH of interstitial fluids. Therefore, keeping normal body/cell function requires maintenance of interstitial fluid pH within a normal range. Energy has to be also supplied to keep normal cell/body function. This process produces organic acids *via* glycolysis and CO_2 *via* TCA cycle (Figure 2). Under physiological conditions, these acids including CO_2 (H^+ produced from CO_2 and H_2O) and organic acids are extruded *via* the lung and the kidney to keep pH of interstitial fluids within a normal range. However, under metabolically pathophysiological conditions such as diabetes mellitus, pH of interstitial fluids would become lower; *i.e.*, interstitial fluids become acidic. For example, severe diabetes mellitus causes ketoacidosis detected as lowered pH (< 7.35) of "arterial" blood even containing strong pH buffers such as hemoglobin and albumin. This suggests that the interstitial fluid pH with little pH buffer in severe diabetes mellitus would be much lower than that in normal persons. The lowered pH of interstitial

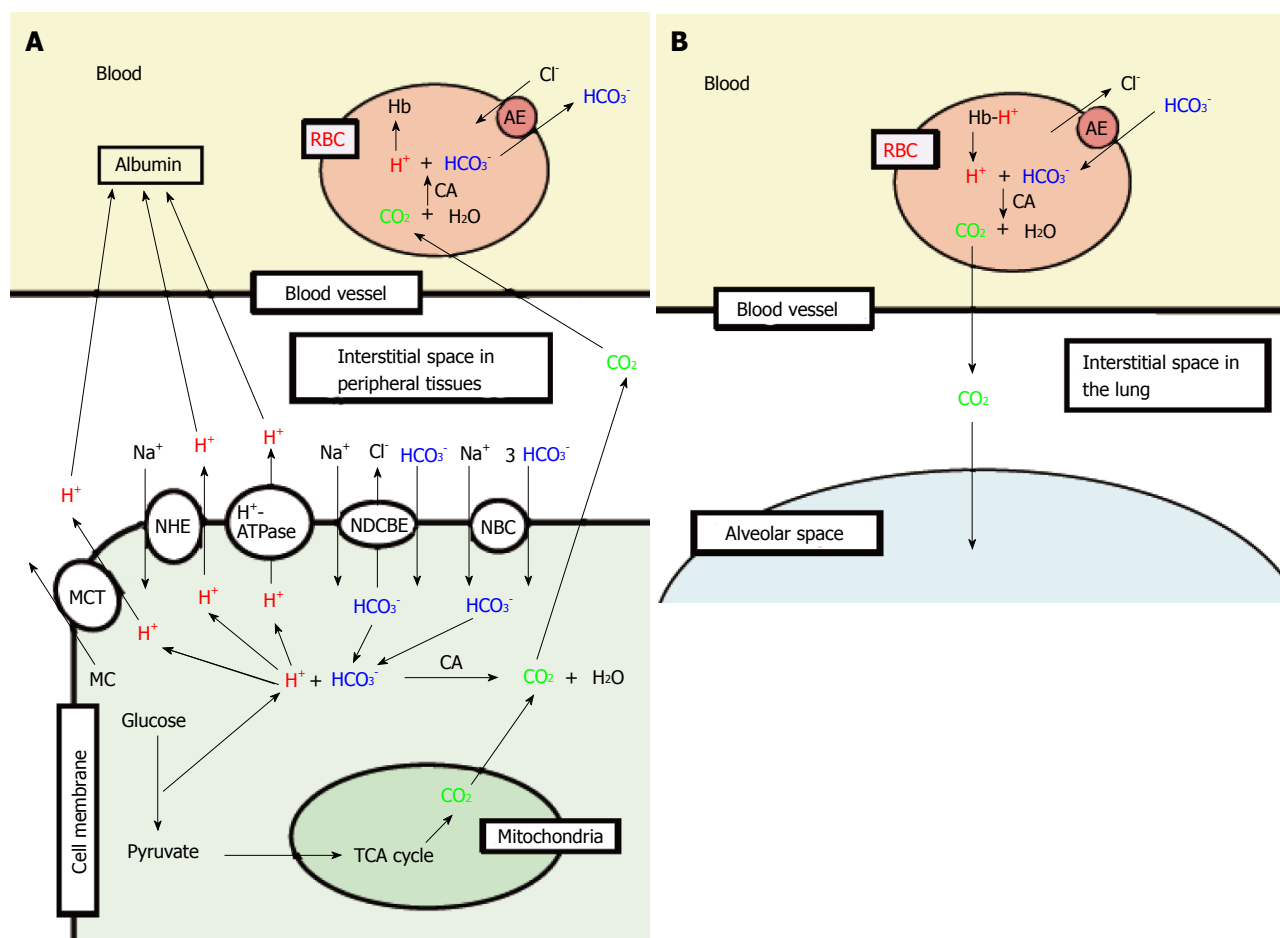


Figure 2 Production of H^+ , and H^+ transporting systems in peripheral tissues (A) and the lung (B). A: In cells of peripheral tissues, H^+ is produced from organic acids generated as metabolites via glycolysis such as lactic acid. H^+ is directly extruded via Na^+/H^+ exchanger (NHE), H^+ -ATPase and H^+ -coupled monocarboxylate (MC) transporter (MCT) from intracellular to extracellular (interstitial) spaces, and moves into blood, and binds to albumin. Further, a part of H^+ produced from metabolites is converted to CO_2 and H_2O consuming HCO_3^- via carbonic anhydrase (CA)-mediated facilitation process. To supply HCO_3^- consumed for conversion of H^+ to CO_2 and H_2O in cells, Na^+ -driven Cl^-/HCO_3^- exchanger (NDCBE) and Na^+ - HCO_3^- cotransporter (NBC) participate in uptake of HCO_3^- into intracellular from extracellular (interstitial) spaces. CO_2 moves into red blood cell (RBC, erythrocyte) in blood via permeation across the plasma membrane of RBC due to high CO_2 permeability of the plasma membrane, and is converted to H^+ and HCO_3^- consuming H_2O via CA-mediated facilitation process. H^+ produced from CO_2 and H_2O via CA-mediated facilitation process in RBC binds to hemoglobin. HCO_3^- produced from CO_2 and H_2O via CA-mediated facilitation process in RBC is extruded from intracellular to extracellular (interstitial) spaces via exchange of Cl^- existing in the extracellular space by anion exchanger (AE): this exchanging step of HCO_3^- extrusion and Cl^- uptake is so called as Cl^- shift; B: In the lung, the reversible process occurs due to low CO_2 circumstances.

fluids under metabolically pathophysiological conditions leads patients to being further worse conditions of the disease^[13,14]. Interestingly, even in pre-disease stages pH is drastically lowered in interstitial fluids around various tissues including the brain^[15-17], developing diseases.

In this review article, I provide a new concept regarding insulin resistance and its improvement; particularly I discuss the role of interstitial fluid pH in cell function in diabetes mellitus.

INTERSTITIAL FLUIDS

Interstitial fluid pH kept within a normal range plays a role as one of the most important key factors in keeping normal cell/body functions and adaptation of body condition as mentioned above. However, unfortunately interstitial fluids have little pH buffers unlike blood. The reason why interstitial fluids have little pH buffers

unlike blood containing hemoglobin and albumin, very strong pH buffers, is as follows. If interstitial fluids have pH-buffering proteins such as albumin, colloid osmotic pressure of interstitial fluids becomes larger than that without pH buffering proteins such as albumin. High colloid osmotic pressure of interstitial fluids leads to disturbance of transport and circulation of nutrition and metabolites between blood and interstitial fluids across walls of blood vessels. Namely, metabolites produced in peripheral tissues are collected into capillaries near veins by the larger colloid osmotic pressure in the capillary than that in the interstitial fluid. This driving force of metabolite collection into capillary becomes low if the colloid osmotic pressure becomes large. Therefore, the fact that interstitial fluids have little pH buffering proteins is a weak point for keeping activity of hormones and enzymes at normal levels, but is essentially required for collection of metabolites produced in peripheral tissues

into capillaries. In general, the pH-buffering capacity in blood is large enough to keep the interstitial fluid pH constant under physiological conditions. Unfortunately, under metabolically pathophysiological conditions, the pH-buffering capacity in blood is not large enough to maintain activity of hormones and enzymes in interstitial fluids, resulting in disorders such as insulin resistance.

REGULATION AND ABNORMALITIES IN pH OF INTERSTITIAL FLUIDS

The pH of mammalian “arterial” blood is accurately maintained at 7.40 ± 0.05 under normal physiological conditions. Severe metabolic disorders cause deviation of “arterial” blood pH more than 0.05 unit from a normal range of 7.35-7.45; *i.e.*, pH < 7.35 is defined as acidosis or pH > 7.45 is defined as alkalosis. Alkalosis, pH > 7.45, is caused by vomiting of gastric juices or diarrhea (metabolic alkalosis), or hyperventilation (respiratory alkalosis), *etc.* However, long-term alkalosis rarely occurs. On the other hand, acidosis (specially long-term acidosis) occurs in various metabolic disorders including diabetes mellitus. Acidosis and alkalosis are well recognized as severe disorders of body conditions, however little information is available on the interstitial fluid pH. Even under the condition with normal pH (7.35-7.45) of “arterial” blood, pH of interstitial fluids would deviate from the normal range. Our previous reports indicate that the pH of interstitial fluids is deviated from the normal range^[15-17] even under conditions maintained at normal “arterial” blood pH. The pH of interstitial fluids is determined by the content of H^+ (proton) provided from organic acids as metabolites produced at ATP synthesis in living cells.

One of typical H^+ sources is lactate, $CH_3-CH(OH)-COOH$ [$CH_3-CH(OH)-COO^- + H^+$], which is converted from pyruvate, $CH_3-CO-COOH$ ($CH_3-CO-COO^- + H^+$), a product from glycolysis. In tissues requiring much energy (ATP) such as skeletal muscles, the anaerobically glycolytic metabolism mediates the conversion of glucose and glycogen into lactic acid *via* production of pyruvate. Under an aerobic condition, pyruvate is used for TCA cycle conducted in mitochondria. Therefore, under physiological conditions little amounts of lactate are generated, and most of the final product of glycolysis followed by TCA cycle is CO_2 , which is facilitated to be converted into H^+ and HCO_3^- by carbonic anhydrase. Of course, CO_2 is one of major sources for H^+ . However, to obtain a fixed amount of ATP, the amount of H^+ generated by organic acids and CO_2 produced in the process for generation of ATP mediated *via* both glycolysis and TCA cycle is much smaller than that produced only by glycolysis. Namely, under conditions with ATP synthesis predominantly mediated *via* glycolysis but not followed by function of TCA cycle, the total amount of produced H^+ is much larger than that under conditions with ATP synthesis *via* glycolysis associated with functional TCA cycle. Patients with diabetes mellitus are suggested to have reduced mitochondria function^[11,18-20].

Based on this suggestion^[11,18-20], the total amount of H^+ produced in patients with diabetes mellitus is much larger than that in healthy persons with normal mitochondrial function. Even in cases that blood pH in patients with diabetes mellitus except severe cases is within a normal range (7.35-7.45), pH of interstitial fluids would be less than 7.35.

Other sources of H^+ are ketone bodies; *i.e.*, metabolism of fatty acids in liver generates beta-hydroxybutyrate, which provides H^+ *via* dissociation into beta-hydroxybutyrate anion and H^+ ($\text{beta-hydroxybutyrate}^- + H^+$)^[21]. Beta-hydroxybutyrate is the major ketone body (approximately 70% of total ketone bodies) produced by TCA cycle in liver mitochondria *via* oxidation of free fatty acids released from adipocytes^[22]. Another major ketone body is acetoacetate, which is converted to beta-hydroxybutyrate. The synthesis of these ketone bodies in liver mitochondria is, in general, occurs in response to an unavailability of blood glucose, contributing to overall energy metabolism. The ketone bodies produced in liver mitochondria are transported *via* blood to extra-hepatic tissues such as skeletal muscles and heart muscles. Under conditions with an unavailability or low availability of blood glucose, the transported ketone bodies such as beta-hydroxybutyrate and acetoacetate to muscles are used as sources of acetyl CoA, which is utilized in TCA cycle in mitochondria of muscles for generation of ATP^[23]. Further, fatty acids can be converted to acetyl CoA without formation of ketone bodies, meaning that fatty acids are sources of ATP in mitochondria. However, when amounts of fatty acids is very large, abundant ketone bodies are produced in liver mitochondria and the amount of ketone bodies exceeds the metabolizing capacity in mitochondria of muscles^[23]. In this case, the body produces a large amount of ketone bodies, leading to elevation of H^+ concentration (lowered pH) as mentioned above. This means that when low utilization of glucose or low mitochondria function occurs, high levels of ketone bodies appear in periphery tissues, providing a large amount of H^+ (low pH).

METABOLITES INFLUENCING pH OF INTERSTITIAL FLUIDS AND pH-BUFFERING SYSTEMS

Lactate is dissociated into $CH_3-CH(OH)-COO^- + H^+$ under physiological conditions of pH (approximately 7.40) much higher than pKa of lactate (3.86), leading to production of H^+ . In addition to lactate, as mentioned above H^+ is also provided from materials such as ketone bodies; for example, metabolism of fatty acids in liver generates beta-hydroxybutyrate, one of typical ketone bodies, provides H^+ *via* dissociation into beta-hydroxybutyrate anion and H^+ ($\text{beta-hydroxybutyrate}^- + H^+$). The H^+ produced in cells *via* metabolism is extruded as a form of H^+ itself or CO_2 *via* various types of H^+ transporter such as Na^+/H^+ exchanger (NHE), H^+ -ATPase, H^+ -coupled monocarboxylate transporter (MCT),

Na^+ -driven $\text{Cl}^-/\text{HCO}_3^-$ exchanger (NDCBE), and Na^+ - HCO_3^- cotransporter (NBC), *etc.*, (Figure 2A)^[24-29]. Namely, extrusion of H^+ as a form of H^+ itself is mediated by NHE, H^+ -ATPase and/or MCT, *etc.*, that directly transport H^+ to the extracellular (interstitial) space. On the other hand, NDCBE or NBC doesn't directly transport H^+ , but uptake HCO_3^- into the intracellular space, resulting in production of $\text{CO}_2 + \text{H}_2\text{O}$ from H^+ and HCO_3^- *via* carbonic anhydrase (CA). CO_2 produced by H^+ and HCO_3^- *via* a CA-facilitated process easily moves to the extracellular space by permeating the plasma membrane, since the plasma membrane of cells has high permeability to CO_2 (Figure 2A). Thus, H^+ produced in the intracellular space is extruded to the extracellular (interstitial) space as a form of CO_2 *via* consumption of HCO_3^- transported from the extracellular (interstitial) space. Because, HCO_3^- originally generated from CO_2 produced *via* TCA cycle in cells associated with H^+ could not be a net source for conversion to CO_2 due to its origin, CO_2 . Thus, H^+ produced from lactate, *etc.*, in the intracellular space is extruded to the extracellular (interstitial) space *via* directly NHE/ H^+ -ATPase/MCT, and indirectly NDCBE/NBC. Anyway, H^+ produced from metabolites such as lactate, *etc.*, consumes HCO_3^- , reducing the intracellular concentration of HCO_3^- associated with a compensatory increase in HCO_3^- uptake *via* NDCBE and NBC. The source of HCO_3^- under anaerobic conditions associated with dysfunction of mitochondria in cells such as muscles is HCO_3^- in blood, which is produced from $\text{CO}_2 + \text{H}_2\text{O}$ *via* facilitated conversion by carbonic anhydrase in other tissues. H^+ is finally extruded from the body *via* the kidney into urine. Therefore, severe overproduction of H^+ spends HCO_3^- , being converted into CO_2 and H_2O (Figure 2). This CO_2 produced from H^+ and HCO_3^- moves into red blood cells (RBC, erythrocytes), and is again converted into H^+ and HCO_3^- *via* a CA-facilitated process (Figure 2A). In red blood cells, H^+ binds to hemoglobin, and HCO_3^- is extruded to the extracellular space in blood *via* anion exchanger (AE) (Figure 2A). Namely, even though HCO_3^- is consumed in cells, HCO_3^- is again produced in red blood cells, suggesting that HCO_3^- is not consumed in peripheral tissues. However, HCO_3^- is transported into red blood cells *via* AE in blood at the lung due to low CO_2 pressure compared with peripheral tissues, and HCO_3^- is converted to CO_2 with H^+ released from hemoglobin (Figure 2B). CO_2 produced in this process is extruded to atmosphere (Figure 2B). Thus, *via* this overall process the concentration of HCO_3^- in blood is reduced under these metabolically pathophysiological conditions. Severe overproduction of H^+ causes metabolic acidosis consuming HCO_3^- , pH of "arterial" blood being less than 7.35. This acidosis has been previously recognized to occur as results from general metabolic disorders, however diabetes mellitus is recently indicated to be associated with mitochondrial dysfunction^[11,18-20]. This mitochondrial dysfunction is one of main causes leading to acidosis. Further, as mentioned above, lowered pH is also caused by H^+ dissociated from ketone bodies

generated in liver provide, which are mainly produced *via* oxidation of free fatty acids released from adipocytes^[22]; representative ketone bodies are beta-hydroxybutyrate, and acetoacetate. As described above, the synthesis of these ketone bodies in the liver mitochondria are generally produced in response to an unavailability of blood glucose in muscles. Therefore, these ketone bodies are utilized as sources of acetyl CoA for generation of ATP *via* TCA cycle in mitochondria of muscles^[23]. Under conditions with mitochondrial dysfunction in muscles, these ketone bodies generated in the liver are not utilized as sources of acetyl CoA for generation of ATP *via* TCA cycle in mitochondria of muscles^[23]. Thus, under these conditions, the ketone bodies provide a lot of H^+ , leading to much lower pH (acidosis) than that under conditions with normal mitochondrial function.

Lactate in intracellular spaces is a useful energy source *via* oxidation as a respiratory fuel^[30,31]. Thus, the cytosolic lactate produced in fast muscles contracting relatively fast at heavy exercise under physiological conditions is extruded to the extracellular (interstitial) space *via* MCTs^[32,33], being delivered to oxidative tissues *via* extracellular lactate shuttle through the blood^[34]. Specially, in diabetes mellitus patients, lactate is generated due to mitochondrial dysfunction even in cases of regular exercise without heavy contraction of fast muscles^[20,35]. In most mammalian cells, MCTs participate in the transport of lactate and other monocarboxylic acids such as pyruvate, beta-hydroxybutyrate and acetoacetate across the cellular membrane^[36-38]. It has been indicated that patients suffering from diabetes mellitus show alteration of MCTs expression^[39,40]. Since MCTs carry monocarboxylate with H^+ , MCTs function as H^+ extrusion coupled with monocarboxylate extrusion (Figure 2A), meaning that MCTs plays important, essential roles in pH and energy balance in patients suffering from diabetes mellitus.

ABNORMAL INTERSTITIAL FLUID pH AND PROGRESSION OF DIABETES MELLITUS

As well known, insulin decreases blood glucose levels by stimulating glucose uptake into skeletal muscle cells *via* glucose transporter 4 (GLUT4), maintaining whole-body glucose homeostasis^[41]. GLUT4 translocation to the plasma membrane from intracellular store sites is the main mechanism of insulin showing its stimulatory action on glucose uptake into skeletal muscle cells and adipocytes. Insulin regulates a dynamic process of GLUT4 trafficking between the plasma membrane and its intracellular store sites^[42]. Insulin binds to its receptor located on the plasma membrane, immediately auto-phosphorylating tyrosine residues of the receptor. This auto-phosphorylation of insulin receptor subsequently induces phosphorylation of tyrosine residues of insulin receptor substrate-1 (IRS-1) phosphorylating (activating) PI3K, which catalyzes 3' phosphorylation of phosphatidylinositol 4,5-diphosphate,

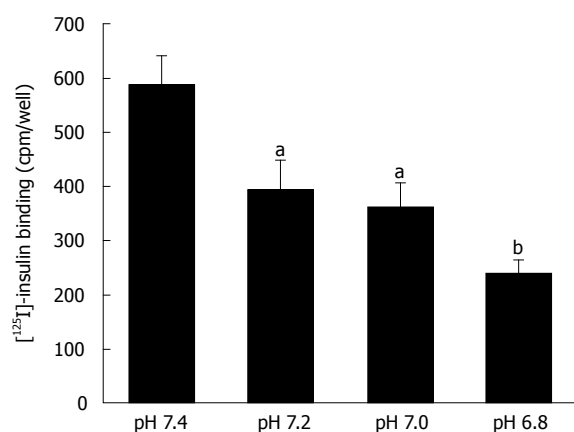


Figure 3 Insulin binding to insulin receptor under various pH conditions. After serum starvation for 4 h, L6 myotubes were treated with 100 nmol/L insulin for 15 min in the HEPES buffer with different values of pH. Proteins expressed on the plasma membrane were biotinylated and precipitated. Differentiated L6 myotubes were treated with [¹²⁵I]-labeled insulin for 15 min in the indicated pH buffers, and the radioactivities were measured after cells were washed and suspended. The values of radioactivity from at least 6 experiments are shown. The values are shown as the mean \pm SEM. ^a $P < 0.05$, ^b $P < 0.01$ vs pH 7.4. Modified from ref.[46] with allowance of non-profit use of figures.

leading to activation of Akt. This PI3K/Akt-mediated signaling pathway in the insulin-induced down-stream pathway stimulates the intracellular translocation of GLUT4 to the plasma membrane, elevating glucose uptake in skeletal muscles. Dysfunction of this insulin signal transduction leads to reduced levels of insulin-stimulated glucose uptake into skeletal muscles in type 2 diabetic patients, and this dysfunction is so-called insulin resistance^[43]. Our recent study has shown that pH of interstitial fluids is lower in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model in type 2 diabetes mellitus, than normal one^[15]. Many epidemiological studies have recently reported the relationship between metabolic acidosis and insulin resistance^[44]. Organic acids-induced acidosis would contribute to early stages in the development of insulin resistance^[15,44-46]. The relationship between production of organic acids and development of insulin sensitivity is an important subject in patients suffering from type 2 diabetes mellitus^[47-49]. Insulin sensitivity and urine pH have negative correlation with body weight and waist size^[34]. Persons with metabolic syndrome are reported to have a significantly lower value of 24-h urine pH than that in normal persons without metabolic syndrome^[48]. Persons with higher amounts of anion gap in metabolic acidosis associated with lower serum HCO₃⁻ show lower insulin sensitivity^[50]. Our recent studies^[15,17] indicate that the interstitial fluid pH in ascites, brain hippocampus and metabolic tissues in Otsuka Long-Evans Tokushima Fatty (OLETF) rats in early developing stages of diabetic mellitus is lower than the normal pH (7.40). Although our studies have not yet clarified the molecular mechanism causing lowered pH of interstitial fluids, these phenomena would be due to dysfunction or hypo-function of mitochondria in diabetes mellitus^[11,19,20]. As mentioned above, the pH-

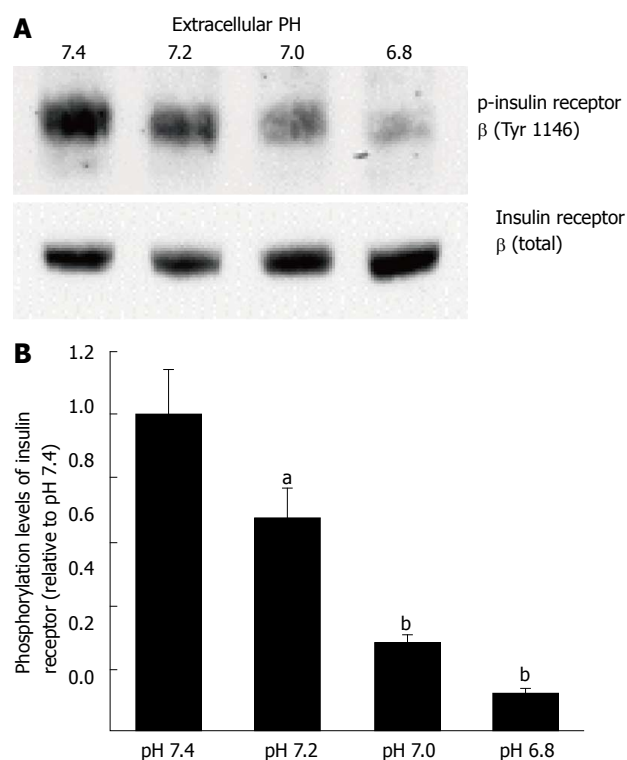


Figure 4 Phosphorylation levels of insulin receptor. After serum starvation for 4 h, L6 myotubes were treated with 100 nmol/L insulin for 15 min in the buffer with different pH. Total cell lysates were isolated and analyzed by Western blotting with indicated antibodies. A: Representative blots are shown; B: The quantitative values of expression of insulin receptor using densitometry from 6 independent experiments using anti-phosphorylated-insulin receptor- β (Tyr 1146) antibody normalized to the level of total insulin receptor compared with that in pH 7.4 buffer. The values are shown as the mean \pm SEM ($n = 6$). ^a $P < 0.05$, ^b $P < 0.01$ vs pH 7.4. Modified from ref.[46] with allowance of non-profit use of figures.

buffering capacity in the interstitial fluid is much lower than that in the cytosol and in the blood, meaning that pH of interstitial fluids in metabolic tissues has valuable values depending on metabolic conditions. Therefore, we have studied if the lowered extracellular (interstitial fluid) pH reduces insulin action on its signaling pathways in rat skeletal model cells^[46,51]. As mentioned above, insulin shows its stimulatory action on glucose uptake in skeletal muscle in a phosphatidylinositol 3-kinase (PI3K)-mediated pathway after binding to its receptor located on the plasma membrane *via* phosphorylation of its receptor. Therefore, we first studied if the insulin binding to its receptor is affected by lowering extracellular (interstitial fluid) pH. We have found that lowered extracellular (interstitial) fluid pH diminishes binding affinity of insulin to its receptor (Figure 3) associated with diminution of insulin receptor phosphorylation (activation) (Figure 4) without any change in expression of insulin receptor on the plasma membrane of skeletal muscle (Figure 5)^[46]. Further, levels of phosphorylated (activated) Akt, a down-stream molecule of insulin signaling pathway, are decreased under conditions with lowered extracellular (interstitial fluid) pH (Figure 6)^[46]. Glucose uptake is also diminished under the condition^[46]. These observations indicate the importance

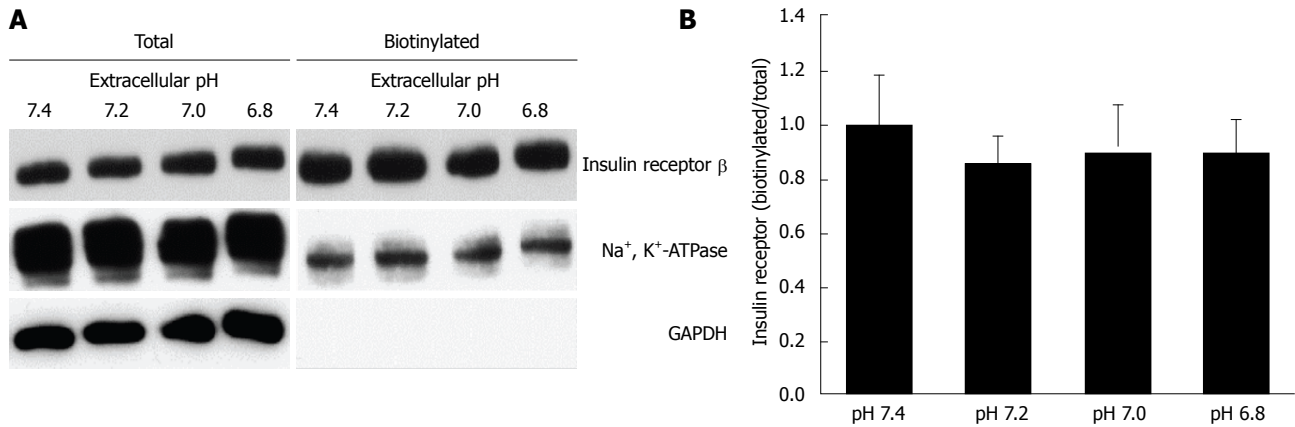


Figure 5 Effects of extracellular pH on the expression of insulin receptor on the plasma membrane. After serum starved for 4 h, L6 myotubes were treated with 100 nmol/L insulin for 15 min in the HEPES buffer with different pH. Proteins expressed on the plasma membrane were biotinylated and precipitated. A: Representative blots of total expression of insulin receptor on the plasma membrane, the Na^+ , K^+ -ATPase, and GAPDH; B: Quantitative data of expression of insulin receptor on the plasma membrane at different pH normalized to that at pH 7.4. The results are presented as the mean \pm SEM ($n = 8$). pH had no significant effects on the expression of insulin receptor on the plasma membrane. Modified from ref.[46] with allowance of non-profit use of figures.

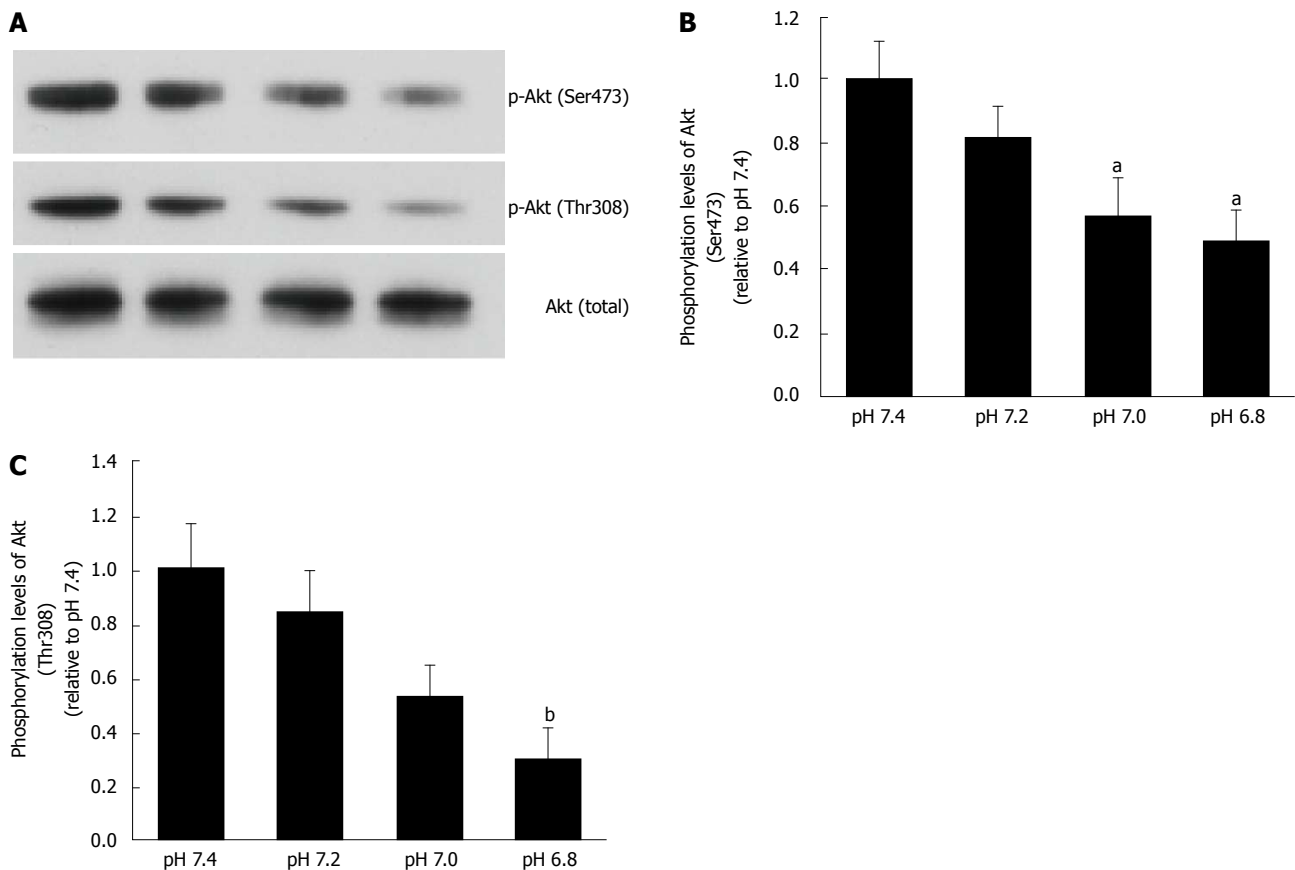


Figure 6 Phosphorylation levels of Akt. L6 myotubes were treated with 100 nmol/L insulin for 15 min in the buffer with different pH after serum starvation for 4 h. Total cell lysates were isolated, and were analyzed by Western blot with the indicated antibodies. A: Representative blots are shown using anti-phosphorylated (Ser473)-Akt, anti-phosphorylated (Thr308)-Akt, and anti-Akt antibodies. Phosphorylation levels of Ser473 (B) and Thr308 (C) are expressed as normalized values to the level of total Akt compared with those in pH 7.4. The values are shown as the mean \pm SEM ($n = 6$). ^a $P < 0.05$, ^b $P < 0.01$ vs pH 7.4. Modified from ref.[46] with allowance of non-profit use of figures.

of interstitial fluid pH in occurrence of insulin resistance.

Our report also indicates an interesting observation regarding effects of propolis on various factors in type 2 diabetes mellitus model rats^[15,16]. Propolis, a natural compound derived from plant resins collected by

honeybees, contains various factors such as amino acids, steroids, phenolic aldehydes, polyphenols, sesquiterpene quinines, and coumarins, *etc.*^[52]. Propolis has been shown to possess anti-oxidant, anti-inflammation, and anti-tumor activities^[53-56]. In addition to these actions, we

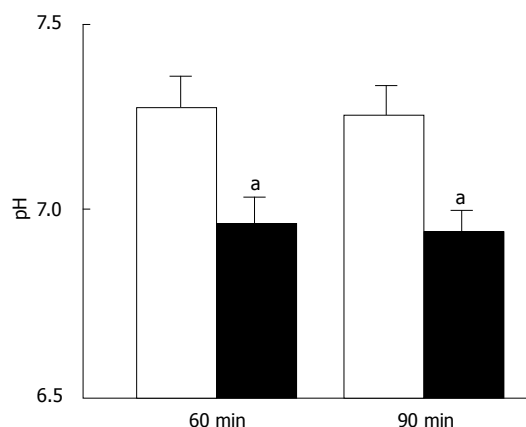


Figure 7 pH of interstitial (extracellular) fluid around the hippocampus of Otsuka Long-Evans Tokushima Fatty and normal (Wistar) rats. The pH value is shown as the mean \pm SEM ($n = 4$). The pH values shown in Figure 7 were measured at 60 and 90 min after antimony pH electrodes reached interstitial (extracellular) fluids around the hippocampus of the Otsuka Long-Evans Tokushima Fatty rats (closed columns) and normal (Wistar) rats (open columns). ^a $P < 0.05$ compared with that in normal (Wistar) rats at each measured time. Modified from ref.[17] with allowance of free use of figures.

have found that propolis improves insulin sensitivity^[15,16]. Propolis improves (elevates) pH of interstitial fluids that is lower in type 2 diabetes mellitus than normal one^[15,16]. Lowered pH of interstitial fluids diminishes binding affinity of insulin to its receptor (Figure 3), causing insulin resistance^[46]. Although we have no information on molecular mechanisms how propolis improves lowered pH of interstitial fluids in type 2 diabetes mellitus at the present stage, these observations indicate us a very interesting point that propolis improves insulin sensitivity by elevating pH of interstitial fluids *via* recovery from diminished insulin binding affinity to insulin receptor in type 2 diabetes mellitus.

FLUIDS SECRETED INTO GASTROINTESTINAL LUMINAL SPACE AND FROM SEWEAT GLAND IN DIABETES MELLITUS

As mentioned above, the interstitial fluid has lower pH in type 2 diabetes mellitus than non-diabetic control. In addition to the interstitial fluid, I discuss about pH of fluids secreted from glands and gastrointestinal fluids. Before discussing about pH of those fluids, I should mention that these fluids are not secreted for maintenance of intracellular ionic conditions unlike the interstitial fluids. As mentioned above, pH of the interstitial fluids is lowered as a result from high production of H^+ due to mitochondrial dysfunction and/or disability of glucose in muscles, neurons and, *etc.* On the other hands, pH of fluids secreted from glands and gastrointestinal fluids is, in general, not directly influenced by high production of H^+ due to mitochondrial dysfunction and/or disability of glucose in muscles, neurons and *etc.* When mitochondrial dysfunction and/or disability of glucose occur in gland

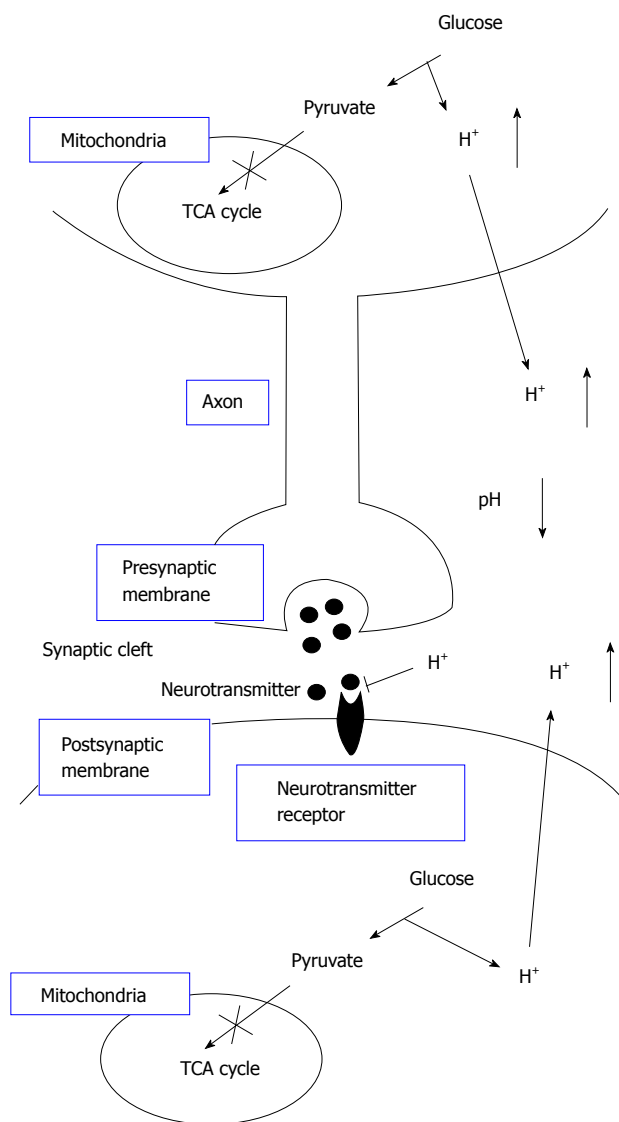


Figure 8 The pH-dependent mechanism of neural cell function in diabetes mellitus with dysfunction of mitochondria. Neural cells with dysfunction of mitochondria synthesize ATP required for maintenance of neural cell function only or mainly *via* glycolysis. Thus, neural cells with dysfunction of mitochondria produces much larger amounts of H^+ than neural cells with normal function of mitochondria. H^+ produced by glycolysis in neural cells with dysfunction of mitochondria^[11,18-20] is released to the extracellular space, lowering pH of interstitial fluids^[15-17] including the fluids in synaptic clefts. Lowered pH of synaptic cleft fluid diminishes the binding affinity of neurotransmitters to their receptors^[67]. Thus, activity of neural cells is diminished at lowered pH of synaptic cleft fluid. Namely, the amount of neurotransmitters released into the synaptic cleft is large enough for generation of action potential under conditions with normal function of mitochondria. However, the amount of neurotransmitters released into the synaptic cleft is insufficient for generation of action potential under conditions with dysfunction of mitochondria, since lowered pH of synaptic cleft diminishes the binding affinity of neurotransmitters to receptors. Modified from ref.[17] with allowance of free use of figures.

or gastrointestinal cells, H^+ produced in these cells is, in general, extruded to the extracellular space across the basolateral membrane (so called interstitial fluid) but not to the luminal space across the apical membrane by H^+ transporter such as NHE, H^+ -ATPase and *etc.* However, a study^[57] indicates that the amounts of acid secreted from gastric gland under the basal condition and in response

to cholecystokinin are larger in diabetes than that in non-diabetic control. This suggests that the intra-gastric pH in diabetes is lower than that in control. On the other hand, HCO_3^- secretion from pancreas in response to secretin shows no difference between diabetes and non-diabetic control^[57]. Further, observations on sweating in diabetes are reported^[58,59]. Most studies on sweating in diabetes are focused on blood flow around sweat glands and blood-flow-dependent amounts of sweat secretion without studies on ionic composition^[58,59], although a study indicates that Cl^- concentration is not changed in diabetes^[60].

ALZHEIMER'S DISEASE IN DIABETES MELLITUS AND pH OF INTERSTITIAL FLUID

Patients with type 2 diabetes mellitus have been suggested to have a high risk of developing dementia and Alzheimer's disease with defective memory functions^[61]. Insulin is suggested to be necessary for neuronal survival within the central nervous system^[62,63]. Fluctuating levels of blood glucose resulting from dysfunction of insulin (insulin resistance) leads neurons including central nervous system to apoptosis, formation of neuritic plaques, neurofibrillary tangles, energy starvation, and altered acetylcholine levels in the hippocampus, which are observed in Alzheimer's disease^[64,65]. Hippocampus is an important region participating in memory function^[66]. We have found that pH of interstitial fluid around hippocampus is lower in type 2 diabetes mellitus model OLETF rats than that in normal ones (Figure 7), suggesting diminution of neuronal activity around hippocampus (Figure 8)^[17,67].

Therefore, we suggest that maintenance of the interstitial fluid pH at the normal level or the recovery of the "interstitial" pH to normal from lowered levels would be a key factor in developing molecular and cellular therapies for metabolic brain disorders including Alzheimer's disease.

CONCLUSION

Interstitial fluids have little pH buffering capacity. Therefore, over production of acid metabolites lower pH of interstitial fluids even when the intracellular and "arterial" blood pH remains normal (Figures 1 and 2). The lowered pH of interstitial fluids causes insulin resistance *via* reduced binding affinity of insulin to its receptor (Figures 1 and 3). Acidic environments due to dysfunction of mitochondria occurring in type 2 diabetes mellitus lead to insulin resistance. Further, the acidic environment occurring in the brain would be related to diminution of neuronal function and onset of Alzheimer's disease (Figure 8).

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Ipragliflozin: A novel sodium-glucose cotransporter 2 inhibitor developed in Japan

Tsuyoshi Ohkura

Tsuyoshi Ohkura, Division of Cardiovascular Medicine, Endocrinology and Metabolism, Department of Molecular Medicine and Therapeutics, Tottori University Faculty of Medicine, Yonago, Tottori 683-8504, Japan

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Correspondence to: Tsuyoshi Ohkura, MD, PhD, Assistant Professor, Division of Cardiovascular Medicine, Endocrinology and Metabolism, Department of Molecular Medicine and Therapeutics, Tottori University Faculty of Medicine, 36-1 Nishi-chou, Yonago, Tottori 683-8504, Japan. ohkura@med.tottori-u.ac.jp

Telephone: +81-859-386517

Fax: +81-859-386519

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glucose excretion increased to 90 g/24 h after 28 d of treatment with ipragliflozin 300 mg/d. Twelve weeks of ipragliflozin 50 mg/d *vs* placebo reduced glycated hemoglobin and body weight by 0.65% and 0.66 kg, respectively, in Western T2DM patients, and by 1.3% and 1.89 kg, respectively, in Japanese patients. Ipragliflozin (highly selective SGLT2 inhibitor) improves glycemic control and reduces body weight and lowers hypoglycemic risk and abdominal symptoms. Ipragliflozin can be a novel anti-diabetic and anti-obesity agent.

Key words: Sodium-glucose cotransporter 2 inhibitor; Type 2 diabetes mellitus; Ipragliflozin; Japan

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Core tip: Ipragliflozin is highly selective for sodium-glucose cotransporter 2 (SGLT2) inhibitor. Twelve weeks of ipragliflozin 50 mg/d *vs* placebo decreased HbA1c and body weight by 0.65% and 0.66 kg, respectively, in Western patients, and by 1.3% and 1.89 kg, respectively, in Japanese patients. The highly selective SGLT2 inhibitor ipragliflozin improves glycemic control and reduces body weight, and lowers hypoglycemic risk and abdominal symptoms. Ipragliflozin has potential as a novel anti-diabetic and anti-obesity agent.

Abstract

Sodium-glucose cotransporter 2 (SGLT2) inhibition induces glucosuria and decreases blood glucose levels in diabetic patients and lowers hypoglycemic risk. SGLT1 is expressed in the kidney and intestine; SGLT1 inhibition causes abdominal symptoms such as diarrhea and reduces incretin secretion. Therefore, SGLT2 selectivity is important. Ipragliflozin is highly selective for SGLT2. In type 2 diabetes mellitus (T2DM), urinary

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and defective insulin secretion^[1]. Hyperglycemia

is caused by glucose influx exceeding glucose outflow from the plasma compartment^[2]. In the fasting state, hyperglycemia is related to increased hepatic glucose production^[2]. In the postprandial state, further glucose excursions result from insufficient glucose output suppression and defective insulin stimulation of glucose disposal in target tissues^[2]. Once the renal tubular transport maximum for glucose exceeds, glycosuria curbs, but does not prevent further hyperglycemia^[2].

Oral hypoglycemic agents include insulin secretagogues [sulfonylureas, meglitinides, and dipeptidyl peptidase-4 (DPP-4) inhibitors] and insulin sensitizers [metformin and thiazolidinediones (TZDs)]^[3]. α -glucosidase inhibitors decrease glucose absorption. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes recommend metformin as the first-line oral therapy^[2,3]. If the glycated hemoglobin (HbA1c) target is not achieved by 3 mo, either sulfonylurea, TZD, DPP-4 inhibitor, GLP-1 receptor agonist, or basal insulin should be combined with metformin^[2].

The ADA recommends lowering HbA1c to < 7.0% to reduce microvascular disease incidence^[4]. However, only approximately half of T2DM patients achieve this^[3,5]. Oral hypoglycemic agents have side effects: hypoglycemia and weight gain (sulphonylureas)^[6]; peripheral edema, weight gain, and fractures (TZDs)^[7]; a possible increased risk of bladder cancer (pioglitazone)^[8]; and abdominal symptoms (metformin and α -glucosidase inhibitors). Metformin can also cause lactate acidosis.

Few insulin sensitizers and anti-obesity agents exist. Mazindol maintains body weight after obesity therapy and treats obesity-related diseases such as diabetes, hypertension, and hyperlipidemia^[9], but has side effects including tremor, nausea, vomiting, and diarrhea. Therefore, novel anti-diabetic and anti-obesity agents are required.

SODIUM-GLUCOSE COTRANSPORTER

TYPE 2

The kidney is important in glucose metabolism; it is a target for therapeutic intervention^[10]. Sodium-glucose cotransporter 2 (SGLT2) mediates glucose reabsorption from the proximal renal tubule^[10]. SGLT2 inhibition induces glycosuria and lowers blood glucose in diabetes, and lowers hypoglycemic risk^[10].

Ipragliflozin is an SGLT2 inhibitor first released in Japan (Figure 1)^[3]. Here studies on ipragliflozin and other SGLT2 inhibitors are reviewed.

PHARMACOLOGY, MODE OF ACTION, AND PHARMACOKINETICS

In vitro SGLT inhibition

Two types of SGLT exist: SGLT1 and SGLT2. SGLT1 is expressed in the kidney and intestine; intestinal SGLT1 inhibition causes abdominal symptoms such as diarrhea.

It is pivotal for intestinal mass absorption of d-glucose and triggers glucose-induced secretion of gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)^[11]. Therefore, SGLT1 inhibition reduces incretin secretion. Miglitol (α -glucosidase inhibitor) suppresses GIP and increases GLP-1, reducing body weight and improving glycemic control^[12], but suppression of GLP-1 reduces insulin secretion^[13]. Therefore, SGLT2 selectivity is important. The selectivity of currently available SGLT2 inhibitors is presented in Table 1^[3,14-19].

Urinary glucose excretion

Healthy Japanese subjects receiving ipragliflozin excreted approximately 70 and 50 g of glucose/24 h after a single 300 mg dose or after multiple 50 or 100 mg doses, respectively^[20]. In healthy European subjects, ipragliflozin dose-dependently increased urinary glucose excretion (UGE) to a maximum of approximately 59 g/24 h (327 mmol/24 h) (dose: 5-600 mg/d) without affecting plasma glucose levels^[21]. In T2DM, ipragliflozin increased UGE to a maximum of approximately 90 g/24 h after 28 d of treatment with 300 mg/d^[22]. Therefore, SGLT2 inhibitors increased UGE more in T2DM patients compared with healthy subjects^[3]. Human exfoliated proximal tubular epithelial cells (HEPTECs) from T2DM patients expressed significantly more SGLT2 and the facilitative glucose transporter GLUT2 than cells from healthy individuals^[23]. Renal glucose uptake in HEPTECs isolated from T2DM patients was markedly increased compared with that in healthy controls^[23]. Therefore, renal glucose transporter expression and activity is increased in T2DM^[23]. In T2DM patients, ipragliflozin increases glycosuria directly proportional to the glomerular filtration rate (GFR) and degree of hyperglycemia, so it can be reliably predicted for individuals^[24]. Although absolute glycosuria decreases with declining GFR, ipragliflozin efficiency is maintained in patients with severe renal impairment^[24].

Effect of ipragliflozin on the pharmacokinetics of other medications

AUCinf or Cmax of single doses of sitagliptin, pioglitazone, or glimepiride^[25] were unaffected by multiple doses of ipragliflozin; the combination was well tolerated in healthy subjects^[25]. Ipragliflozin (300 mg qd) and metformin together were well tolerated in T2DM patients; the addition of ipragliflozin did not result in a clinically relevant change in the pharmacokinetic properties of metformin^[26]. Dose adjustments may not be required when ipragliflozin is administered with other glucose-lowering drugs^[25].

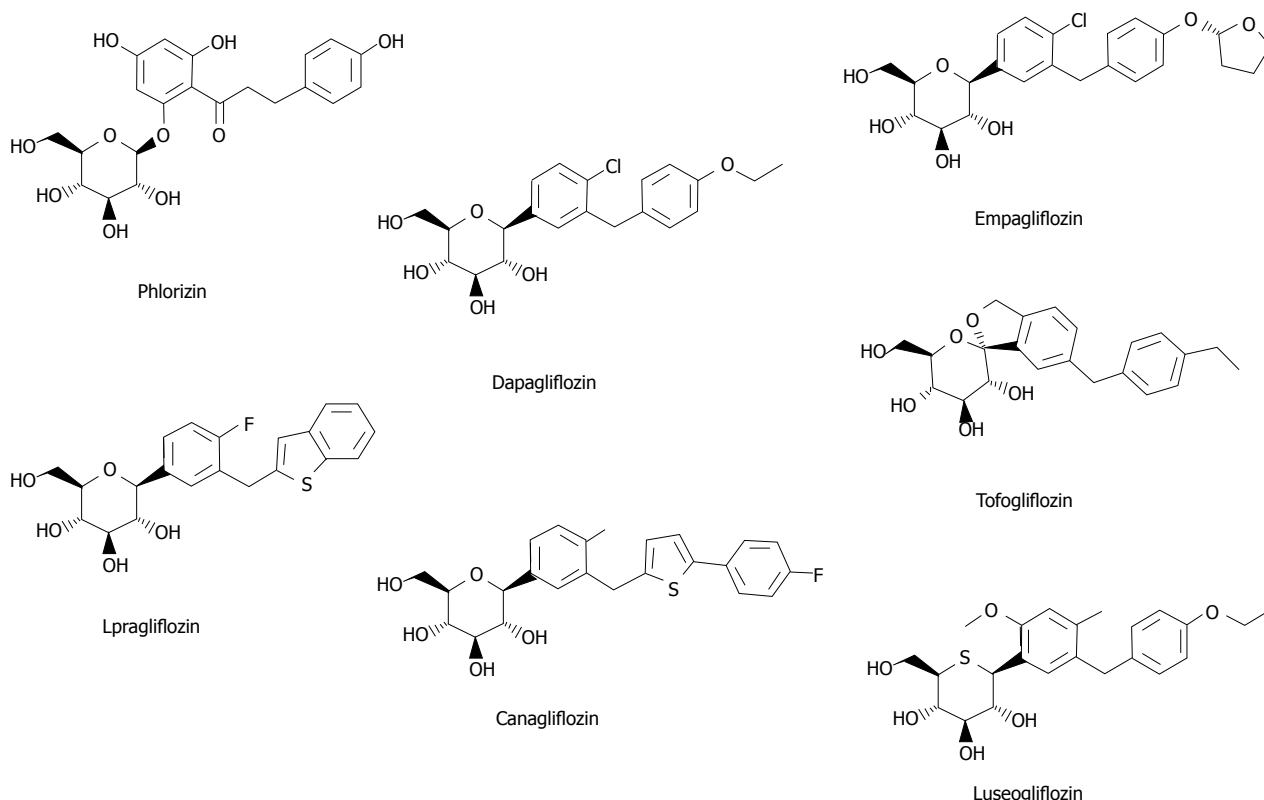
Effect of moderate hepatic impairment on the pharmacokinetics of ipragliflozin

Moderate hepatic impairment had no clinically relevant effects on the single-dose pharmacokinetics of ipragliflozin and its major metabolite^[27]. A single oral dose of ipragliflozin 100 mg was well tolerated in healthy subjects

Table 1 Sodium-glucose cotransporter 2 selectivity of sodium-glucose cotransporter 2 inhibitors

Company	IC ₅₀ for human SGLT1/SGLT2 (nmol/L)	SGLT2 selectivity (fold)
Phlorizin	210/34.6	6
Ipragliflozin	1876/7.38	254
canagliflozin	684/4.4	155
Dapagliflozin	1391/1.12	1242
Empagliflozin	8300/3.1	2680
Tofogliflozin	8444/2.9	2912
luseogliflozin	3990/2.26	1770

SGLT2 selectivity was calculated by using the following formula: IC₅₀ value for SGLT1/IC₅₀ value for SGLT2. IC₅₀: Half maximal (50%) inhibitory concentration; SGLT: Sodium-glucose cotransporter.

**Figure 1 Chemical structure of sodium-glucose cotransporter 2 inhibitors in late-stage clinical trials.**

and those with moderate hepatic impairment^[27].

EFFICACY AND COMPARATOR STUDIES WITH OTHER SGLT2 INHIBITORS

HbA1c

In Western T2DM patients, a 12-wk treatment with ipragliflozin 12.5, 50, 150, and 300 mg/d reduced HbA1c by 0.49%, 0.65%, 0.73%, and 0.81%, respectively, compared with placebo treatment (Figure 2)^[28]. In Japanese patients, 12-wk treatment with ipragliflozin 12.5, 25, 50, and 100 mg/d reduced HbA1c by 0.61%, 0.97%, 1.29%, and 1.31%, respectively, compared with placebo treatment^[29].

Canagliflozin 50, 100, 200, 300 mg/d and 300 mg

twice daily for 12 wk significantly reduced HbA1c by 0.79%, 0.76%, 0.70%, 0.92%, and 0.95%, respectively, compared with reductions of 0.22% for placebo (all $P < 0.001$), and 0.74% for sitagliptin^[30]. The adjusted mean difference in HbA1c between placebo and 100 mg canagliflozin was -0.54%^[30] (Figure 2). Dapagliflozin 2.5, 5, and 10 mg reduced HbA1c by 0.67%, 0.70%, and 0.84%, respectively^[31]. Empagliflozin 5, 10, and 25 mg for 12 wk reduced HbA1c by 0.4%, 0.5%, and 0.6% compared with placebo (+0.09%)^[32]. Ipragliflozin reduced HbA1c levels when added to metformin (-0.87 ± 0.66), pioglitazone (-0.64 ± 0.609), or sulfonylurea (-0.83 ± 0.717)^[3,33].

Fasting plasma glucose

In Western T2DM patients, 12-wk of ipragliflozin

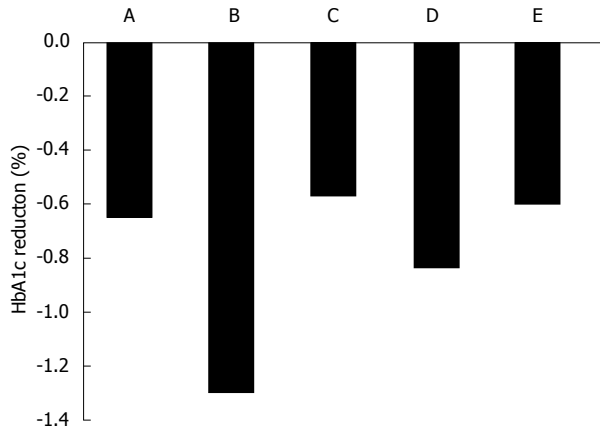


Figure 2 The adjusted mean difference in HbA1c from baseline to 12 wk between placebo and the standard dose of sodium-glucose cotransporter 2 inhibitors. A: 50 mg ipragliflozin in Westerners; B: 50 mg ipragliflozin in Japanese; C: 100 mg canagliflozin in Westerners; D: 10 mg dapagliflozin in Westerners; E: 25 mg empagliflozin in Westerners.

treatment at 12.5, 50, 150, and 300 mg/d decreased fasting plasma glucose (FPG) by 0.84, 1.10, 1.30, and 1.68 mmol/L, respectively compared with placebo^[28]. In Japanese T2DM patients, 12.5, 25, 50, and 100 mg ipragliflozin decreased FPG from baseline by 15.6, 23.7, 34.1, and 46.9 mg/dL (0.87, 1.32, 1.89 and 2.60 mmol/L) compared with +12.0 mg/dL for placebo^[29].

Body weight

In T2DM patients, SGLT2 inhibitors ipragliflozin, dapagliflozin, and canagliflozin reduced body weight by approximately 2 kg^[3] (Figure 3). In Western individuals, the standard dose of 50-mg ipragliflozin for 12 wk reduced body weight by 0.66 kg^[28]. In Japanese T2DM patients, 12-wk of placebo or 12.5-100 mg ipragliflozin treatment reduced body weight by 0.39 kg and 1.46-2.10 kg, respectively^[29]. Twelve-weeks of canagliflozin 100 mg^[30], dapagliflozin 10 mg^[34], or empagliflozin 25 mg^[32] reduced body weight by 2.28, 2.7, and 2.06 kg, respectively.

Most weight loss in patients receiving dapagliflozin is related to visceral and subcutaneous fat loss^[3,35]. After 24-wk of dapagliflozin treatment at 10 mg/d, placebo-corrected changes were -2.08 kg body weight, -1.52 cm waist circumference, -1.48 kg total body fat mass, -258.4 cm³ visceral adipose tissue, and -184.9 cm subcutaneous adipose tissue^[3,35]. Compared with placebo, 26.2% more patients achieved weight reduction of at least 5%^[3,35].

Blood pressure

SGLT2 inhibitors decrease blood pressure *via* osmotic diuresis induced by glucose in the urine earlier during treatment^[3]. Ipragliflozin 50 mg for 16 wk reduced systolic blood pressure by 3.2 mmHg and diastolic blood pressure by 2.5 mmHg, without hypotension^[36]. Dapagliflozin for 12 wk reduced systolic blood pressure by 2.6-6.4 mmHg, with no clear dose-dependent relationship, but changes in diastolic blood pressure and heart rate were small and

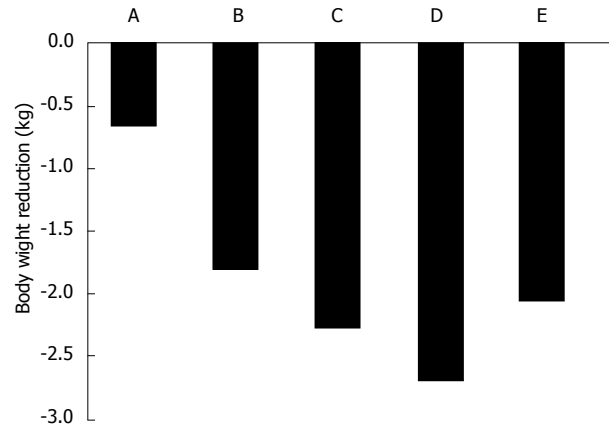


Figure 3 The adjusted mean difference in body weight from baseline to 12 wk between placebo and the standard dose of sodium-glucose cotransporter 2 inhibitors. A: 50 mg ipragliflozin in Westerners; B: 50 mg ipragliflozin in Japanese; C: 100 mg canagliflozin in Westerners; D: 10 mg dapagliflozin in Westerners; E: 25 mg empagliflozin in Westerners.

inconsistent^[34]. Small dose-related increases in 24-h urine volumes were observed (107-470 mL above baseline volumes of 1.8-2.2 L)^[34].

Canagliflozin 100 and 300 mg for 26 wk significantly reduced systolic BP by 3.7 and 5.4 mmHg, respectively, compared with placebo (both $P < 0.001$)^[37]. Diastolic BP was also reduced by 1.6 and 2.0 mmHg, respectively^[37]. Minimal changes in heart rate were observed with canagliflozin 100 and 300 mg compared with placebo (-1.6, -0.5, and +1.4 beats/min, respectively)^[37]. Empagliflozin 25 mg for 12 wk decreased systolic blood pressure by 3.4 mmHg, and diastolic blood pressure by 1.7 mmHg, but there was no significant difference compared with placebo^[32]. Overall, SGLT2 inhibitors reduced blood pressure by approximately 2-6 mmHg.

Beta-cell function

Chronic hyperglycemia induces β -cell dysfunction and insulin resistance^[38]. SGLT2 inhibitors improve glucose toxicity and glycemic control^[3]. There are no clinical reports on effect of ipragliflozin on β -cell function but ipragliflozin increased insulin content in the pancreas and suppressed the loss of insulin-positive cells in islets of db/db mice, an animal model of T2DM^[3,39].

Compared with placebo, canagliflozin 100 mg/d for 12 wk significantly improved β -cell function as assessed by homeostasis model assessment 2 (HOMA2)-%B (measure of fasting insulin secretion)^[30]. Another study reported improvements in β -cell function following 26-wk treatment with canagliflozin 100 and 300 mg compared with placebo, with increases in HOMA2-%B of 12.4 and 22.8, respectively^[37].

Proinsulin/insulin (PI/I) ratio reflects β -cell dysfunction associated with the onset and progression of T2DM^[40,41]. Mitiglinide improved the postprandial insulin secretion profile, suppressed the postprandial glucose spike, and improved the PI/I ratio in T2DM patients with low insulin resistance and low triglyceride levels^[42].

Dose-related decreases in proinsulin/insulin ratio of 0.5 and 0.8 pmol/mIU were observed with canagliflozin at 100 and 300 mg, respectively, compared with placebo, and decreases in proinsulin/C peptide ratio were also seen with both doses of canagliflozin^[37]. These results suggest that SGLT2 inhibitors improve β -cell function.

Insulin resistance

To date, there are no clinical reports on effect of ipragliflozin on insulin resistance. However, reductions in HOMA2 insulin resistance after dapagliflozin treatment at 2.5 and 10 mg for 12 wk were significantly larger compared with placebo^[43]. The most precise method to assess insulin resistance is the glucose clamp technique^[44]. Results of a hyperinsulinemic-euglycemic clamp study demonstrated that within 3 d of completing 2-wk of dapagliflozin treatment, Zucker diabetic fatty rats displayed improved glucose utilization accompanied by reduced glucose production and enhanced glucose influx into liver tissue^[16]. In a clamp study of T2DM patients, 12-wk of dapagliflozin treatment increased glucose disposal rates^[3,45]. There are few glucose clamp studies of SGLT2 inhibitors because the method is complex and expensive^[46]. Recently, a novel insulin resistance index “20/(fasting C-peptide \times fasting plasma glucose),” to estimate the insulin resistance index was derived from the glucose clamp method^[46]. This index will evaluate insulin resistance in clinical studies.

SAFETY, EFFICACY, AND TOLERABILITY

Genito-urinary tract infections

A meta-analysis of 45 clinical trials indicated that SGLT2 inhibitors increased the risk of urinary and genital tract infections [odds ratios, 1.42 (95%CI: 1.06-1.90) and 5.06 (95%CI: 3.44-7.45)], respectively, probably a result of glucosuria^[47].

In ipragliflozin phase 3 trial, treatment-emergent urinary tract infections (UTIs) were reported in 32/412 patients across all treatment groups, including placebo^[28]. Infections were symptomatic and asymptomatic in 9 and 23 patients, respectively^[28]. A total of 14 patients experienced treatment-emergent genital tract infections but there was no evidence that the frequency was related to the dose of ipragliflozin^[28]. All events were treated with antifungal or antibacterial agents and were resolved prior to the final study visit (except three)^[28]. In canagliflozin phase 3 trial, the incidence of genital mycotic infections, UTIs, and osmotic diuresis-related adverse events was higher in the treatment group^[37]. UTIs were observed in 5%-12% of dapagliflozin-treated patients (with no clear dose relationship) compared with 6% of placebo-treated patients and 9% of metformin-treated patients^[34]. Genital infections were observed in 2%-7% of dapagliflozin treated patients, 0% of placebo-treated patients, and 2% of metformin-treated patients^[34]. Therefore, SGLT2 inhibitors might increase the risk of UTIs.

Hypoglycemia

In a multi-center Japanese study of 361 patients randomized to receive either ipragliflozin (12.5, 25, 50, or 100 mg/d) or a placebo for 12 wk, a single mild symptomatic hypoglycemic event (not confirmed by plasma glucose measurement) occurred in one patient in the 100-mg ipragliflozin group^[29]. In ipragliflozin phase 3 trial, only one patient in each of the ipragliflozin 50 mg (67 patients) and 300 mg (68 patients) dose groups experienced treatment-emergent hypoglycemia^[29]. In T2DM patients, ipragliflozin did not significantly increase the incidence of hypoglycemic events compared to placebo, even in combination with other hypoglycemic agents^[3,48]. Hypoglycemic events were reported in 6%-10% of patients treated with dapagliflozin, with no dose-dependent relationship, compared with 4% and 9% for placebo and metformin, respectively^[34]. There were no symptomatic hypoglycemic events with a fingerstick glucose of ≤ 50 mg/dL^[34]. In canagliflozin phase 3 trial, the incidence of hypoglycemia was similar for canagliflozin 100 and 300 mg and placebo (3.6%, 3.0%, and 2.6%, respectively), with no report of severe hypoglycemia^[37]. Therefore, these data suggest that SGLT2 inhibitors lowers hypoglycemic risk.

Osmotic diuretic effect

Ipragliflozin caused a mild 1.5%-2.0% increase in hematocrit at all doses^[29]. Similarly, blood urea nitrogen (BUN) was also mildly increased by 1.0-2.2 mg/dL compared with placebo^[29].

Cancer risk

An increased incidence of bladder and breast cancer was indicated in patients receiving dapagliflozin compared with controls^[47]. Data on bladder and breast cancer were retrieved from regulatory databases and other sources to produce a pool of 5501 patients (at least 5000 patient-years of exposure to dapagliflozin), and a total of 3184 patients (at least 2350 patient-years of exposure to placebo or an active comparator)^[49,50]. Nine cases of bladder cancer were identified in patients treated with dapagliflozin compared with one case in patients receiving placebo^[49,50]. The number of observed cases exceeds the expected number in the general diabetic population^[47]. UTIs may increase the risk of bladder cancer. However, early detection after short exposure and potential detection bias related to frequent urinalysis mitigate against a causative relationship^[47]. Therefore, no robust conclusions can be drawn, pending accumulation of long-term data^[47].

There were 9 cases of breast cancer in the dapagliflozin group (2223 patients) compared with one case in the placebo group (1053 patients), diagnosed within the first year of the study^[47]. These figures were higher than the predicted number of 7.1 cases based on the Surveillance Epidemiology and End Results (SEER) program^[51]. It remains uncertain whether the use of dapagliflozin is associated with an increased risk of breast cancer and further studies are needed^[47]. There are no reports

indicating that other SGLT2 inhibitors are associated with an increased risk of cancer^[3].

Safety and tolerability of metformin combination therapy

A meta-analysis of 20 randomized, double-blind studies demonstrated SGLT2 inhibitors administered with metformin significantly decreased the incidence of diarrhea^[52]. However, the addition of SGLT2 inhibitors increased the risk of genital infection^[52]. Despite some limitations, SGLT2 inhibitors have a favorable safety profile, and combination therapy with metformin is well tolerated^[52].

Patient-focused data on quality of life, satisfaction, and acceptability

One study investigated effect of ipragliflozin on quality of life^[28]. Outcomes were assessed using the European Quality of Life-5 Dimensions (EQ-5D)^[53], Audit of Diabetes-Dependent Quality of Life (ADDQoL)^[54], and Diabetes Medication Satisfaction (Diab-MedSat) questionnaires^[55]. No differences were observed in EQ-5D domains or ADDQoL scores at week 12^[28]. However, mean changes in EQ-5D visual analogue scale scores from baseline to week 12 showed positive changes in the treatment groups, suggesting improvements in perceived health status^[28]. Changes in Diab-MedSat scores for burden and symptoms were small and similar across all treatment groups, but changes in the efficacy score from baseline to week 12 were greater for the ipragliflozin groups^[28]. Another study reported that changes from baseline to week 12 in EQ-5D domains and ADDQoL scores were small across all treatment groups but with a non-statistically significant trend for improvement in the ipragliflozin treatment groups^[33]. These results suggest that the SGLT2 inhibitor ipragliflozin may improve the quality of life in T2DM patients.

Ethnic differences

There are no clinical reports on ethnic differences in effects of ipragliflozin. However, past reports imply that ipragliflozin reduces HbA1c more in Japanese patients compared with Western patients (Figure 2)^[28,29]. The mechanism is unclear, but a meta-analysis reported that DPP-4 inhibitors were associated with a reduction in HbA1c of 0.65% in non-Japanese randomized, controlled trials (RCTs; 55 patients), compared with 1.67% in Japanese RCTs^[56]. There may be pharmacogenetic or cultural lifestyle differences that contribute to the larger reduction in HbA1c in Japanese patients. Japanese people have a greater amount of abdominal visceral fat relative to abdominal subcutaneous fat compared with Caucasians^[57]. Dapagliflozin reduced visceral adipose tissue more than subcutaneous adipose tissue^[35]. Therefore, the difference in visceral adipose tissue between Japanese and Western T2DM patients may contribute to the difference in effect of SGLT2 inhibitors.

Japanese and Asian patients often show reduced β -cell function^[46] and East Asians may have a limited innate

capacity for insulin secretion^[58,59]. The body mass index (BMI) of Japanese T2DM patients was significantly correlated with insulin secretion ability in a meal tolerance test; the insulin secretion ability diminished in patients with BMI < 20 kg/m²^[60]. Other reported complications associated with familial renal glucosuria include episodes of ketosis, UTIs, and natriuresis^[61]. SGLT2 inhibitors increase blood ketone bodies^[62]. Low insulin secretion ability and lean stature in Asian patients receiving SGLT2 inhibitors may increase the risk of ketosis; therefore, caution is required.

CONCLUSIONS AND PLACE IN THERAPY ALONGSIDE OTHER SGLT2 INHIBITORS

SGLT2 inhibitor ipragliflozin improves glycemic control and reduces body weight, especially in Japanese T2DM patients. Furthermore, ipragliflozin lowers hypoglycemic risk and abdominal symptoms and can be safely used with sulphonylureas, metformin, pioglitazone, and DPP4 inhibitors. SGLT2 inhibitors are likely to improve β -cell function and insulin sensitivity. They offer great potential as novel anti-diabetic and anti-obesity agents. Ipragliflozin is particularly effective for Japanese T2DM patients with a greater abdominal visceral fat relative to abdominal subcutaneous fat than Caucasians. Ipragliflozin is a highly selective SGLT2 inhibitor, and lower hypoglycemic risk and abdominal symptoms.

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Pathological consequences of C-peptide deficiency in insulin-dependent diabetes mellitus

Ahmad Ghorbani, Reza Shafiee-Nick

Ahmad Ghorbani, Reza Shafiee-Nick, Pharmacological Research Center of Medicinal Plants, School of Medicine, Mashhad University of Medical Sciences, 91375-3316 Mashhad, Iran

Reza Shafiee-Nick, Department of Pharmacology, School of Medicine, Mashhad University of Medical Sciences, 91375-3316 Mashhad, Iran

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Correspondence to: Reza Shafiee-Nick, Pharm D, PhD, Department of Pharmacology, School of Medicine, Mashhad University of Medical Sciences, Pardis Campus, Azadi Square, 91375-3316 Mashhad, Iran. shafieer@mums.ac.ir

Telephone: +98-51-38002256

Fax: +98-51-38828567

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diabetes mellitus (type-1 diabetes). In this metabolic syndrome, in addition to decrease of endogenous insulin, the plasma level of connecting peptide (C-peptide) is also reduced due to beta cell destruction. Studies in the past decade have shown that C-peptide is much more than a byproduct of insulin biosynthesis and possess different biological activities. Therefore, it may be possible that C-peptide deficiency be involved, at least in part, in the development of different complications of diabetes. It has been shown that a small level of remaining C-peptide is associated with significant metabolic benefit. The purpose of this review is to describe beneficial effects of C-peptide replacement on pathological features associated with insulin-dependent diabetes. Also, experimental and clinical findings on the effects of C-peptide on whole-body glucose utilization, adipose tissue metabolism and tissues blood flow are summarized and discussed. The hypoglycemic, antilipolytic and vasodilator effects of C-peptide suggest that it may contribute to fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. Therefore, C-peptide replacement together with the classic insulin therapy may prevent, retard, or ameliorate diabetic complications in patients with type-1 diabetes.

Key words: C-peptide; Diabetes; Insulin; Nephropathy; Neuropathy

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Core tip: In type-1 diabetes, in addition to decrease of endogenous insulin, the plasma level of connecting peptide (C-peptide) is also reduced due to beta cell destruction. Therefore, it may be possible that C-peptide deficiency be involved in the development of diabetic complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. In this paper, beneficial effects of C-peptide replacement on pathological features associated with type-1 diabetes

Abstract

Diabetes is associated with several complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. Currently, insulin is the main used medication for management of insulin-dependent

are described. Also, experimental and clinical findings that support the hypoglycemic, antilipolytic and vasodilator effects of C-peptide are discussed.

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INTRODUCTION

Diabetes mellitus is still an increasing health problem in both developing and developed countries. World Health Organization reported (August, 2011) that, 346 million people have diabetes worldwide and 3.4 million patients died from diabetes-related complications in the year 2004. Diabetes is generally classified into two main types: insulin-dependent diabetes mellitus [type-1 diabetes (T1D)] which is a state of insulin deficiency because of destruction of islet beta cells, and non-insulin-dependent diabetes mellitus [type-2 diabetes (T2D)] which is characterized by resistance to the action of insulin^[1].

Poor control of diabetes is associated with several complications such as nephropathy, retinopathy, neuropathy and cardiovascular diseases^[1]. Currently, insulin is the main used medication for management of T1D^[2]. Even though early-onset complications may be controlled by insulin therapy, it remains difficult to achieve normal glycemic control and late-onset complications occur in many of diabetic patients^[3,4]. In addition to decrease of endogenous insulin, the level of connecting peptide (C-peptide) is also reduced in the plasma of patients with T1D due to autoimmune destruction of beta cell^[5]. Although for many years C-peptide has been considered as a byproduct of insulin biosynthesis, data from several lines of studies reveals the beneficial actions of C-peptide replacement in prevention of metabolic changes and structural alterations in T1D^[6]. Therefore, one cannot rule out the possibility that C-peptide deficiency may also be involved, at least in part, in the development of some pathological features associated with T1D. This article reviews the current understanding of biological effects of C-peptide and the beneficial actions of C-peptide replacement on preventing or ameliorating the T1D-related complications.

C-PEPTIDE SYNTHESIS AND SECRETION

In pancreatic beta cells, proinsulin is transferred in vesicles from rough endoplasmic reticulum to Golgi apparatus, where the vesicles are directed into a regulated secretion. During this transition of vesicles, three peptidases participate in proinsulin posttranslational processing to generate insulin and C-peptide^[7]. First, proinsulin is cleaved by prohormone convertase type 2

at the A-chain/C-peptide junction or by prohormone convertase type 1/3 at the B-chain/C-peptide junction. Then, carboxypeptidases H removes two pairs of amino acids located at both cleaved junctions providing the *des* forms of proinsulin (*des*-31,32 and *des*-64, 65). Finally, endopeptidase type 1/3 and type 2 recognizes *des*-64,65 proinsulin and *des*-31, 32 proinsulin, respectively, leading to release of insulin and C-peptide from proinsulin. C-peptide facilitates the correct folding of proinsulin to allow form two disulfide bridges between A- and B-chains of insulin and therefore plays an essential role in biosynthesis of insulin^[6,8]. In most species only one form of proinsulin has been described. However, in rats and mice two proinsulin isoforms I and II have been found^[9].

Increase of blood glucose leads to secretion of an equimolar amount of insulin and C-peptide into the portal circulation^[7]. However, the liver rapidly uptakes insulin because of single pass effect and only 50% of insulin reaches to the systemic circulation with a half-life about 4 min. On the other hand, C-peptide is primarily metabolized by kidney and has a circulating half-life about 30 min which is the reason for its higher plasma concentration than insulin^[5,7]. The level of C-peptide in fasting and postprandial conditions varies between 0.3-1 nM and 1.5-2.5 nmol/L, respectively^[10].

Since C-peptide and insulin are secreted from beta cells in equimolar concentrations, measuring serum C-peptide is an estimate of residual beta cell function and can be used to differentiate between patients with T1D and T2D^[6]. However, the mean C-peptide concentration is higher in diabetic patients with renal diseases insufficiency compared with those have normal renal function. Therefore, in severe renal failure, the serum C-peptide assay is unreliable for assess residual beta cells^[6,11,12].

BIOLOGICAL EFFECTS OF C-PEPTIDE

Effects of C-peptide on glucose utilization

Experimental studies on diabetic rats showed that C-peptide prolongs the hypoglycemic effect of insulin^[13] and increases whole-body glucose utilization^[9,14,15]. The glucose lowering effect of C-peptide was also investigated in human. Hoogwerf *et al*^[16] have shown no effect by C-peptide on blood glucose level in healthy subjects or patients with T1D. However, Johansson and coworkers demonstrated that infusion of physiological concentrations of C-peptide to patients with T1D augments whole body glucose utilization by approximately 25%^[17]. Also, Oskarsson *et al*^[18] showed that C-peptide hasten the insulin-induced hypoglycemia in diabetic patients. Activation of glucose metabolism by short time C-peptide infusion in healthy controls and in patients with T1D was also reported by Wilhelm *et al*^[19].

The augmented whole body glucose utilization is most probably a result of increased muscle glucose uptake rather than inhibition of hepatic gluconeogenesis^[20]. In normal rats, we observed that adipose tissue glucose consumption was not affected by C-peptide^[21]. Direct

examinations under *in vitro* condition confirmed that C-peptide stimulates the rate of glucose transport to muscle strips obtained from healthy subjects or patients with T1D^[22]. Also, Zierath *et al*^[23] showed that C-peptide dose-dependently increases glucose uptake into human skeletal muscle through a mechanism shared partly with insulin. Although the exact pathway involved in this effect of C-peptide is still unknown, incubation of isolated muscle strips with a cAMP analogue abolishes the C-peptide-stimulated glucose transport.

Regarding metabolic actions of C-peptide, it should be considered that although this peptide at low physiological concentrations mimics insulin effects, however in the presence of high level of insulin (*e.g.*, in the postprandial condition) the concomitant elevated level of C-peptide may blunt the insulin's peripheral effects^[21,24]. It is possible that high levels of C-peptide induce a desensitization processes which may be recovered after a period of its absence.

Effect of C-peptide on adipose tissue

Soon after discovery of C-peptide, Solmon *et al*^[25] examined the effects of pork and beef C-peptide on adrenocorticotropin-induced lipolysis in rats, but no significant effects were found. Subsequently, Yu and coworkers tested the effect of supraphysiological concentrations of porcine C-peptide on the lipolysis in isolated adipocytes from rats and found an insignificant antilipolytic effect^[26]. Using an *ex-vivo* organ culture method, we observed a similar insignificant reduction in basal lipolysis of rat retroperitoneal adipose tissue^[21]. Because it has been reported that some effects of C-peptide appear only in diabetes condition^[9,27,28], we examined whether C-peptide alters lipolysis in diabetic rats. Our data showed that C-peptide like insulin significantly inhibits isoproterenol-stimulated lipolysis^[29]. Therefore C-peptide may act, conditionally, as an antilipolytic hormone and may be involved in fine-tuning of lipid metabolism.

Effects of C-peptide on circulation

Patients with T1D show reduced tissues blood flow despite intensive insulin therapy and good management of glucose control^[30]. C-peptide has been shown to enhance blood flow of kidney^[17], nerve^[31], skeletal muscle^[32], myocardium^[30,33] and skin^[34]. The vasodilator effect of C-peptide is mediated by stimulation of nitric oxide release from endothelial cells^[35-37]. Wallerath *et al*^[35] reported that physiological postprandial concentration of C-peptide is able to activate endothelial nitric oxide synthase (eNOS) and stimulating nitric oxide production. Forst *et al*^[38] showed that intravenous infusion of C-peptide to patients with T1D increases plasma concentration of cGMP, as an index of nitric oxide activity^[38]. This finding is in agreement with earlier report that in diabetic rats the C-peptide induced glucose utilization is sensitive to eNOS inhibition^[9].

OTHER BIOLOGICAL EFFECTS OF C-PEPTIDE

Interaction with insulin

In the presence of C-peptide, insulin hexamers in solution becomes undetectable. Also, subcutaneous injection of an insulin and C-peptide mixture to diabetic patients accelerates the increase of insulin levels in plasma and in comparison with injection of insulin alone utilizes more glucose. Therefore, it seems that C-peptide increases disaggregation of insulin by binding to insulin oligomers and thereby enhances the availability of monomeric (biologically active form) insulin^[39].

Protection of endothelium

It has been reported that C-peptide is able to inhibit leukocyte-endothelium interaction induced by thrombin or by NG-nitro-L-arginine methyl ester. This effect of C-peptide may be important in protection of vasculature against inflammatory disorders such those observed in T1D^[40].

EFFECTS OF C-PEPTIDE ON DIABETIC COMPLICATIONS

Effects on diabetic nephropathy

Different aspects of the diabetic renal pathogenic abnormalities can be improved by C-peptide in T1D (Figure 1). In diabetic rats, C-peptide decreases urinary protein excretion^[41-43], reduces glomerular hyperfiltration rate and restores the renal functional reserve^[42-44]. These beneficial effects have also been demonstrated in insulin-dependent diabetic patients^[17,45]. In a clinical study, patients were administered insulin alone or in combination with C-peptide by subcutaneous infusion pump for 4 wk. While combination therapy led to decrease of glomerular filtration rate and protein excretion after 2 wk, the insulin alone was ineffective^[45]. Johansson *et al*^[46] extended the period of C-peptide therapy to 3 mo and reported a significant decrease in the rate of protein excretion in patients receiving combination of insulin and C-peptide. In line with these findings, Zerbini *et al*^[47] found a decreased C-peptide/creatinine ratio in the plasma of T1D patients with nephropathy when compared with those without albuminuria^[47]. Microscopic examinations have showed that in diabetic rats, C-peptide reduces the hypertrophy of mesangial matrix in glomeruli of the kidneys^[48]. Several mechanisms are postulated for beneficial effects of C-peptide on renal function including inhibition of apoptosis, increase of Na⁺, K⁺-ATPase activity and interaction with the signaling pathway of growth factors^[49,50]. Activation of the key signaling molecules such as phospholipase C and protein kinase C followed by phosphorylation of extracellular-signal-regulated kinase and c-Jun N-terminal kinase have been shown in human renal tubular cells treated with

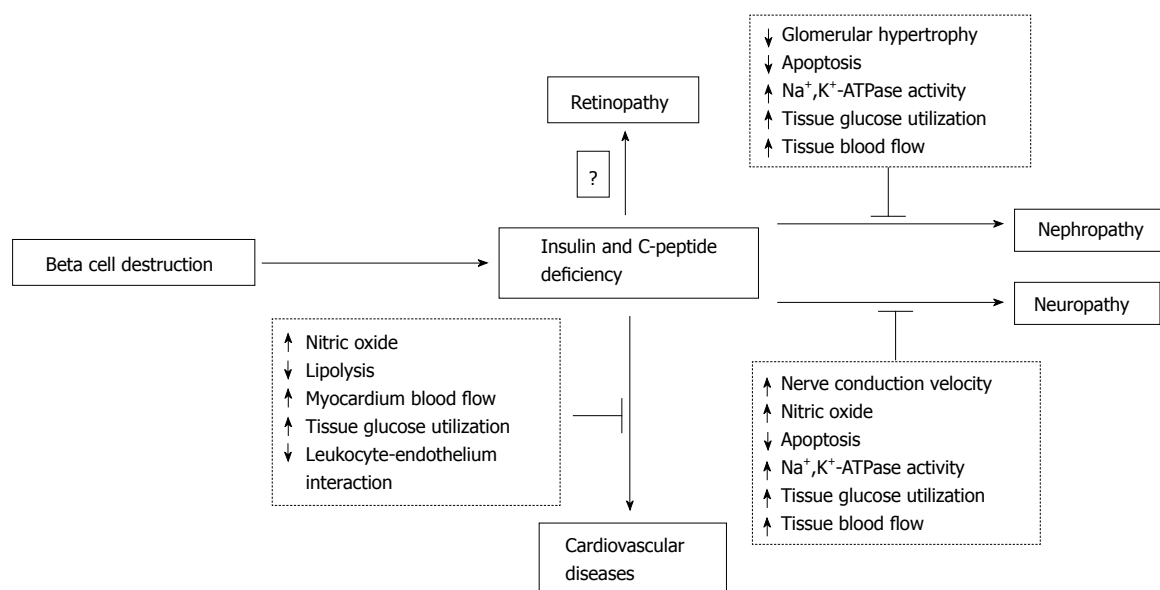


Figure 1 Proposed mechanisms (dashed rectangles) by which C-peptide may prevent, retard, or ameliorate diabetic complications in patient with type-1 diabetes. ↓: Decrease; ↑: Increase.

C-peptide^[49]. Regarding beneficial effects of C-peptide, we should emphasize that some of the C-peptide beneficial effects are limited to animals or patients who show very low or missing C-peptide plasma levels^[9,27,28]. Therefore, the nephroprotective effect of C-peptide may represent a therapeutic goal for patients with T1D.

Effects on diabetic neuropathy

Accumulating evidence suggests that C-peptide can prevent, retard, or ameliorate neuropathy in T1D (Figure 1)^[51-57]. Decreased level of Na^+ , K^+ -ATPase activity and reduced nitric oxide formation are considered as contributor factors to pathogenesis of diabetic neuropathy. It has been shown that C-peptide prevents the neural Na^+ , K^+ -ATPase defect and the nerve conduction velocity reduction^[52]. In study of Cotter *et al.*^[31] C-peptide at physiologic doses improved sensory and motor nerve conduction velocity in STZ-induced diabetic rats through increase of nitric oxide release. Ekberg *et al.*^[58] demonstrated that C-peptide improves vibration perception in patients with T1D^[58]. C-peptide also may prevent cognitive dysfunction by its antiapoptotic effect in the brain particularly in the hippocampus^[51-54]. The antiapoptotic property was also confirmed by Li *et al.*^[59] who showed that C-peptide, in the presence of insulin, inhibits high glucose-induced apoptosis in neuroblastoma cells. There are also clinical evidence that autonomic dysfunction can be ameliorated by C-peptide replacement. Infusion of C-peptide to patients with T1D increases the heart rate variability during deep breathing and the heart rate brake index after tilting^[53]. In contrast to insulin alone, administration of a combination of C-peptide and insulin improves heart rate during deep breathing in T1D patients^[46].

CONCLUSION

According to data presented in this paper, C-peptide is much more than a byproduct of insulin synthesis and has several biological actions such as hypoglycemic, antilipolytic and vasodilator effects. These biological effects suggest that it may act as a hormone to contribute in fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. In T1D diabetes, in particular, it was found that patients who conserve low but sustained secretion of endogenous C-peptide show better metabolic control and less retinopathy, nephropathy and neuropathy than patients who have become fully C-peptide and insulin deficient^[56-60]. These beneficial effects are demonstrated only in T1D models. It is possible that in physiological conditions, C-peptide produces its maximum effect and induces some levels of desensitization processes in phosphorylation mediated actions, especially nitric oxide-dependent pathways. Recovering the C-peptide mechanism of action during a period of its absence is in good agreement with the experimental results in T1D models. Therefore, present data suggest the possibility of a clinically applicable role for C-peptide replacement, together with the classic insulin therapy, to prevent, retard, or ameliorate diabetic complications in patient with T1D.

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Adiponectin: Probe of the molecular paradigm associating diabetes and obesity

Kakali Ghoshal, Maitree Bhattacharyya

Kakali Ghoshal, Maitree Bhattacharyya, Department of Biochemistry, University of Calcutta, Kolkata 700019, India
 Author contributions: Ghoshal K and Bhattacharyya M equally contributed to this paper.

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Correspondence to: Maitree Bhattacharyya, Professor, Department of Biochemistry, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India. bmaitree@gmail.com

Telephone: +91-33-24614712

Fax: +91-33-24614849

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Abstract

Type 2 diabetes is an emerging health challenge all over the world as a result of urbanization, high prevalence of obesity, sedentary lifestyle and other stress related factors compounded with the genetic prevalence. The health consequences and economic burden of the obesity and related diabetes mellitus epidemic are enormous. Different signaling molecules secreted by adipocytes have been implicated in the development of obesity and associated insulin resistance in type 2 diabetes. Human adiponectin, a 244-amino acid collagen-like protein is solely secreted by adipocytes and

acts as a hormone with anti-inflammatory and insulin-sensitizing properties. Adiponectin secretion, in contrast to secretion of other adipokines, is paradoxically decreased in obesity which may be attributable to inhibition of adiponectin gene transcription. There are several mechanisms through which adiponectin may decrease the risk of type 2 diabetes, including suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and stimulation of insulin secretion. To date, no systematic review has been conducted that evaluate the potential importance of adiponectin metabolism in insulin resistance. In this review attempt has been made to explore the relevance of adiponectin metabolism for the development of diabetes mellitus. This article also identifies this novel target for prospective therapeutic research aiming successful management of diabetes mellitus.

Key words: Adiponectin; Obesity; Dyslipidemia; Type 2 diabetes mellitus; Insulin resistance

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Core tip: Diabetes mellitus and related metabolic disorders like obesity, dyslipidemia are emerging as major global health challenges in recent era. Adiponectin, an adipokine demands profound importance in the field of metabolomics due to its potential role in all these complications. Plasma adiponectin concentration is remarkably lower in subjects with metabolic disorders predicting its significant role as an important biomarker in disease prognosis. We have attempted to enlighten adiponectin function stretching its role as a modulator associating these metabolic obstacles. We believe, this article will surely contribute to the fundamental and clinical research in the field of diabetes and related complications.

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INTRODUCTION

Rapid urbanization and change in life style has intensified the prevalence of obesity and dyslipidemia which plays crucial role in developing diabetes mellitus across the globe. Diabetes mellitus is a major public health concern with 382 million individuals being affected worldwide in 2013. Type 2 diabetes mellitus (T2DM) constitutes one of the major forms of diabetes disease burden associated with remarkably accelerated rates of microvascular obstacles and macrovascular disorders. Obesity and its association with developing type 2 diabetes is an interesting area of research for scientists in recent years. Insulin resistance is one of the earliest hallmarks of the pre-diabetic state and results from a complex interplay between obesity-favoring environmental factors, such as unrestricted supply of high-caloric foods and markedly increased sedentary lifestyle combined with a permissive genetic background. The high incidence is attributed to a combination of genetic susceptibility plus adoption of a high-calorie, low-activity lifestyle mainly by urban population.

Adipose tissue was long been identified as an energy storage organ but in recent times extensive studies revealed the role of adipose tissue as an important endocrine organ with a number of metabolic activities; thus its function as a storage organ is now far from reality^[1]. Adiponectin, an adipose tissue derived hormone, is lower in obese subjects than their lean counterparts^[2]. Epidemiological studies revealed that patients with diabetes and cardiovascular disease (CVD) has lower amount of adiponectin in their serum^[3,4], and low serum adiponectin level can be an excellent predictor of developing type 2 diabetes and associated CVD in later stage^[5-8]. Thus the role of adiponectin hormone as a potential biomarker for predicting the occurrence of type 2 diabetes is evolving as an interesting area in the study of metabolomics. In this review we aimed to highlight the potential beneficiary function of adiponectin in type 2 diabetes, dyslipidemia and obesity considering both genetic and biochemical approach.

OBESITY AND DIABETES: MAJOR GLOBAL THREATS OF THIS MILLENNIUM

In modern times rapid urbanization and change in lifestyle has increased the prevalence of obesity in manifold, especially the young generation has modified their food habits with high calorie junk foods. Furthermore rapid development of technology has increased the tendency

of uptaking sedentary lifestyle with less or no work at all, increasing the chances of getting obese. Obesity which is a major global threat virtually affecting both developed and developing countries. In Central America easy access to high calorie food and adoption of sedentary lifestyle has increased the prevalence of both diabetes and obesity^[9] where in developing countries like countries in Latin America^[10] and East Asia rise in income has shifted the mass from low calorie whole grain diet to high calorie processed foods which is far energy dense affecting not only the adults but the children and adolescents as well. BMI or body mass index and WC or Waist Circumference is two major parameters to measure obesity^[11]. Higher value of BMI (30 kg/m²) and WC increases the risk of type 2 diabetes, high cholesterol, high blood pressure and heart disease.

Type 2 is the most prevalent form of diabetes accounting 90%-95% of the cases, especially in developed countries^[12]. According to recent estimates of the International Obesity Task Force, up to 1.7 billion people of the world's population are at a heightened risk of weight-related, non-communicable diseases such as type 2 diabetes which is majorly a lifestyle disorder (International Diabetes Federation, 2004). According to International Diabetes Federation India accounts for the largest number of people (50.8 million) suffering from diabetes in the world, followed by China (43.2 million) and United States (26.8 million). This metabolic syndrome is closely associated with different macro and microvascular disorders^[13] (Table 1). The most prevalent diabetic macrovascular complication is Cardiovascular disorder (CVD)^[14], which in turn is associated with environmental risk factors as well as genetic predisposition. Antidiabetic drug metformin is particularly useful for overweight and obese diabetic patients. Our earlier report indicates that metformin is particularly useful to restore the antioxidant status of cells hampered in type 2 diabetes stress^[15].

Therefore type 2 diabetes and obesity interplays together to exert more deteriorating effect incorporating other metabolic syndromes such as CVD, dyslipidemia and hypertension^[16-25].

ADIPONECTIN: STRUCTURE AND RECEPTORS

The correlation between rapidly emerging type 2 diabetes and obesity still remained a major question for researcher. It was hypothesized that metabolic dysfunction may cause to acquire obesity which in turn can develop type 2 diabetes. Adipocyte, the major energy storing cell is a storage site of a number of hormones as well whose prime function remains to govern lipid metabolism. Major adipocyte derived hormones are adiponectin, leptin, resistin and visfatin^[26]. Leptin and adiponectin exerts positive effect on lowering blood glucose whereas resistin tends to increase blood glucose levels (Figure 1).

Reported for the first time by Scherer *et al*^[27], 1995,

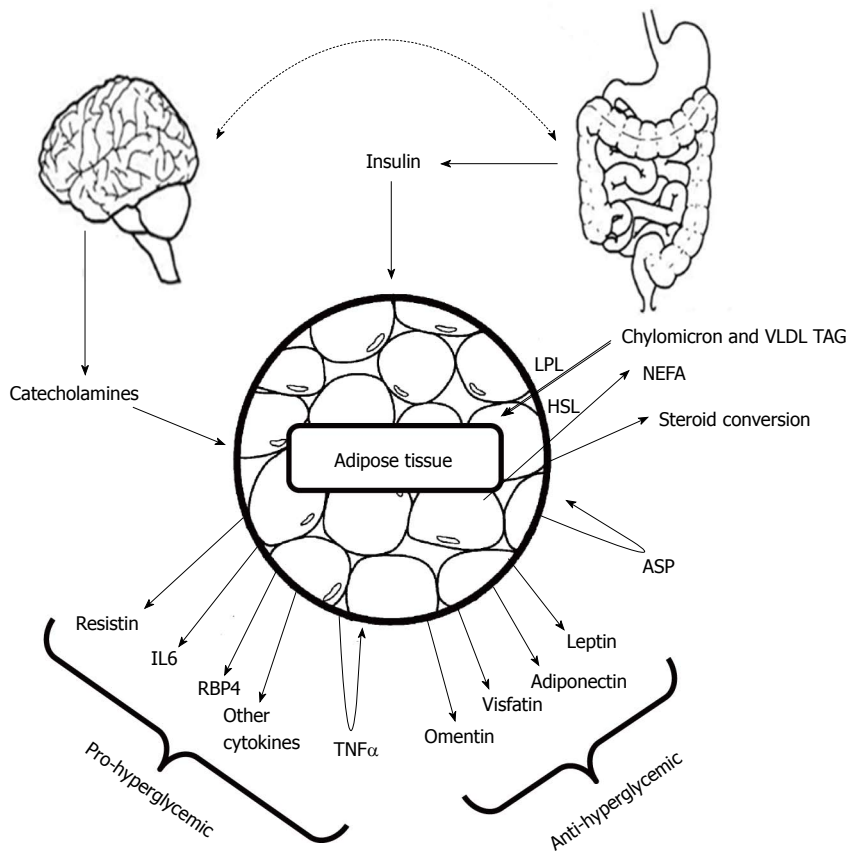


Figure 1 Adipocyte-derived proteins with anti-diabetic actions include leptin, adiponectin, omentin and visfatin; other factors tend to raise blood glucose including resistin, Tumor necrosis factor- α and Retinol-binding protein 4. (Adapted from Mohamed-Ali *et al.*^[31] and Rosen *et al.*^[32]). LPL: Lipoprotein lipase; HSL: Hormone-sensitive lipase; NEFA: Non-esterified fatty acids; ASP: Acylation stimulating protein; TAG: Triacylglycerol; TNF- α : Tumor necrosis factor α ; RBP4: Retinol-binding protein; IL6: Interleukin 6.

Table 1 Vascular complications in type 2 diabetes

Microvascular complications prevalence	Macrovascular complications prevalence
Retinopathy 23.7%	Cardiovascular disease 11.4%
Background 20.0%	Peripheral vascular disease 4.0%
Proliferative 3.7%	Cerebrovascular accidents 0.9%
Nephropathy 5.5%	Hypertension 38.0%
Peri-neuropathy 27.5%	

Adiponectin, also known as Acrp30 (adipocyte complement-related protein of 30 kDa) is a protein exclusively secreted by adipocyte having huge structural similarity with C1q^[27]. Three monomers (30 kDa) associate together at the globular domain to form the adiponectin trimer, where four to six trimers associate through their collagenous domains to form the high order structure. Monomeric adiponectin has not been observed in the plasma and it is believed to remain within adipocyte^[28]. Human adiponectin is encoded by the *ADIPOQ* gene on the chromosomal locus 3q27 consisting three exons and two introns^[29], involved in regulating glucose levels as well as fatty acid breakdown^[1]. Mouse adiponectin is a 247 amino acid long protein where human adiponectin is a protein product of 244 amino acids consisting of four domains, an amino-terminal signal sequence, a variable region, a collagenous domain (cAd) consisting of 22 Gly-X-Y repeats, and a carboxy-terminal globular domain (gAd)^[27]. It is the most abundant adipokines with its serum concentration ranging from 5 to 30 $\mu\text{g/mL}$ ^[30].

Structure of single-chain globular domain adiponectin (sc-gAd) is reported (Figure 2), where globular domain is composed of three part A, B and C respectively^[30]. The adiponectin protein can undergo proteolytic cleavage and can form the globular form of adiponectin, where the globular head domain has been reported to increase the fatty acid oxidation^[33]. Acrp30 is found in two forms in serum; one is low molecular weight (LMW) trimer-dimer where the other one is high molecular weight complex. Oligomer formation of Acrp30 depends on the formation of disulfide bond mediated by Cys-39. Mutation of Cys-39 results in the trimers which can easily undergo proteolytic cleavage in the collagenous domain^[34].

Yamauchi *et al.*^[35] reported for the first time about the two adiponectin receptors which can successfully increase AMP kinase and PPAR- α ligand activities as well as can accelerate fatty acid oxidation and glucose uptake by adiponectin. These receptors are named as AdipoR1 which is abundantly expressed in skeletal muscle and AdipoR2 which is mainly expressed in the liver (Figure 3)^[35,36].

They first successfully performed the cloning of complementary DNAs encoding adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) by expression cloning^[35]. AdipoR1 and AdipoR2 mRNA expression in the liver and skeletal muscle increases after fasting and re-feeding can rapidly restore these to levels equal to the original fed state (Figure 4)^[35,36]. Both of these receptors contain seven transmembrane domains but they are structurally

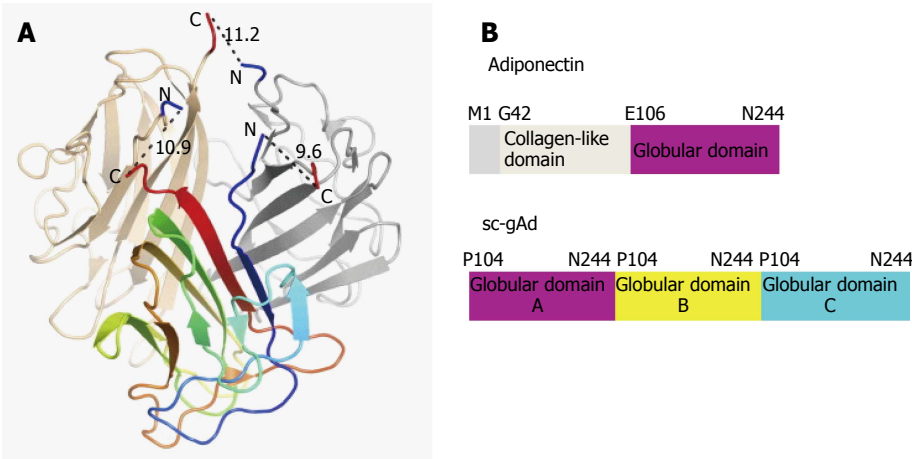


Figure 2 Structure of single-chain globular domain adiponectin (sc-gAd). A: Base region of mouse gAd structure where blue arrow determines the N terminus and red arrow determines the C terminus; B: Domain organization of human adiponectin and the sc-gAd, where there are three domains A, B and C respectively. (Adapted from Min *et al*^[30]).

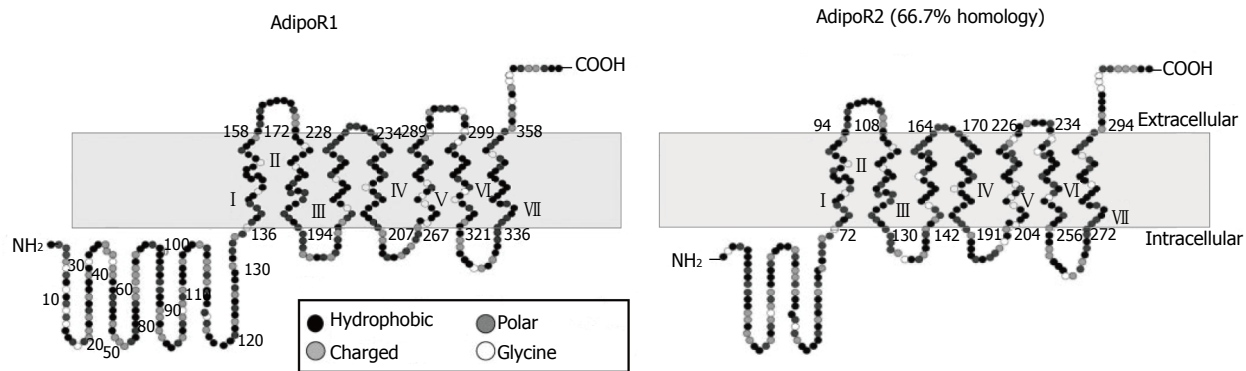


Figure 3 Proposed structure of adiponectin receptors (Adapted from Kadowaki *et al*^[36]).

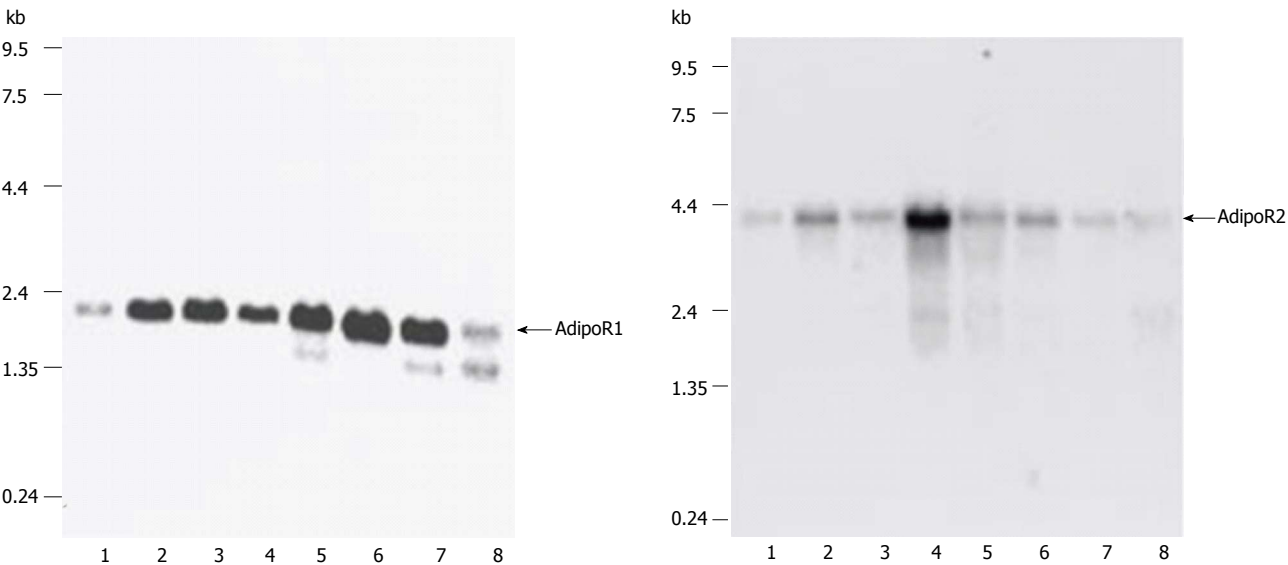


Figure 4 Northern blot analysis of AdipoR1 (top panel) and AdipoR2 (bottom panel) mRNA in mouse tissues (lanes: 1, brain; 2, heart; 3, kidney; 4, liver; 5, lung; 6, skeletal muscle; 7, spleen; 8, testis)^[35]. AdipoR: Adiponectin receptors.

and functionally completely distinct from G-protein-coupled receptors. Mild insulin resistance has been observed in both *adipoR1* and *adipoR2* knocked out mice, but complete abolition of adiponectin activity has been observed in *adipoR1/R2* double knockout mice, resulting in increased tissue triglyceride content, inflammation and

oxidative stress^[37].

It has been observed by one research group (Figure 5) that abolition of AdipoR2 eradicates β cell replication and neogenesis, thus in presence of high energy diet although it shows moderate insulin sensitivity initially and shows moderate body mass, in later state it tends to

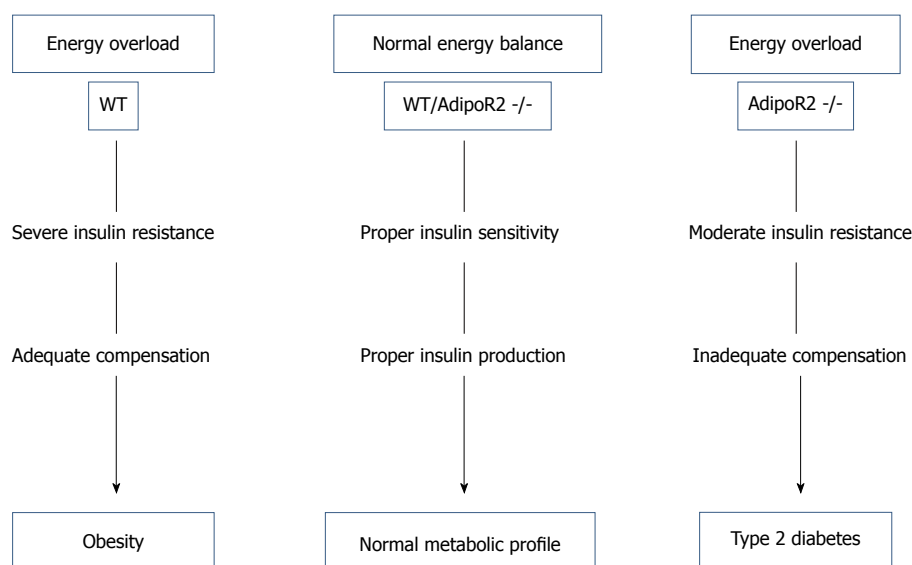


Figure 5 Diagram depicting the metabolic profile of wild type and AdipoR2 $-/-$ mice (Adapted from Liu *et al*^[38]). WT: Wild type; AdipoR 2: Adiponectin receptors 2.

develop type 2 diabetes^[38]. Insulin resistance consuming high energy diet increases obesity in wild type (WT) mice, where normal energy diet in both WT and AdipoR2 double knocked out mice (AdipoR2 $-/-$) shows normal metabolism, but AdipoR2 $-/-$ mice with energy overload although shows moderate insulin resistance initially but in later stage develops type 2 diabetes.

In increased oxidation of fatty acids such as in Nonalcoholic steatohepatitis (NASH) the expression levels of AdipoR1/R2 and insulin receptor substrate isoforms 2 (IRS-2) were significantly decreased, whereas IRS-1 was significantly increased^[39].

ADIPONECTIN AND ITS ROLE IN OBESITY AND DIABETES

Although it circulates in high concentrations, adiponectin levels are lower in obese subjects than their lean counterparts. Apart from negative correlations with measures of adiposity, adiponectin levels are also reduced in association with insulin resistance and type 2 diabetes^[40]. Epidemiological studies in different ethnic groups revealed that low level of plasma adiponectin, especially its HMW form can be an important key factor for type 2 diabetes, hypertension, atherosclerosis and myocardial infarction^[41]. Other than preventing insulin resistance and adipose tissue inflammation, adiponectin has been associated to exert several cardioprotective roles through direct actions on heart as well as on other vascular cells (Figure 6)^[42]. Adiponectin has negative correlation with insulin resistance, along with it maintains negative correlation with plasma triglyceride and low density lipoproteins (LDLs) where it has positive correlation with high density lipoproteins (HDLs)^[43]. In this review we will try to elucidate the role of adiponectin in acquiring adiposity in various aspects, *i.e.*, from the metabolomic view to genetic predisposition.

Adipocyte derived adiponectin can modulate the functions of cardiomyocytes, endothelial cells, endothelial progenitor cells, macrophages, leukocytes, and vascular smooth muscles in both endocrine and paracrine manner (Figure 6). Here we will discuss the possible roles of this adipokine in type 2 diabetes mellitus, obesity and dyslipidemia.

Studies in Japan showed that hypertension has a major effect on atherosclerosis and CVD events in persons with high body mass index with T2DM^[16]. Adiponectin and its association with lipid metabolism and increased obesity are studied well in many populations.

Mode of actions of this potential biomarker

Adiponectin serves as a central regulatory protein in many metabolic pathways playing crucial role in many metabolic disorders. Its importance as a potential biomarker in type 2 diabetes is increasing rapidly. The major way to estimate plasma adiponectin is by Sandwich ELISA. Lower plasma adiponectin level ($< 5 \mu\text{g/mL}$) is associated with increasing obesity and acquiring of metabolic disorders.

As a key factor of the metabolic pathway: Adiponectin has multifunctional roles in metabolic synchronization (Figure 7). Adiponectin (ADIPOQ) an adipocyte derived hormone activates ADIPOR1 and ADIPOR2, the two adiponectin receptors; it also activates PPAR γ ultimately increasing the rate of β oxidation which is a major pathway for lipid metabolism. ADIPOR1 increases the action of number of genes including NF- κ B, TNF α , IL1, IL4. NF- κ B^[41] furthermore decreases VCAM1, ICAM1 and IL18 levels; these are important genes involved in inflammation. ADIPOR1 also activates p38MAPK, another gene involved in transcriptional machinery. The action of PI3K is indirectly regulated by ADIPOR1. PI3K acts on HSP90 which again increases the action of

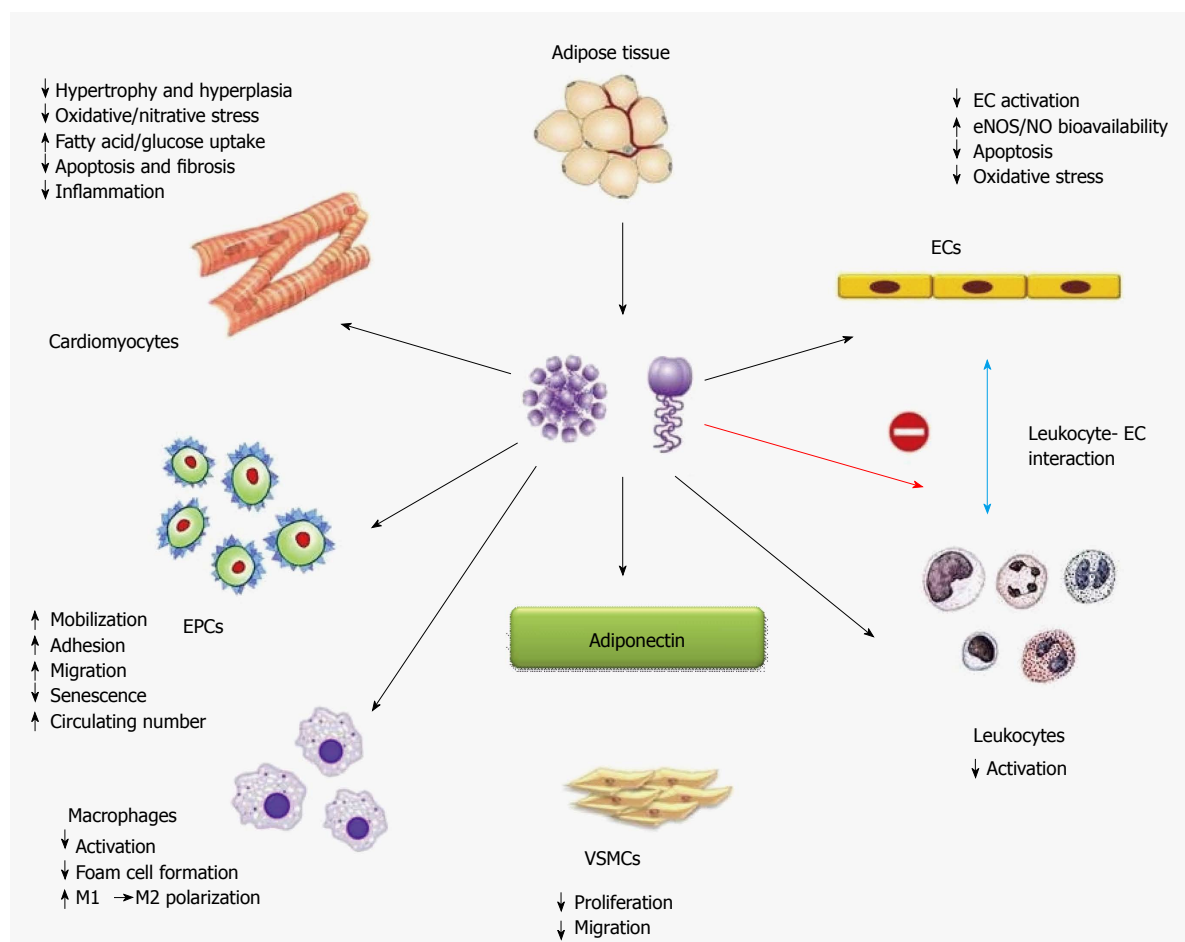


Figure 6 Actions of adiponectin in different cell line (Adapted from Xu *et al.*^[42]). EC: Endothelial cell; EPCs: Endothelial progenitor cells; VSMC: Vascular smooth muscles; eNOS: Endothelial nitric oxide synthase.

endothelial nitric oxide synthase (eNOS), which is related to oxidative stress. ADIPOR2 activates APPL1 which up regulates AMP-activated protein kinase 1 (AMPK1) which again up regulates eNOS^[40,41] increasing the production of nitric oxide. Elevated AMPK also increases the action of PEPCK ultimately increasing gluconeogenesis. APPL1 works on Akt which increases Glut4 translocation ultimately elevating glucose uptake of the cell^[42] (Figure 7). Derived from adipocyte it comes in contact with blood plasma and directly acts on Adipo R1/R2 receptors which further activates/inhibits the downstream genes related to oxidative stress and inflammation. Plasma and hemolysate of patients of type 2 diabetes contains elevated level of protein carbonyl content, which indicates increased oxidative stress^[44].

T-cadherin (CDH13) localizes adiponectin to the vascular endothelium. It has been reported that T-cadherin deficiency by siRNA knockdown prevented the ability of adiponectin to promote cellular migration and proliferation^[45]. T-cadherin protects from stress-induced pathological cardiac remodeling by binding with adiponectin and activating its cardioprotective functions in mice^[46].

Mechanisms of action: Adiponectin exhibits two major mechanisms of action by which it inhibits obesity and

type 2 diabetes, one by increasing insulin sensitivity and the other way is to increase fatty acid oxidation.

APPL1, stimulated by adiponectin can interact with both adiponectin receptors and can mediate the downstream events such as lipid oxidation and membrane translocation of glucose transport 4 (GLUT4), thus increasing glucose uptake (Figure 7), providing a platform for increased insulin sensitization^[47]. APPL1 also acts as a mediator of adiponectin signaling pathways by interacting directly with ADIPOR1/ADIPOR2 or signaling proteins, thereby playing critical roles in cell proliferation, apoptosis, cell survival, endosomal trafficking, and chromatin remodelling^[48]. APPL1 modulates the insulin signalling pathway by acting with Akt and PI3K^[49] (Figure 7).

The major form of storing and transporting fatty acids is triglycerides. Adiponectin has been reported to decrease tissue triglyceride content by increasing the expression of CD36, a fatty acid transporter^[50]. Increased tissue TG content activates PI3K and Glut4 increasing glucose uptake, elevating insulin resistance^[51]. Thus, lowering of tissue triglyceride content promotes insulin sensitivity. Along with adiponectin has been also reported to increase the expression of PPAR α which further lowers the tissue triglyceride content^[50]. Some researcher

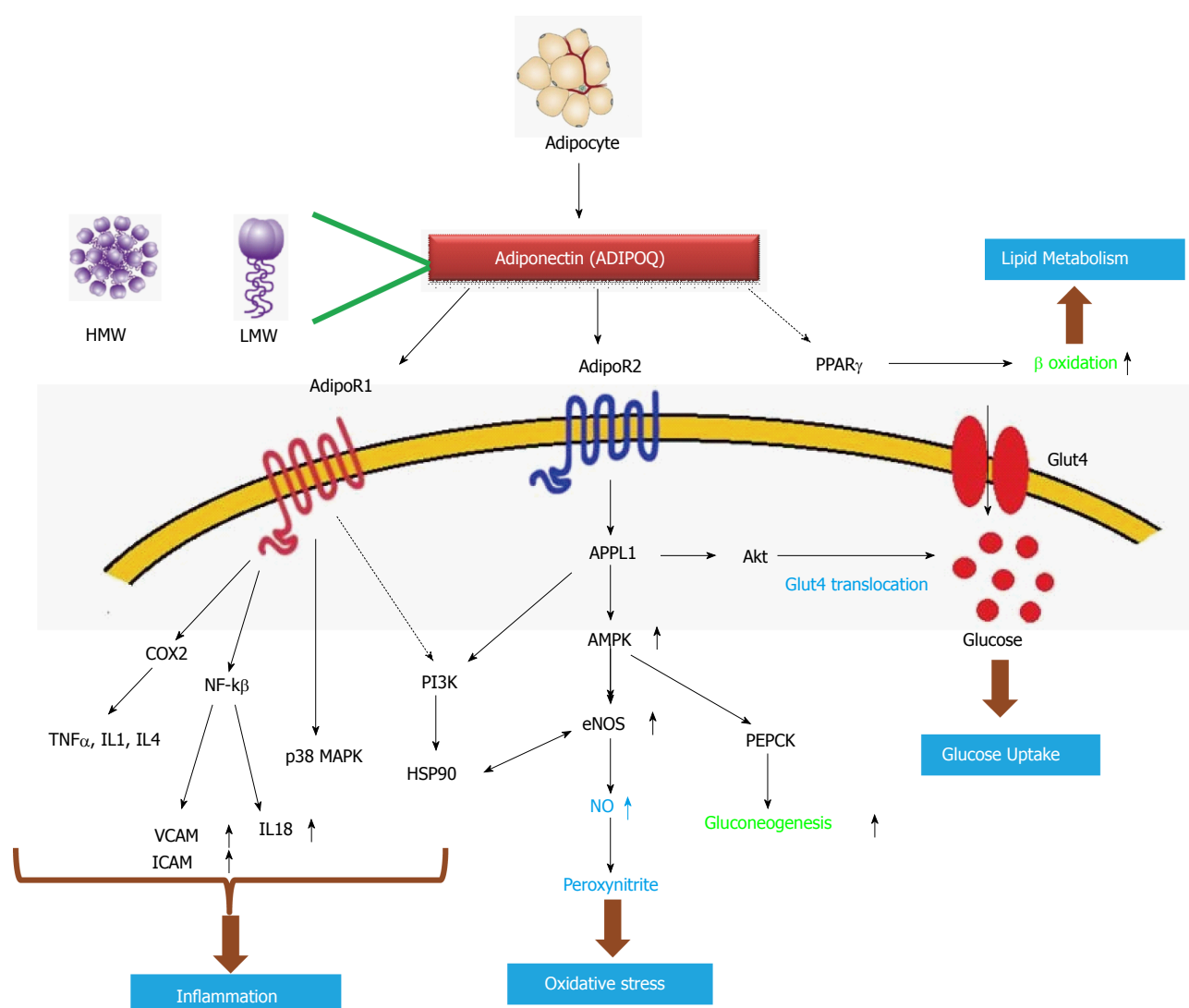


Figure 7 A proposed model of adiponectin metabolic pathway and associated genes. HMW: High molecular weight; LMW: Low molecular weight AdipoR1: Adiponectin receptor 1; PPAR γ : Peroxisome proliferator-activated receptor gamma; Glut4: Glucose transporter type 4; APPL1: Adaptor protein, phosphotyrosine interaction, pH domain and leucine zipper containing 1; Akt: Protein kinase B; COX2: Cyclooxygenase 2; AMPK: Adenosine monophosphate-activated protein kinase; PI3K: Phosphoinositide 3-kinase; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF α : Tumor necrosis factor alpha; IL: Interleukin; p38 MAPK: p38 mitogen-activated protein kinase; HSP90: Heat shock protein 90; eNOS: Endothelial nitric oxide synthase; PEPCK: Phosphoenolpyruvate carboxykinase; NO: Nitric oxide; VCAM: Vascular cell adhesion protein; ICAM: Intercellular adhesion molecule.

group has demonstrated the role of adiponectin in activating AMPK which can stimulate β oxidation and glucose up taking^[52].

It has been established that adiponectin enhances insulin-stimulated IRS-1 tyrosine and Akt phosphorylation. Activation of the LKB1/AMPK/TSC1/2 pathway alleviates the p70S6 kinase-mediated negative regulation of insulin signaling, providing a mechanism by which adiponectin increases insulin sensitivity in cells^[53].

Other than playing a crucial role as an insulin sensitizer, adiponectin also defeats obesity and obesity onset type 2 diabetes by increasing fatty acid oxidation. Increased fatty acid oxidation in turn also elevates insulin sensitivity. As stated earlier, adiponectin associated activation of AMPK phosphorylation which in turn implements major role in fatty acid oxidation. In cultured myotubes C2C12, adiponectin treatment has been

associated with increased PPAR α activity; expression of some downstream genes such as suchasacyl-CoAoxidase and carnitinepalmitoyltransferase1 has been also reported, thus promoting fatty acid oxidation^[54]. Adiponectin induces fatty acid oxidation in muscle cells by sequential activation of AMPK, p38 MAPK (mitogen activated protein kinase) and PPAR α ^[54]. It has been studied in humans that LDL activity is correlated positively with plasma adiponectin level, thus LPL may represent a link between low adiponectin levels and dyslipidemia in both nondiabetic individuals and patients with type 2 diabetes^[55] where plasma TGs is negatively correlated with LDL activity and positively with diabetic state^[56].

It has been well postulated that subjects with type 2 diabetes has reduced mitochondrial content and decreased electron transport chain activity^[57]. Adiponectin has been reported to increase mitochondrial biogenesis

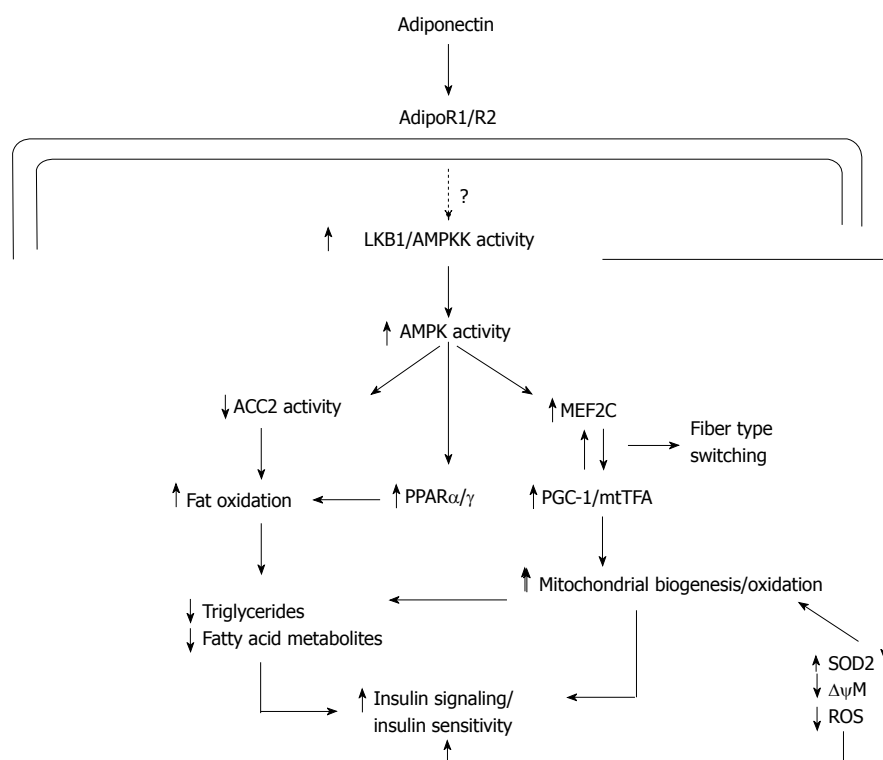


Figure 8 Hypothetical scheme of adiponectin signaling and the regulation of mitochondrial function in skeletal muscle (Adapted from Civitarese *et al.*^[59]). AdipoR1: Adiponectin receptor 1; PPAR γ : Peroxisome proliferator-activated receptor gamma; LKB1: Liver kinase B1; AMPKK: Adenosine monophosphate-activated protein kinase kinase; AMPK: Adenosine monophosphate-activated protein kinase; ACC2: Acetyl-CoA carboxylase 2; MEF2C: Myocyte-specific enhancer factor 2C; PPAR α : Peroxisome proliferator-activated receptor alpha; PGC-1: Peroxisome proliferator-activated receptor gamma coactivator 1; mtTFA: Mitochondrial transcription factor A; SOD2: Superoxide dismutase 2; $\Delta\psi$ M: Mitochondrial membrane potential; ROS: Reactive oxygen species.

and oxidative capacity in mice which in turn is favorable for glucose metabolism as well as fatty acid oxidation as mitochondria is the major cellular site of metabolism^[58]. In human model also the adiponectin stimulated mitochondrial biogenesis has been observed^[59] (Figure 8).

Adiponectin binds to its receptors which activates AMPK and stimulates the phosphorylation of ACC2 which in turn increases fatty-acid oxidation. As previously discussed adiponectin can activate peroxisome proliferative activated receptor- α (PPAR α) stimulating transcription of genes in the fatty-acid oxidation pathway and decreasing triglyceride content in muscle, thus promoting fatty acid oxidation (Figure 8) and improving insulin sensitivity^[50,54]. Independent of changes in transcription and mitochondrial mass, the improvements in lipid oxidation occur in less than 6 h in mice^[52]. Adiponectin activation of AMPK by upstream kinase AMPK kinase activates transcription of myocyte enhancer factor 2C and phosphorylation of peroxisome proliferative activated receptor γ coactivator 1- α (PGC1 α), which in turn increases mitochondrial content, oxidative capacity, and oxidative-fibre type composition. Central to the development of mitochondrial dysfunction is reactive oxygen species (ROS) production, which reacts with DNA, protein, and lipids leading to oxidative damage. ROS production is inversely related to mitochondrial content. Activation of the adiponectin pathway reduces the generation of ROS by two processes: (1) Increasing mitochondrial content, which in turn decreases the workload for each mitochondrion leading to reduced membrane potential ($\leftarrow\Delta\psi$) and lower ROS production; and (2) adiponectin increases PGC1 α activity which increases the transcription/activity of the antioxidant

enzyme SOD2 that decreases super oxide radical (O_2^{\bullet})^[60].

Oxidative stress is a major consequence of type 2 diabetes and obesity related disorders. Previously in our laboratory we had established that hyperglycaemic condition increases the oxygen releasing capacity of haemoglobin which in turn boosts the effect of oxidative stress in diabetes and CVDs^[44]. Oxidative stress which is a major indicator of inflammation correlates significantly with adiponectin metabolic pathway. Study by a research group demonstrates that lower adiponectin level is significantly associated with higher inflammatory state^[61].

Other than decreasing circulating free fatty acid and lowering triglyceride content adiponectin has been also observed to exert anti-inflammatory and anti-atherogenic effects by reducing TNF α -induced monocyte attachment to endothelial cells and inhibiting platelet derived growth factor-BB to minimize vascular smooth muscle cell proliferation^[62]. Most adipokines can exert pro inflammatory effects, among which adiponectin is increasing its importance as a potential inflammatory marker. Obesity is characterized by low grade systemic inflammation^[63]. Adiponectin inhibits the action of TNF α which is a key pro inflammatory cytokine in both vascular and cardiac tissue^[64]. This novel cytokine has been also reported to decrease the secretion of IL 8 from human aortic endothelial cells (HAEC) stimulated with TNF α , along with it also inhibits IL8 mRNA expression induced by TNF α . Phosphorylation of I κ B α is decreased by adiponectin, but phosphorylation of ERK, SAPK/JNK, and p38MAPK remains unaffected^[65]. Adiponectin also increases intra-cellular cAMP levels in HAEC and increases PKA activity^[65]. The inverse relationship of adiponectin with inflammatory marker CRP has been

discussed in the next paragraph of this review.

Thus adiponectin exerts several multitasking roles and combat the prevalence of metabolic disorders like diabetes and obesity. In first step it works as a fascinating insulin sensitizer and in second step it increases fatty acid oxidation. Simultaneously in all above mentioned mode of actions it acts as an important inflammatory marker while playing significant role in minimizing oxidative stress. Thus adiponectin plays affluent role to protect the metabolic harmony of the system through various metabolic pathways and considered as one of the potential biochemical and inflammatory biomarker in metabolic disorders.

Correlation with other adipocyte derived hormones

Adipocyte is involved with the releasing of another three hormones playing some roles in metabolism; these are leptin, resistin and visfatin. Where low plasma adiponectin has been observed in obesity, leptin levels become significantly higher, having an inverse correlation with adiponectin. Increased subcutaneous fat has been a major determinant of leptin levels. The action of leptin remains to decrease appetite, thermogenesis and increase fatty acid oxidation^[66]. The leptin signal is transmitted by the Janus kinase, signal transducer; and activator of transcription pathway decrease glucose, and reduce body weight and fat^[66]. One research group showed that adiponectin is more influenced by visceral adipose tissue where leptin is by subcutaneous adipose tissue^[62] where fasting glucose, insulin, HOMA-IR and triglyceride has an inverse correlation with adiponectin and leptin maintaining a fairly positive association with these parameters^[67]. It is reported that leptin/adiponectin ratio alters in type 2 diabetes as this alteration increases insulin resistance^[68]. Another research group reported the plasma leptin/adiponectin ratio as an important atherogenic index^[69]. Thus it can be concluded that where adiponectin is a proinflammatory adipokine giving proatherogenic effect, leptin serves as an antiinflammatory molecule giving a direct antiatherogenic effect.

The plasma level of resistin, a cysteine rich adipokine has been observed to increase in type 2 diabetes but this increase in level is not correlated with insulin resistance and adiposity^[70]. Another research group found a decrease in serum resistin value in patients with type 2 diabetes^[71]. Where adiponectin level is significantly associated with lipid profile, BMI, resistin levels seem to level independent of these attributes in patients with type 2 diabetes mellitus^[72]. Thus the association of resistin with type 2 diabetes, obesity and dyslipidemia is still a new field to explore; and the association of this adipokine with adiponectin is poorly understood.

Visfatin, another adipokine maintains a direct relationship between plasma visfatin levels and type 2 diabetes mellitus. Visfatin binds to the insulin receptor at a site distinct from that of insulin and causes hypoglycaemia by reducing glucose release from liver cells and stimulating glucose utilization in adipocytes and

myocytes. Visfatin is upregulated by inflammation and hyperglycaemia and downregulated by insulin^[73]. Where the association of visfatin with diabetes mellitus has been well studied its correlation with adiponectin is poorly known. Although it has been postulated in one article that adiponectin maintains a fairly inverse relationship with visfatin^[74]. Thus activity of other adipokines with adiponectin is still remained a major field to explore in metabolic syndrome.

Association with other important diabetic biomarker

Adiponectin which is increasing its importance as a potential biomarker maintains some association with other diabetic biomarkers such as fasting insulin, C-reactive protein (CRP) and homocysteine. Fasting insulin and CRP has been observed to maintain an inverse correlation with adiponectin level^[75]. A data observed on Asian Indian obese men revealed that serum adiponectin level is inversely related with fasting insulin and CRP^[76]. Both adiponectin and CRP is strongly associated with insulin sensitivity where CRP is more dependent on adiposity^[77]. One study group found no significant correlation between plasma homocysteine level and adiponectin in patients with type 2 diabetes^[78]. Although an inverse relationship was found between adiponectin and homocysteine in patients with type 1 diabetes but no significant association has been reported in type 2 diabetes^[79].

Genetic variants and expression of genes in adiponectin metabolic pathway

Genetic polymorphisms in ADIPOQ gene and the genes of its receptors has been a major reason for functional defect of this novel adipokine. Genetic polymorphisms of the other genes present in adiponectin metabolic pathway may also alter the functional properties of adiponectin and thus promoting the progression of insulin resistance, dyslipidemia and atherogenesis. These genetic polymorphisms have seen in many ethnic groups. ADIPOQ gene polymorphisms were associated with the risk of T2DM in Chinese Han population^[80]. It has been observed that rs2241767AG genotype increases the risk of T2DM in obesity group^[80]. A study in south Indian population implies ADIPOQ gene +276 G/T and -3971 A/G polymorphisms are associated with generalized obesity and +349 A/G with central obesity^[81].

The polymorphism -1131 T/C in apolipoprotein A5 gene is associated with postprandial hypertriglycerolemia, elevated small, dense LDL concentrations and oxidative stress in non-obese Korean men^[82] and dyslipidemia in Brazilian subjects^[83] (Table 2). A significant association of -11391 G/A adiponectin gene polymorphism with waist circumference in diabetic patients has been observed^[84]. In white Europeans, +276 G/T was associated with higher serum adiponectin concentrations where -10066 G/A was associated with lower serum adiponectin concentrations^[85]. Genetic polymorphisms of ADIPOR1 and ADIPOR2 are also

Table 2 List of SNPs found in the genes of adiponectin pathway in metabolic disorders such as type 2 diabetes, obesity, dyslipidemia and cardiovascular disorders (courtesy to <http://www.genecards.org/> for providing the information regarding SNP location)

Ref.	Gene	SL No.	Location	Variation	SNP ID
Blech <i>et al</i> ^[96]	PPAR γ	1	Intron 1	Pro12Ala	rs1801282
Blech <i>et al</i> ^[96] ; Ramya <i>et al</i> ^[81]	ADIPOQ	1	5' flanking region	-11365 C/G	rs266729
		2	Intron 1	-4522 C/T	rs822393
		3	Intron 1	-3971 A/G	rs822396
		4	Intron 1	+276 G/T	rs1501299
		5	Exon 1 coding synonymous	+45 T/G	rs2241766
		6	Intron 1	+349 A/G	rs2241767
		7	Intron 1	+712 G/A	rs3774261
		8	5' flanking region	-11391 G/A	rs17300539
		9	Exon 3 splicing enhancers	Y111H T/C	rs17366743
Wang <i>et al</i> ^[97]	ADIPOR1	1	Intron 1	+5646 A/G	rs1342386
		2	Intron 1	+5843 A/G	rs1342387
		3	Intron 1	-101 T/G	rs2275737
		4	5' transcription factor binding site	-8503C/T	rs6666089
Vaxillaire <i>et al</i> ^[98]	ADIPOR2	1	Exon 3 splicing enhancers	+33371 C/T	rs12342
		2	Intron 1	+26314 A/G	rs767870
		3	5' flanking region	-64241 T/G	rs1029629
		4	Intron 1	+8645 G/C	rs1468491
		5	Intron 1	+14645 A/T	rs4766415
		6	Intron 1	-35361 G/A	rs10773982
Thameem <i>et al</i> ^[99]	eNOS/NOS3	1	Exon 3 splicing enhancers	Glu298Asp	rs1799983
		2	Intron 1	-786 T/C	rs2070744
Zhang <i>et al</i> ^[100]	NF-kB	1	5' flanking region	-94 insertion/deletion	rs28362491
Rees <i>et al</i> ^[101]	PEPCK	1	5' flanking region	-232C/G	rs2071023
Jang <i>et al</i> ^[82] and Ferreira <i>et al</i> ^[83]	Apolipoprotein A5 gene (APOA5)	1	5' flanking region	-1131T/C	rs662799
Ol <i>et al</i> ^[102]	COX-2	1	5' flanking region	-765G/C	rs20417
Ho <i>et al</i> ^[103]	IL4	1	5' flanking region	-590 C/T	rs2243250

IL-6: Interleukin 6; AdipoR: Adiponectin receptors; eNOS: Endothelial nitric oxide synthase; PPAR γ : Peroxisome proliferator-activated receptor gamma; COX2: Cyclooxygenase 2; NF-k β : Nuclear factor kappa-light-chain-enhancer of activated B cells.

involved in altered function of adiponectin and have been observed by many groups (Table 2). Polymorphisms of other pathway genes like eNOS, NF-kB, PEPCK, IL4 (Table 2) has been also reported to play roles in development of type 2 diabetes, thus they may correlate with adiponectin and regulate its function.

Adiponectin gene function is not solely dependent on gene polymorphisms rather expression levels of certain genes may modulate its function significantly. Both in type 2 diabetic patients and in animal models of insulin resistance it has been observed that the mRNA expression and secretion of adiponectin is significantly decreased^[86,87]. Very low calorie diet has been reported to raise adiponectin mRNA level, whereas re-feeding significantly decreases the mRNA level in morbidly obese women^[88]. AdipoR2 mRNA expression in subcutaneous tissue is negatively associated with insulin resistance and metabolic parameters independently of obesity may mediate the improvement of insulin resistance in response to exercise^[89]. PPAR γ agonist thiazolidinedione has been reported to increase adiponectin level in animal models and human patients^[90]. A single nucleotide polymorphism in Pro12Ala in PPAR γ is reported to be involved in type 2 diabetes. PPAR γ has been found to undergo obesity-induced and protein kinase cdk5-mediated phosphorylation at Ser²⁷³ which mediates obesity-induced down-regulation of adiponectin in white

adipose tissue^[91].

There are certain evidences that adrenomedullin (ADM) may modulate the expression of adiponectin gene. One group of scientists postulated that a genetic variant in ADM gene (rs182052) alters the expression of adiponectin gene and minimizes plasma adiponectin levels^[92]. A variation in CDH13 (rs4783244) showed strong associations with total adiponectin and HMW adiponectin in East Asian population where people with this variation have significantly lower adiponectin plasma level, but adiponectin sensitivity tends to increase, eventually maintaining a better metabolic profile^[46].

Glucocorticoids are also reported to regulate adiponectin gene expression in human adipocytes, where TNF α does not seem to directly inhibit adiponectin synthesis in human adipocytes^[93]. SIRT1 and Foxo1, two important genes involved in insulin sensitivity whose low expression leads to impaired Foxo1-C/EBP α complex formation, has been reported to decrease adiponectin expression in obesity and type 2 diabetes^[94]. CRP has been reported to suppress adiponectin gene expression partially through the PI3K pathway where decreased production of adiponectin might represent a mechanism by which CRP regulates insulin sensitivity^[95].

Genetic polymorphisms which supposed to be a screening tool of adiponectin metabolic disorder may be overpowered by the altered gene expression of

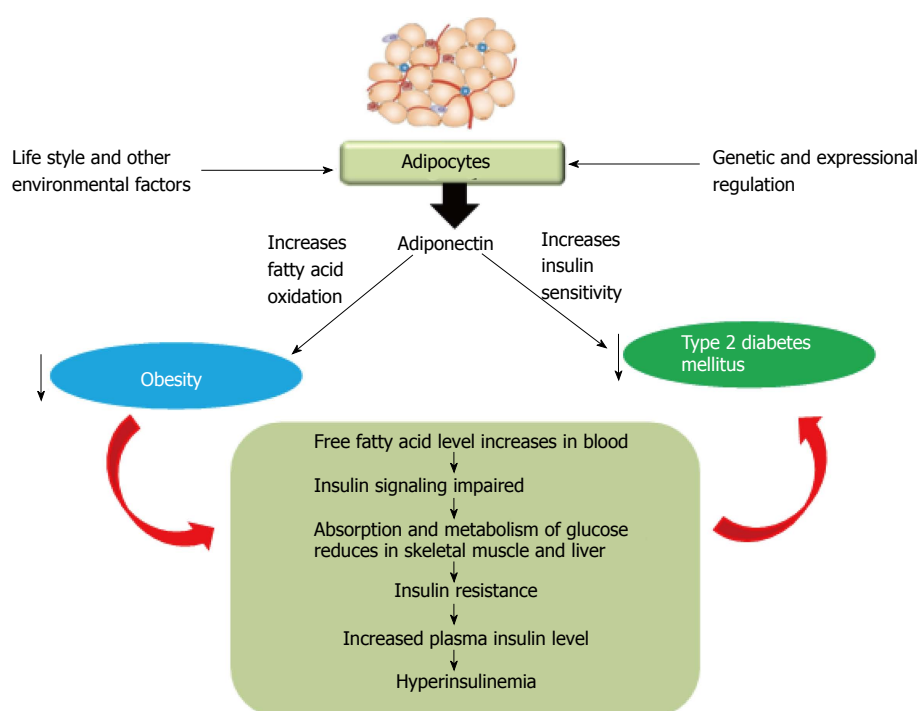


Figure 9 Hypothetical model showing the interrelation between adiponectin, obesity and type 2 diabetes mellitus.

adiponectin and related genes. Both of these actions may significantly be associated with low expression of adiponectin which in turn is positively correlated with insulin resistance increasing the prevalence of diabetes and obesity.

Adiponectin and epigenetics

Epigenetic association of adiponectin expression is remained a big question to answer. DNA methylation can partly explain the link between the early exposures to a detrimental fetal environment, where the mother is hyperglycemic which may in turn increase the risk to develop obesity and diabetes later in life^[104]. One group found significant correlation between the mother blood glucose level and placental DNA methylation at cytosines located at *ADIPOQ* gene proximal promoter CpG islands^[105]. Expression and methylation of *ADIPOR1* gene isolated from skeletal muscle cells has been modified after an exercise period of 6 mo in subjects who are first degree relatives of type 2 diabetes patients^[106]. But still there are few evidences of the epigenetic modulation of adiponectin and remains a promising field to explore.

Clinical aspects

Balanced diet with adequate exercise can combat obesity and type 2 diabetes in manifold. Although genetic predisposition is a main key factor of these disorders by still maintaining a well-balanced energy is still a beneficiary supplements in preventing these disorders. Exercise can fairly maintains plasma adiponectin levels and thus promoting insulin sensitivity. One study shows that aerobic exercise increases insulin sensitivity among diabetic patients mediated by adiponectin^[107], although

drug treatment may be required to normalize plasma adiponectin levels. Adiponectin replenishment therapy is yet not possible as biologically active recombinant adiponectin proteins are inherently unstable and difficult to produce^[108]. Certain drug classes such as antidiabetic drugs glitazones and sulfonylureas, and angiotensin receptor blockers, ACE inhibitors and nicotinic acid exert beneficial effects on insulin resistance partly by increasing plasma adiponectin levels. Others such as tetrahydrobiopterin or certain antioxidants are also promising in normalizing plasma adiponectin levels^[109]. Omega-3 polyunsaturated fatty acids has been reported to increase plasma adiponectin to leptin ratio in stable coronary artery disease, thus playing a cardioprotective role, might in turn be beneficiary for diabetes and obesity^[110]. Thus a healthy life style with some oral supplements may increase adiponectin levels in patients with type 2 diabetes.

CONCLUSION

Adiponectin, the novel adipocyte has been demonstrated well to play crucial role in obesity and type 2 diabetes mellitus (Figure 9). It is increasing its importance as a potential biomarker in above mentioned diseased state as: (1) It increases insulin sensitivity; (2) It increases fatty acid oxidation; (3) It correlates significantly with oxidative stress; and (4) It acts as an important inflammatory biomarker and up/down regulates many genes in various metabolic pathways. Thus adiponectin could be a novel target for the therapeutic approach to treat diabetes mellitus in near future. Recombinant adiponectin is not effective thus altered expression of adiponectin or related

pathway genes could be an effective tool for researchers to mediate its function which in turn may minimize the prevalence of obesity, type 2 diabetes or other metabolic disorders.

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Rare complications of pediatric diabetic ketoacidosis

Shara R Bialo, Sungeeta Agrawal, Charlotte M Boney, Jose Bernardo Quintos

Shara R Bialo, Sungeeta Agrawal, Charlotte M Boney, Jose Bernardo Quintos, Division of Pediatric Endocrinology of Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI 02903, United States

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Correspondence to: Jose Bernardo Quintos, MD, Division of Pediatric Endocrinology of Rhode Island Hospital, Warren Alpert Medical School of Brown University, 593 Eddy Street, MPSII, Providence, RI 02903, United States. jose_bernardo_quintos@brown.edu
Telephone: +1-401-4445504

Fax: +1-401-4442534

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sequela of diabetic ketoacidosis that warrants close monitoring. The medical literature details various other complications in children with diabetic ketoacidosis, including hypercoagulability leading to stroke and deep vein thrombosis, rhabdomyolysis, pulmonary and gastrointestinal complications, and long-term memory dysfunction. We review the pathophysiology, reported cases, management, and outcomes of each of these rare complications in children. As the incidence of T1D continues to rise, practitioners will care for an increasing number of pediatric patients with diabetic ketoacidosis and should be aware of the various systems that may be affected in both the acute and chronic setting.

Key words: Type 1 diabetes; Diabetic ketoacidosis; Complications; Pediatric; Review

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Core tip: Diabetic ketoacidosis is highly prevalent in pediatric patients with both newly diagnosed and established type 1 diabetes. The most common rare complication is cerebral edema, which is the leading cause of death in youth with diabetes. However, several other complications involving multiple systems have been described and can cause significant morbidity in cases of pediatric diabetic ketoacidosis, thus warranting awareness and targeted monitoring.

Abstract

The incidence of type 1 diabetes (T1D) among youth is steadily increasing across the world. Up to a third of pediatric patients with T1D present with diabetic ketoacidosis, a diagnosis that continues to be the leading cause of death in this population. Cerebral edema is the most common rare complication of diabetic ketoacidosis in children. Accordingly, treatment and outcome measures of cerebral edema are vastly researched and the pathophysiology is recently the subject of much debate. Nevertheless, cerebral edema is not the only

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INTRODUCTION

Approximately 1 in 300 youth have type 1 diabetes (T1D)^[1], and the incidence in the pediatric population is

increasing by almost 3% each year in the United States^[2] and worldwide^[3]. Despite the burgeoning statistics and awareness, the prevalence of diabetic ketoacidosis (DKA) remains as high as 30% in children presenting with T1D^[4]. DKA is defined by the American Diabetes Association^[5], the European Society for Paediatric Endocrinology, and the Pediatric Endocrine Society^[6] as hyperglycemia (plasma glucose > 200 mg/dL or approximately 11 mmol/L) and venous pH < 7.3 and/or bicarbonate < 15 mmol/L. DKA is the most common cause of death in children with T1D^[7,8], and the most common rare and primary fatal complication of DKA is cerebral edema^[7]. The treatment and prevention of cerebral edema is, therefore, the subject of extensive medical research and attention. However, cerebral edema is not the only complication of DKA worthy of close monitoring during patient care. In this review article we will examine cerebral edema as well as the vascular, musculoskeletal, pulmonary, gastrointestinal, and cognitive complications of pediatric DKA, which are less common but can result in acute and long-term morbidity.

CEREBRAL EDEMA

Many children who present with DKA have some degree of altered mental status. Typically the altered status is due to acidosis or hyperosmolarity, although some studies show that subclinical cerebral edema occurs in the majority of patients in DKA^[9,10]. Approximately 0.5%-1% of children in DKA develop frank cerebral edema^[11-13]. Morbidity related to cerebral edema is approximately 13%-35% and mortality 24%-28%^[12,14]. Risk factors for the development of cerebral edema during DKA include new onset T1DM, low bicarbonate, low partial pressure of CO₂, and high BUN^[13,15].

Conventional thinking attributes the mechanism of injury in cerebral edema to swelling from an influx of fluid into the brain^[15-17]. This influx is thought to be due to the rapidly declining serum osmolarity caused by overly aggressive fluid resuscitation; however, data reveals the only treatment-related risk factor to be administration of bicarbonate^[15]. The association between high fluid infusion rates and development of cerebral edema trends toward, but does not reach, statistical significance^[13]. Radiographic confirmation of cerebral edema in patients with DKA prior to initiation of fluid therapy further discredits the association^[13,15]. Also, many children have normal brain imaging at the onset of clinical cerebral edema and do not develop radiographic signs of edema until hours or days later, suggesting that edema is a consequence rather than the cause of injury^[11].

A more plausible hypothesis is that cerebral edema is caused by cerebral hypoperfusion, which leads to cytotoxic edema (cell swelling and death) at presentation followed by vasogenic edema (breakdown of the blood brain barrier leading to capillary leakage) during treatment^[9]. There is supporting evidence for this mechanism, including the association between cerebral

hypoperfusion and the risk factors associated with the development of cerebral edema, including high BUN, low bicarbonate, and low partial pressure of CO₂^[13,15]. Additionally, Lam *et al.*^[18] show that untreated DKA in rats is associated with changes on diffusion-weighted Imaging Magnetic Resonance (DWI MR) consistent with cytotoxic edema. When the DKA is treated, the DWI MR images demonstrate slight changes that suggest advancement to vasogenic cerebral edema. MR DWI changes consistent with vasogenic edema have also been shown in children during treatment of DKA^[16]. These studies support the model that DKA-related cerebral edema stems from early ischemic brain damage followed by reperfusion injury during treatment.

COAGULOPATHIC COMPLICATIONS

Abnormalities of hemostasis have been identified in patients with poorly controlled diabetes, although the mechanism is not entirely understood^[19,20]. Likewise, clinical studies of both adult and pediatric patients with T1D with DKA have described a variety of transient changes in coagulation factors, such as increased platelet activation, fibrinolytic activity, and endothelial activation^[21,22]. A prospective study of adolescents with T1D and DKA demonstrated low levels of free protein S, which facilitates activated protein C in inactivating von Willebrand factor^[23]. Accordingly, the levels of von Willebrand factor activity were increased. Protein C activity was decreased in DKA but normalized following treatment.

DKA is also characterized by elevated levels of inflammatory markers (CRP), cytokines (IL6, IL1beta, TNF alpha), and complement activation^[24]. This inflammatory state, combined with the disruption of the normal coagulation cascade, can place patients at increased risk of thrombosis and stroke during acute episodes of DKA.

Deep vein thrombosis

Deep vein thrombosis (DVT) is not uncommon in critically ill children who require central venous catheter placement as they introduce a foreign body, cause endothelial damage, and impair blood flow^[25]. Children and adolescents with DKA, however, appear to be at increased risk of DVT when they undergo placement of a central venous catheter^[26,27]. This increased risk of thrombosis likely stems from shock compounded by DKA, as severe dehydration activates the coagulation cascade and causes venous stasis and DKA itself confers a hypercoagulable state. Gutierrez *et al.*^[26] published the first report to describe this observation in a retrospective case-matched control series. It details that 4 of 8 children with DKA who underwent placement of a femoral central venous catheter developed DVT compared to 0 of the 16 of control patients who underwent central venous catheter placement without diabetes or DKA^[26]. A retrospective cohort study published by Worly *et al.*^[27] found similar observations with evidence of femoral

DVT on Doppler ultrasound within 48 h of the central catheter placement for treatment of DKA. Patients in that series with DKA and DVT had significantly higher serum glucose, corrected sodium concentrations, and lower pH and serum bicarbonate than their age-matched cohorts with shock and central venous catheters. DVT in children with DKA and catheter placement is also more common in those less than 3 years of age, which may be due to smaller vessel diameter and greater severity of illness at presentation^[26].

Children with DKA and DVT require low-molecular weight heparin until ultrasound confirmation of DVT resolution, which can take up to 6 mo^[27]. Given the increased risk of DVT and associated morbidity, use of central venous catheters should be avoided in children with DKA when possible. If placement is required, the central venous catheters should be removed as soon as possible and use of prophylactic anticoagulation therapy should be considered in cases of prolonged use.

Cerebral venous thrombosis

In general, the incidence of cerebral sinovenous thrombosis is 0.67 cases per 100000 children per year^[28]. Central venous thrombosis in association with pediatric DKA is reported twice in the medical literature^[29,30]. The first published case report is a 5-year-old girl with known T1D who presented with emesis, lethargy, and mild DKA who then neurologically decompensated 12 h into treatment, as evidenced by unconsciousness, response to painful stimuli only and limb rigidity^[29]. A CT scan demonstrated a thrombosis in the straight sinus and the vein of Galen with ischemic changes in the thalamus. She was anticoagulated with Heparin for 48 h followed by Warfarin for three months, and her baseline neurological status two years later was remarkably normal aside from mild learning difficulties.

The second case reported was in an 8 years old boy on first presentation of T1D with severe DKA with hyperosmolar state with serum glucose of 1668 mg/dL^[30]. Two hours into treatment he became unconscious and with sluggish pupillary response. A CT demonstrated thrombosis in the superior sagittal sinus and vein of Galen, as well as large infarctions in both cerebral hemispheres. Long-term follow-up information is not available for this case.

Stroke

The overall incidence of pediatric stroke is estimated at 2-13 per 100000 children^[31]. Hemorrhagic or ischemic brain infarction accounts for approximately 10% of intracerebral complications of DKA, and not all cases of stroke in DKA are associated with cerebral edema^[32]. The procoagulant state of DKA places patients at increased risk of ischemic brain injury as well as subsequent hemorrhagic conversion arising from hypoxia and vascular injury^[24]. Diagnosis of stroke during an episode of acute DKA is difficult as there is considerable overlap of signs, symptoms, and laboratory data^[33]. Early signs and symptoms of CNS injury include nonspecific findings

such as headache, confusion, lethargy, and unexpected changes in heart rate, respiratory rate, or blood pressure^[34]. Focal neurological signs allow clinicians to rapidly identify stroke victims; however, less than 30% of patients with DKA-associated stroke have characteristic focal neurologic deficits^[24]. It is also often difficult to differentiate whether cerebral edema in DKA is the cause or the effect of acute cerebral infarction, as stroke itself may cause cerebral edema. Arterial ischemic and hemorrhagic strokes have been documented in children and youth with DKA in a wide variety of cerebral locations, including single or multiple infarctions or thrombi over unilateral or bilateral lobes. The pathologic tissue findings of acute cerebral infarction related to DKA are not expected to be different from those of a nondiabetic child who has suffered a stroke.

Management and outcomes of pediatric stroke associated with DKA

Treatment guidelines for children and adults with diabetic ketoacidosis and stroke are lacking, including the optimal rehydration rate, parameters for use of thrombolytics and other medications, and monitoring schedules^[35]. In general, pediatric patients with suspected stroke should receive prompt neurological imaging and neurologic consultation while managed in an intensive care setting. Thrombolysis for the treatment of pediatric stroke remains controversial without supportive data, although children have achieved successful outcomes when administered intravenous tissue plasminogen activator for acute treatment of ischemic stroke^[36,37].

The first large-scale prospective outcome study on children with ischemic stroke or sinovenous thrombosis found 41% to have moderate or severe deficits on neurologic examination after a mean of 2.1 years^[38]. A recent cross-sectional outcome study of pediatric patients with ischemic stroke and cerebral sinovenous thrombosis a mean of 10.8 years after onset found that 37% were normal and 15% suffered severe deficits^[39]. The authors found a strong predictor of long-term outcomes to be functional status at 1 year post-stroke.

Few data are available regarding the long-term effects of pediatric stroke secondary to DKA, and the cases available are largely dependent on the anatomic site affected. Foster *et al*^[24] reviewed the outcomes of 28 case reports of arterial ischemic stroke, cerebral venous thrombotic stroke, and hemorrhagic stroke associated with DKA in youth and noted full recovery in only 14%. The majority of patients were left with varying degrees of residual neurologic deficit and 29% of cases resulted in death or persistent vegetative state. These grim outcomes highlight the need for large, randomized clinical trials of pediatric stroke during DKA treatment in order to help achieve the most positive outcomes.

RHABDOMYOLYSIS

Rhabdomyolysis is the breakdown of skeletal muscle leading to leakage of cell contents and resulting in muscle

pain, weakness, and potential acute renal injury^[40,41]. Biochemical changes include elevated creatinine kinase and myoglobinuria. The most common causes of rhabdomyolysis in children are viral myositis, trauma, medications, and underlying metabolic diseases. While rhabdomyolysis is more frequently described in patients with hyperosmolar hyperglycemic syndrome (HHS), it is also a well-documented phenomenon in DKA^[42]. Rhabdomyolysis in the setting of diabetes is often subclinical, with risk factors being low pH and high serum glucose, BUN, creatinine, sodium, and osmolality^[42-45].

The mechanism by which rhabdomyolysis occurs is unclear, although is thought to be secondary to the changes in electrolyte and glucose concentration across the muscle cell combined with the presence of insulin^[42,46,47]. These changes may lead to increased intracellular calcium which, in turn, can activate proteases and lead to muscle cell leakage.

The incidence of rhabdomyolysis in adults with DKA is approximately 10%^[47]. A study of children presenting with new onset T1DM found urine myoglobinuria in 10%^[44]. Several case reports detail rhabdomyolysis in pediatric DKA^[42,44,48,49]. These patients, who ranged in age from 15 mo to 12 years, all presented with a mixed HHS and DKA picture as they had acidosis, a blood glucose > 600 mg/dL, and hyperosmolality. They were also significantly dehydrated with elevated BUN or creatinine, consistent with the risk factors for developing rhabdomyolysis during DKA.

The presence of rhabdomyolysis in adults greatly increases mortality, likely secondary to decreased renal function^[43]. While there are no studies looking at the morbidity and mortality of rhabdomyolysis in children presenting in DKA, the incidence of acute renal failure in all children with rhabdomyolysis is 5%^[40]. Other serious complications include severe hyperkalemia and hypocalcemia, which can both lead to cardiac arrest^[50,51]. Fluid therapy and bicarbonate administration to alkalinize the urine are the gold standard treatment to prevent kidney injury.

PULMONARY COMPLICATIONS

Pneumomediastinum

Pneumomediastinum is a rare event that occurs secondary to alveolar rupture after a change in pressure gradients in the alveoli^[52]. These changes can occur secondary to mechanical ventilation, vomiting, coughing, and the valsalva maneuver^[52-54]. Patients with DKA are at increased risk of developing pneumomediastinum in the presence of emesis and Kussmaul breathing, which can generate alveolar pressures of 20-30 mmHg^[55-57]. There are over 50 documented cases of pneumomediastinum in the setting of DKA^[54,55,58] and analysis of the series found a male preponderance (71% male), an average age of 20 years old, and an average blood glucose of 638 mg/dL^[55]. All patients had significant acidosis with respiratory compensation, supporting hyperpnea as a mechanism for

the development of pneumomediastinum. Complications include pneumothorax as well as pneumopericardium, which can lead to cardiac tamponade.

Pneumomediastinum classically presents with chest pain and/or dyspnea. Patients often have a positive Hamman's sign, which is crepitus over the precordium that is synchronized with systole^[53,59,60], and subcutaneous emphysema may also develop. However, many patients are asymptomatic and pneumomediastinum is only found incidentally^[55,59]. Additional treatment is not usually required for cases of pneumomediastinum as the leaked air is often reabsorbed without incident^[53,56].

Pulmonary edema

Pulmonary edema is another rare complication of DKA found in both children and adults^[44,61,62]. While the edema can be subclinical, some children develop hypoxemia requiring supplemental oxygenation or intubation. To determine the incidence of pulmonary edema in the setting of DKA, Hoffman *et al*^[63] performed CT scans on children on presentation of DKA, 6-8 h into treatment, and on discharge. They found increased pulmonary density on presentation that worsened during treatment and self-resolved by discharge. While none developed hypoxemia, P02 values trended low during treatment in the majority of patients.

The edema is thought to be secondary to a decrease in capillary colloid osmotic pressure during intravenous fluid treatment with 0.45% normal saline^[61,64,65]. A concomitant fall in the hematocrit with fall in colloid pressure supports fluid administration rather than increased capillary permeability and leakage as the cause of edema. Hoffman *et al*^[63] also found a negative correlation between lung density and hematocrit, supporting this mechanism.

The development of pulmonary edema in the setting of DKA can be difficult to manage, as it often requires fluid restriction while DKA requires substantial fluid administration to correct total body water losses^[61]. Pulmonary edema in pediatric DKA is rare and general outcomes are not well described, although all of the children in case reports recovered without significant pulmonary sequelae^[44,61,62].

GASTROINTESTINAL COMPLICATIONS

Pancreatitis

Acute pancreatitis occurs in 2% of children and 11% of adults with DKA^[66,67]. It can be difficult to diagnose with concomitant DKA as abdominal pain is a common complaint and non-specific elevation of both lipase and amylase are noted with DKA. Nair *et al*^[66] conducted CT scans on 100 adult patients admitted with DKA and found 11 to have acute pancreatitis, as evidenced by pancreatic enlargement, necrosis, or fluid collections. Elevated serum amylase had a positive predictive value of 69%, elevated lipase 52%, and abdominal pain only 30%.

Haddad *et al*^[67] conducted a prospective study looking at pancreatic enzyme levels of children with new onset

Table 1 Incidence of complications of pediatric diabetic ketoacidosis

System	Dysfunction	Incidence
Vascular	Deep vein thrombosis	50% with central venous catheter placement ^[26,27]
Neurological	Cerebral edema	0.5%-1% ^[11-13]
	Cerebral venous thrombosis	Rare (2 known cases)
	Hemorrhagic or ischemic brain infarction	10% of intracerebral complications ^[32]
Musculoskeletal	Rhabdomyolysis	Unavailable; 10% of adults with DKA ^[43]
Respiratory	Pneumomediastinum	Unavailable; 50 documented cases over pediatric and adult populations ^[54,55,58]
	Pulmonary edema	Unavailable; described in study of 7 pediatric patients with DKA ^[63]
Gastrointestinal	Pancreatitis	2% ^[67]
	GI Bleed	No documented cases in children; 9% in adults with DKA ^[74]
Neurological	Memory dysfunction	Unavailable; described in study of 33 pediatric patients with remote history of DKA ^[79]

DKA: Diabetic ketoacidosis.

T1DM with and without DKA. Of those with DKA, 40% had elevated amylase and/or lipase levels and 40% had hypertriglyceridemia. Conversely, only 1 of 12 patients (8%) without DKA had mildly elevated lipase. Thirteen percent of patients with DKA had a lipase level that was elevated more than 3 times normal range and reported persistent abdominal pain after the DKA resolved, although their CT scans remained negative. Only one patient's symptoms recurred with increasing enzyme levels and her repeat imaging was positive for pancreatitis. This study demonstrates that non-specific enzyme elevation is common in children with DKA.

The etiology of non-specific elevation of lipase and amylase during DKA may be secondary to non-pancreatic sources of the enzymes, an insult to the pancreas itself causing enzyme leakage, and decreased renal clearance^[67-70]. In cases of acute pancreatitis during DKA, transient hypertriglyceridemia is postulated to be the primary etiology^[68,71] through both increased blood viscosity and increased levels of free fatty acids in the pancreas secondary to triglyceride lipolysis, ultimately leading to pancreatic ischemia and injury^[72,73]. The child who developed pancreatitis in Haddad's study did not have hypertriglyceridemia, however, leading the authors to propose that severe acidosis may play a role in the development of acute pancreatitis^[67].

Management of acute pancreatitis during DKA involves aggressive fluid administration, as pancreatitis can worsen intravascular dehydration^[66]. Care must be taken when resuming oral intake as it may exacerbate pancreatitis. The cases of pancreatitis described were mild and all resolved without complications^[67,68,72].

Upper gastrointestinal bleeding

There is a 9% incidence of upper gastrointestinal (GI) bleeding in adults with DKA^[74], although there are no documented reports in children. The most common manifestation is coffee ground emesis, with hematemesis or melena also noted. Faigel *et al* conducted a retrospective review of 25 patients who developed upper GI bleeding during DKA; all 8 who underwent endoscopy were found to have esophagitis. Additionally, 63% had esophageal

erosions or ulcerations and one (13%) had a Mallory-Weiss tear. Conversely, only 33% of patients with DKA without bleeding had evidence of esophagitis on endoscopy and only 11% had erosions. Acute esophageal necrosis, which is characterized by a black-appearance of the distal esophageal mucosa, is a rare cause of upper GI bleeding in DKA that was not found in Faigel's study^[75-77].

Use of ulcer medications, including proton pump inhibitors and H2 receptor antagonists, longer duration of diabetes, and diabetic complications including nephropathy, retinopathy and gastroparesis are clinical risk factors associated with upper GI hemorrhage^[74]. Laboratory values associated with an increased risk of hemorrhage include elevated BUN, creatinine, and glucose; arterial pH and coagulation tests did not differ between the two groups. Acute hyperglycemia in particular has been shown to delay gastric emptying^[78], which causes esophageal mucosal damage secondary to acid reflux and ultimately leads to a GI bleed^[74].

Only 32% of those with upper GI bleeding in Faigel's study underwent endoscopy. While 27% of patients with hemorrhage required blood transfusions, none required invasive therapy and GI bleeding did not directly result in mortality. However, those with GI bleeds had a mortality rate of 15% from other causes, compared to 4% in those without GI bleeds. This higher mortality rate is attributed to greater illness severity with greater likelihood of being admitted to the ICU.

COGNITIVE COMPLICATIONS

Even in the absence of symptoms suggesting cerebral injury, children with diabetic ketoacidosis can exhibit long-term cognitive complications. Ghetti *et al*^[79] assessed for memory deficits in 33 children with T1D who had suffered at least one episode of DKA and 29 children with T1D who had never experienced DKA. Interestingly, the children with DKA history had a significantly lower ability to recall events in association with specific details, as tested by event-color and event-spatial position associations. The average time since the last episode of DKA was 2.54 years, although varied from 0.11 to 14.54 years, and memory performance

was worse in children whose DKA was in the more distant past. This retrospective study also demonstrated that, aside from DKA, reduced memory performance was associated with male sex, young age at onset of diabetes, and severe hypoglycemia. The authors hypothesize that cerebral edema-related hypoxic/ischemic injury to the hippocampus is responsible for these specific, long-term cognitive deficits, as similar outcomes are observed in both clinical and animal studies of hypoxic injury^[80,81].

Animal models allow for a more controlled assessment of DKA-related cognitive dysfunction. Rats with streptozotocin-induced diabetes who are subjected to only one episode of DKA have longer mean latency times on maze testing after DKA recovery compared to rats with streptozotocin-induced diabetes without DKA^[82]. This measurable decrease in neurocognitive function raises concern for similar effects in people with DKA, although the underlying mechanism was not examined further *via* imaging or gross dissection. A recent prospective study of patients ages 6-18 years with and without DKA at diagnosis of T1D demonstrated cerebral white matter changes on MRI that, despite resolution over the first week, resulted in persistent alterations in attention and memory for up to 6 mo later^[83]. The greatest risk factors for these changes in cerebral structure were degree of acidosis and younger age at presentation, further highlighting the need for improved DKA prevention.

CONCLUSION

The most common cause of acute deterioration in children with DKA is cerebral edema, the pathogenesis of which remains under active investigation and discussion. Other rare complications of pediatric DKA include acute changes in coagulation, pulmonary function, musculoskeletal and gastrointestinal health as well as long-term cognitive outcomes (Table 1). These findings are rare and require a high index of clinical suspicion, but early recognition and treatment may help avoid permanent deficits. More data related to the presentation, treatment and outcomes of these complications in pediatric DKA patients is still needed, therefore, avoidance of DKA in children and adolescents through public and professional awareness is paramount to preventing these acute and chronic complications.

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Transcriptional factors, Mafs and their biological roles

Mariko Tsuchiya, Ryoichi Misaka, Kosaku Nitta, Ken Tsuchiya

Mariko Tsuchiya, Ryoichi Misaka, Institute of Geriatrics, Tokyo Women's Medical University, Tokyo 150-0002, Japan

Kosaku Nitta, Ken Tsuchiya, Department of Medicine IV, Tokyo Women's Medical University, Tokyo 162-8666, Japan

Author contributions: Tsuchiya M was the principle investigator; Misaka R and Nitta K revised the article; Tsuchiya K was organizing the maf relating project, analyzing data and drafting the article.

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Correspondence to: Ken Tsuchiya, MD, PhD, Clinical Professor, Department of Medicine IV, Tokyo Women's Medical University, 8-1, Kawada-cho, Shinjuku-ku, Tokyo 162-8666,

Japan. tsuchiya@kc.twmu.ac.jp

Telephone: +81-3-33538111

Fax: +81-3-33560293

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factors. The large Maf subgroup consists of four proteins, designated as MAFA, MAFB, c-MAF and neural retina-specific leucine zipper. In particular, MAFA is a distinct molecule that has been attracting the attention of researchers because it acts as a strong transactivator of insulin, suggesting that Maf transcription factors are likely to be involved in systemic energy homeostasis. In this review, we focused on the regulation of glucose/energy balance by Maf transcription factors in various organs.

Key words: Cell; Insulin; MAFA; Microarray; siRNA

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Core tip: This manuscript demonstrates that Maf transcription factors are likely to be involved in the regulation of hormonal systems related to glucose metabolism, with regulation by Maf transcription factors likely occurring near the start of the cascade or acting directly on the expression of genes in coordination with other factors in multiple organs and tissues. The Maf family plays diverse roles as transcription factors in the establishment of energy balance in peripheral organs, such as the pancreas, liver, and adipose tissue.

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Abstract

The Maf family of transcription factors is characterized by a typical bZip structure; these transcription factors act as important regulators of the development and differentiation of many organs and tissues, including the kidney. The Maf family consists of two subgroups that are characterized according to their structure: large Maf transcription factors and small Maf transcription

INTRODUCTION

Maf is a family of oncogenes that were first discovered in the genome of the avian transforming retrovirus, AS42^[1,2]. Maf-related proteins have been identified in many species and exhibit a universally recognized DNA binding site, enabling the proteins to act as transcription

factors. These Maf transcription factors are well known to play active roles in many organs, tissues, and cells for the development, differentiation and establishment of specific functions, including effects in the pancreas^[3], lens^[4], myeloma cells^[5], and cartilage^[6,7].

The Maf family has two distinct subgroups that are categorized according to their molecular size: small Maf transcription factors (150-160 amino acids: MAFF, MAFK, and MAFG), and large Maf transcription factors (240-340 amino acids: MAFA, MAFB, c-MAF, and NRL). Small Maf transcription factors lack a transactivation domain, and these protein products form homodimers or heterodimers within the Maf family or with other transcription factors, inducing transactivation factors^[2,8]. A complex regulatory network is known to link small Maf transcription factors with other regulatory proteins^[9,10]. On the other hand, large Maf proteins consist of a family of transcription factors characterized by a typical bZip structure, which is a motif for protein dimerization and DNA binding^[11,12]. Several reports have revealed that Maf proteins are involved in the essential functions of developing, differentiating and establishing the function of cells, tissues and organs. These transcription factors reportedly regulate several distinct developmental processes, cell differentiation, and the establishment of cell functions; for example, the mouse *Mafb* gene is responsible for the segmentation of the hindbrain^[13], while *c-Maf* has been identified in the liver, renal tubules^[14], adipocytes, and muscle. In this review, we will mainly focus on large Maf transcription factors and their roles in the regulation of various organs, as well as their effects on energy balance.

MAF TRANSCRIPTION FACTORS AND PANCREATIC β CELLS

One of the large Maf transcription factors, transcription factor MAFA, is an interesting molecule among the Maf family members since it promotes the differentiation of pancreatic β cells^[15,16]. Several reports have also indicated that *Mafa* activates the insulin gene C1 element, contributing to β cell function and differentiation^[17,18]. The formation of β cells has been described in detail in several reports and has been summarized in reviews. Two types of large Maf transcription factors, MAFA and transcription factor MAFB, are known to coordinate with each other and with other transcription factors and related genes to induce the generation and differentiation of β cells^[3,19]. MAFB is known to function as a transcription factor in many tissues and organs and has been detected in the pancreas. MAFB was initially identified as a transactivator of β cells, acting on the glucagon gene G1 element. Further studies subsequently revealed that MAFB can be detected in both α and β cells during the early phase of development, followed by a reduction in expression and then a switch to mainly MAFA expression^[20-22]. An additional study has demonstrated that the loss of *Mafa* causes a decrease in insulin gene expression in glucotoxic

β cells^[23], while MAFA deficient mice could not activate insulin transcription, even though the insulin content of the β cells was not significantly diminished^[24]. Recently, Hang *et al.*^[25] described the collaboration of MAFA and MAFB in the development of pancreatic β cells in greater detail^[25,26]. As for the transcription factor c-MAF, which is known to play a role in hematopoietic cell differentiation, its expression has been confirmed in the pancreas and is thought to be involved in α cell differentiation and function^[27].

Previously, Maf transcriptional factors could be shown to stained in premature and mature pancreas tissue in our report^[28]. Cells that stained positive for Maf transcription factors were diffusely localized in premature pancreas tissue, with some cells exhibiting double staining. The staining pattern for each Maf protein was different: unlike, MAFA-positive cells, which exhibited a diffuse staining pattern, MAFB and c-MAF were stained prominently in the branching ducts and acinar buds. Subsequently, MAFA and MAFB were stained more intensely in the islet areas of adult pancreas tissue, suggesting that Maf transcription factors are involved in the differentiation and acquisition of pancreatic endocrine cells, coordinating with each other in some situations (Figure 1). In contrast, non-endocrine composite cells of the pancreas, such as acinar cells and ductal cells, may also be affected by several Maf transcriptional factors during their maturation and differentiation process. More interesting observation is that cells positive stained for Maf transcription factor continued to be detected not only in the islets but around the ductal and interstitial area after maturation.

ACTIVITIES OF MAF TRANSCRIPTION FACTORS BEYOND β CELLS

Despite the details that have been revealed regarding the activities of Maf transcription factors in β -cell function and differentiation, the precise mechanism and coordination of Mafs and other transcriptional factors regarding the regulation of insulin production and its activity remain unknown. The precursors of pancreatic endocrine cells and the mechanism of β cell replication in the islets have been reported^[29-31]. However, several types of Maf transcription factors are likely to be implicated in both the pancreatic endocrine cell lineage and interaction with other transcription factors. Each Maf transcriptional factor were often co-stained in one endocrine cell in immature pancreas.

The network of targeted genes and transcription factors, including several Maf transcription factors, needs to be clarified as part of efforts to accelerate β cell regeneration or preparation for cell therapy. Maf transcriptional factors are reportedly expressed in other tissues and cells, for example, epithelial cells and lymphocytes^[32,33], where they accelerate specific cell function and differentiation. Lumelsky *et al.*^[34] reported the development of embryonic stem cells into insulin-producing cells in the pancreas^[34], while Kawai *et al.*^[1] described the mechanism of β cell replication in islets.

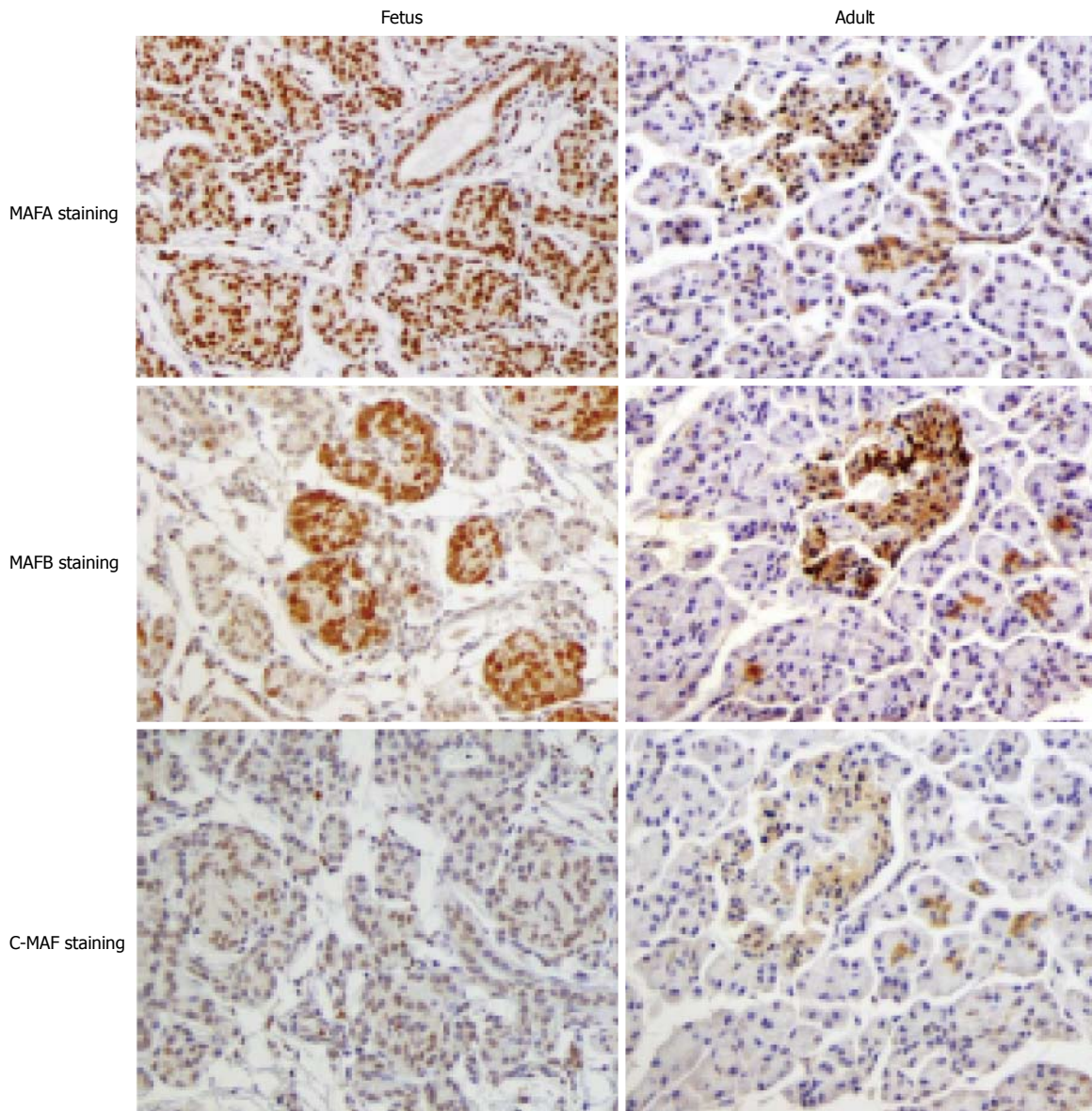


Figure 1 Immunostaining for MAFA, MAFB, and c-MAF in fetal and adult human pancreas tissues. An immunohistochemical analysis was performed using primary antibodies against MAFA (BL1069; Bethyl Laboratories, Inc.), MAFB (P20; Santa Cruz Biotechnology, Inc.), and c-MAF (M153; Santa Cruz Biotechnology, Inc.). The details are described in reference^[28]. Samples of human normal fetal tissue (female, 20 wk, catalog No. T2244188, Lot No. A607380) and adult pancreas tissue (male, 23 years, catalog No. T2234188, Lot No. A604382) were purchased from BioChain. The fetal pancreas tissues were diffusely stained for the Maf transcription factors, and characteristic histological differences were observed between the fetal and adult tissues, with a more intense staining pattern observed in the islet areas of the adult pancreas tissue.

In addition, several reports have described the existence of tissue-specific stem cells in the pancreas^[35,36]. Recently, several reports have discussed the more efficient production of β cells (glucose-sensitive and insulin-secreting cells) through the introduction of a combination of transcription factors, including Maf transcription factors, or the use of induced pluripotent stem cells^[37,38].

Large Maf transcription factors have been identified during the development of the pancreas, and the expressions of these large Maf transcription factors exhibited different localizations in newborn and adult pancreas tissues, which differ in their endocrine characteristics. Thus, Maf transcription factors may contribute to establish all the cells in pancreatic tissue, including cells involved in endocrine

cell differentiation, such as α and β cells, exocrine cells, and ductal cells.

MAF TRANSCRIPTION FACTORS AND THE KIDNEY

In the kidney, large Maf transcription factors may be implicated in both normal development and pathophysiological processes responsible for kidney disease. We previously reported the expression profiles of large Maf transcription factors in the kidney. We have reported the expression of *c-Maf* mRNA levels in mouse kidney tissue from embryonic day 12 (E12) until 1 or 4 wk after birth.

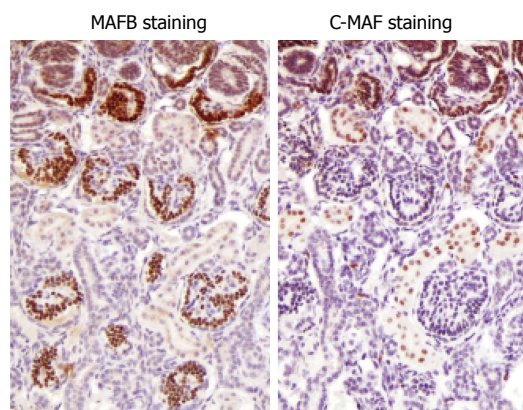


Figure 2 Immunostaining for MAFB and c-MAF in fetal human kidney tissue. An immunohistochemical analysis was performed using primary antibodies against MAFA (BL1069; Bethyl Laboratories, Inc.), MAFB (P20; Santa Cruz Biotechnology, Inc.), and MAF c-Maf (M153; Santa Cruz Biotechnology, Inc.). The details are described in reference^[40]. A sample of human normal fetal kidney tissue (male, 25 wk) was purchased from BioChain (catalog No. T8244431, Lot No. A606275). Glomerular podocyte lesions stained positive for MAFB, and while the proximal tubules stained positive for c-MAF.

c-Maf mRNA was firstly expressed at E16 in the proximal tubules and continued to be expressed until 4 wk after birth. Meanwhile, MAFB expression has been identified in the glomeruli (Figure 2)^[39,40].

The *Mafb* mouse gene (also known as Kreisler and *Krmf*) is known for its role in hindbrain patterning. Sadl *et al.*^[41] showed that mice homozygous for the *kr* (*enu*) mutation develop renal disease, in which the glomerular podocytes are affected, resulting in nephrotic syndrome. The fusion and effacement of the podocyte foot processes were observed histologically, and MAFB was shown to be essential for the cellular differentiation of the podocytes. Since the podocytes of the *kr* (*enu*) homozygotes differentiated abnormally, the homozygotes exhibited proteinuria, as is observed in nephrotic syndrome. The authors speculated that MAFB acted during the final stages of glomerular development, *i.e.*, the transition between the capillary loop and the mature stages, and downstream of the Pod1 basic domain helix-loop-helix transcription factor^[41]. In *Mafb*-knockout mice, renal dysgenesis with abnormal podocyte differentiation and tubular apoptosis were prominent, accompanied by the suppression of F4/80 expression in mature macrophages^[42].

A prominent phenotypic feature of *c-Maf*-knockout mice is a small cell volume of the kidney proximal tubules and hepatocytes^[40]. The precise mechanism underlying this dysregulation of cell structure formation has not been clarified, but the *c-Maf* transcriptional factor has been suggested to contribute to the embryonic cell development and differentiation of at least the proximal tubules and hepatocytes. The mRNA expression profile in kidney tissue from *Maf*-knockout mice, as evaluated using a DNA microarray, showed that the plasma level of glutathione peroxidase 3 (GPx-3) was predominantly downregulated. Since GPx-3 is an antioxidant enzyme,

C-MAF may be related to the antioxidant system mediating the modulation of GPx-3 in the kidney^[14]. Recently, c-Maf-inducing protein (also known as c-Mip; protein designation, CMIP), a pleckstrin homology (PH) and leucine-rich repeat (LRR)-domain-containing protein, has been identified; CMIP inactivates GSKbeta and interacts with RelA, a key member of the NF-kappaB family. Interestingly, the expression of CMIP (c-Maf-inducing protein) was increased in the podocytes of patients with idiopathic nephrotic syndromes^[43]. Membrane nephropathy is characterized by nephrotic-range proteinuria in clinical and subepithelial deposits of immune complex in the basement membrane of the glomerulus. The primary cause of the disease has not been clarified, but antibodies against podocytes located on the outer layer of the basement membrane of the glomeruli form complexes that lead to deposits. A recent report has shown that CMIP was overexpressed in podocytes in an experimental glomerulonephritis rat model exhibiting heavy proteinuria and membranous nephropathy in human. This overexpression was suppressed by immunological treatment resulting in a reduction of proteinuria; thus, while the role and significance of CMIP in podocytes and how it induces massive proteinuria have not yet been elucidated, CMIP or c-MAF-related transcriptional activities may deregulate podocyte function and cause proteinuria^[44].

The expression of MAFA in the kidney is uncertain; however, based on the UniGene databank, human MAFA is also expressed in the kidney, lung, and blood. One interesting report described transgenic mice with a disease in which the hybridized gene complex resulted in MAFA deficiency and MAFK overproduction in pancreatic β cells. The phenotype of these transgenic mice was severe diabetes with large amount of proteinuria. A histological examination showed a reduction in β cells in the pancreas and typical histological diabetic nephropathy accompanied by a characteristic nodular lesion in the glomeruli.

The combination of several Maf dysfunctions generate diabetes with diabetic nephropathy, suggesting one possible mechanism for the onset of diabetic nephropathy. Consequently, these mouse models mice may suggest a mechanism for disease onset and could be useful in investigations of treatments for diabetic nephropathy^[45].

MAF TRANSCRIPTION FACTORS AND THE CENTRAL NERVOUS SYSTEM

Energy balance in humans is well regulated by multiple organized systems, and the central nervous system (CNS) is likely to be involved and to play important roles in these systems^[46]. The CNS contributes to the maintenance of energy balance in part by controlling feeding behavior and also by changing biological conditions and the homeostasis of intra-body conditions. Systemic adjustments of the metabolic state are achieved by the coordination of

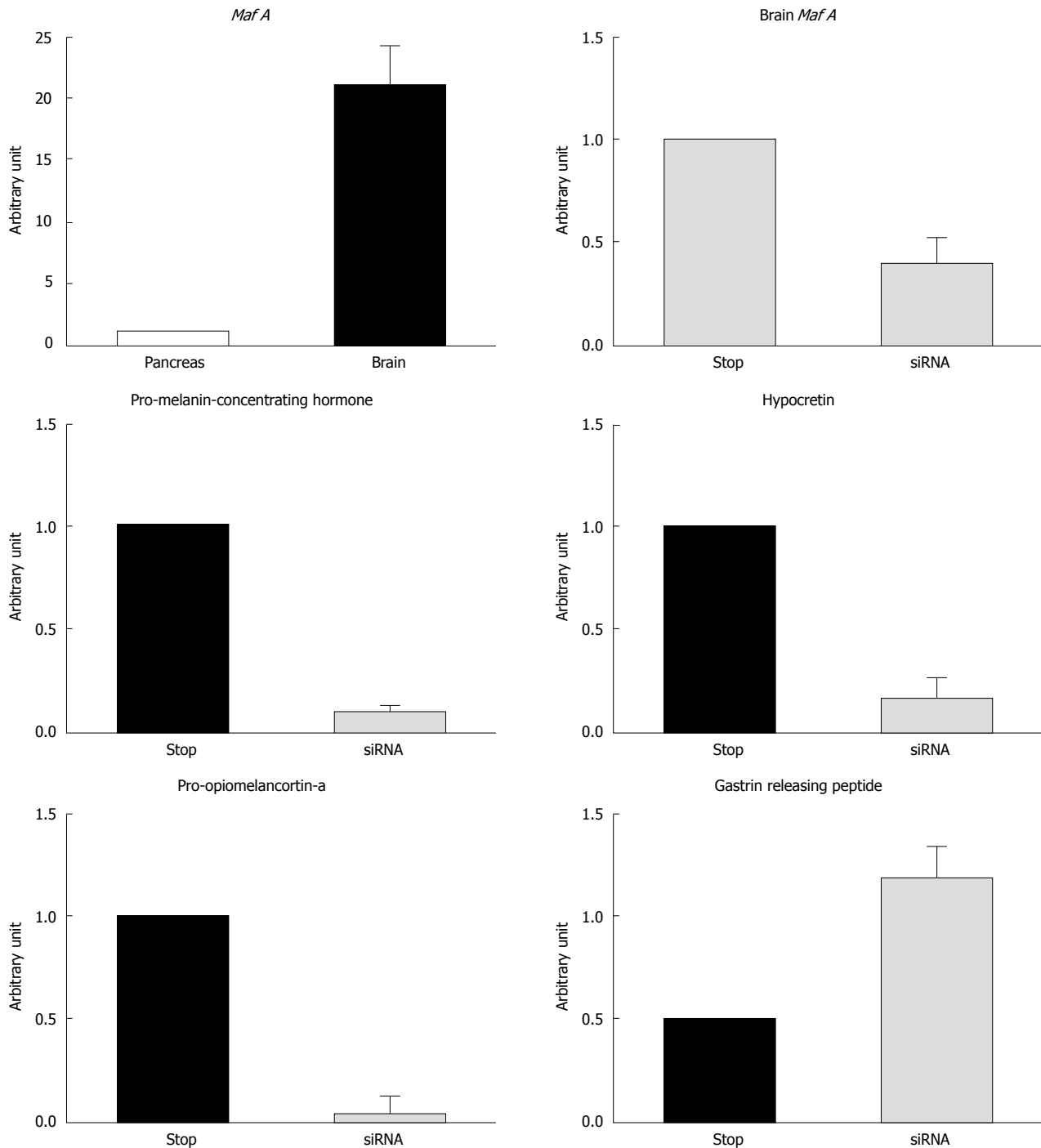


Figure 3 Suppression of *MafA* mRNA by siRNA in the brain and resulting alternation of related genes. A designed small interfering RNA (siRNA) oligomer for mouse *Mafa* was intravenously injected using the hydrodynamic method according to a procedure described by Hamar *et al.*^[58]. A DNA microarray analysis was then performed using Affymetrix GeneChip technology. The mRNA levels were quantified using real-time PCR. The details of the experiment have been described previously. Expression level of *Mafa* mRNA in the brain. The expression level of *Mafa* mRNA in the brain was 20 times higher than that of *Mafa* mRNA in the pancreas, as assessed using real-time PCR. Suppression of *Mafa* in mice using siRNA in the brain. The mRNA expression level taken out in the brain tissue are shown. The *Mafa* mRNA expression level was significantly downregulated by the siRNA. Pro-melanin-concentrating hormone, Hypocretin, and Pro-opiomelanocortin-a were downregulated, and Gastrin-releasing peptide was upregulated, as assessed using real-time PCR with specific primers.

the CNS and peripheral effector organs. In the CNS, transcription factors are involved in the regulation of behavior and intra-body biological conditions^[47]. Calorie intake is sensed in the CNS, altering the expression of signal transduction-mediating transcription factors. These responses are then translated into intra-CNS hormones

(resulting in changes in eating behavior) and peripheral hormones taken out including insulin and leptin, which function to regulate energy balance by direct effects on peripheral organs in coordination with calorie intake and consumption balance.

In our previous mouse study, the *Mafa* mRNA level

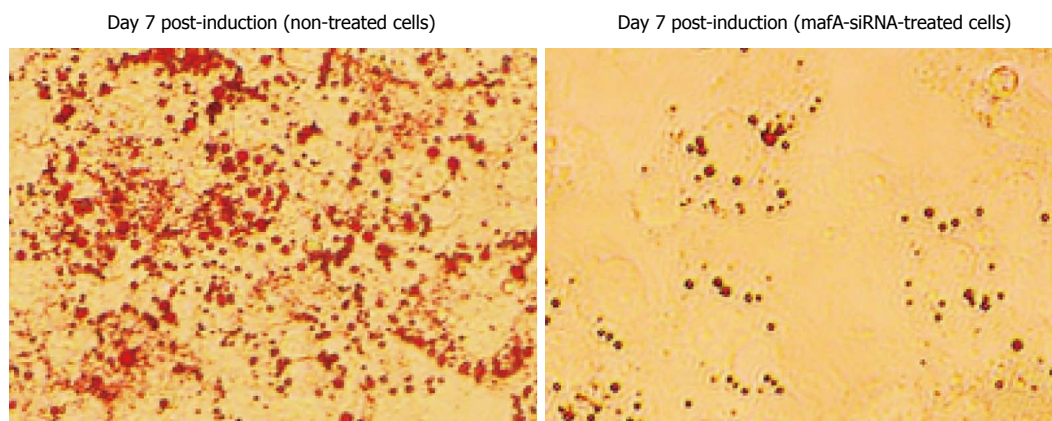


Figure 4 Comparison between histological changes and the Oil-Red-O staining of stop-*mafA*-siRNA- and *mafA*-siRNA-treated cells. Mouse 3T3-L1 pre-adipocytes were induced to differentiate, and *Mafa* siRNA was transfected using a transfection reagent. The morphological appearances of the pre-adipocyte culture before induction and 7 d after induction were then compared. The morphology of the 3T3-L1 cells was directly observed, and lipid droplets were stained using Oil Red O. Oil Red O staining was compared between untreated and *Mafa*-siRNA-treated cells. Intracellular lipid staining was not observed in the *Mafa*-siRNA-treated cells.

was significantly downregulated in brain tissue *in vivo*, as observed using a siRNA technique, and changes in the gene profile in the CNS were screened^[48] (Figure 3). The results showed distinct effects on gene expressions in brain tissue, and some of the affected genes were related to eating behavior and energy consumption, such as growth hormone and arginine vasopressin. In addition, several interesting genes and related gene products were identified in this profiling. Pro-melanin-concentrating hormone (Pro-MCH) regulates body weight^[49], and changes in its expression can alter the susceptibility to fat metabolism^[50,51]. Orexin is a topical neuronal peptide that regulates arousal and sleep^[52], and defective orexin producing neuronal cells cause narcolepsy. Orexin may work in the brain-gut network, which regulates appetite during wakefulness^[53]. In addition, pro-opiomelanocortin- α (an α -melanocyte stimulating hormone) and gastrin-releasing peptide, which are important neuropeptides regulating eating behavior and modifying the excretion of several hormones required for food digestion, have also been implicated in the above-mentioned network.

Thus, MAFA is a strong transactivator of insulin in peripheral organs and pancreas, while the modulation of *MAFA* mRNA expression in the CNS induces change in related genes resulting in upregulation and downregulation of neuropeptides that influence appetite, behavior, arousal, and sleep.

MAF TRANSCRIPTION FACTORS AND ADIPOCYTES

Adipocytes develop from mesenchymal stem cells in adipose tissue and various other tissues. Mesenchymal stem cells destined to become adipocytes develop and differentiate into mature adipocytes as a result of transcriptional regulation. PPAR γ is known to coordinate with members of the C/EBP family to exert well-documented and important functions at different time points during adipocyte differentiation^[54]. Siersbæk *et al*^[55]

also reported transcriptional networks for adipogenesis take out in which two waves of transcriptional cascades composed the adipogenetic pathway. Maf transcription factors are likely to be involved in this process, and Serria *et al*^[33] reported that the expression of c-MAF is downregulated during 3T3-L1 cell differentiation and proliferation. Furthermore, an age-related decrease in the expression of c-MAF in mesenchymal cells has been reported, and present evidence indicates that c-MAF regulates mesenchymal cell bifurcation into osteoblasts and adipocytes. A role of c-MAF in osteogenesis and adipogenesis was also observed in *c-Maf*-knockout mice^[56].

As discussed previously, MAFA may be involved in the differentiation of both adipocytes and pancreatic β cells. To explore the role of MAFA in adipose tissue, alterations in the expressions of MAFA-related genes in a cultured adipocyte cell line, 3T3-L1, were observed after *Mafa* mRNA interference had been induced^[57]. *Mafa* mRNA suppression induced morphological changes in 3T3-L1 cells during differentiation. As shown in Figure 4, the cytoplasm of spindle-shape cells expanded after differentiation and lipid droplets formed in mature adipocytes, as revealed by the presence of red droplets of Oil Red O stain in the cytoplasm. This morphological change was not observed during *Mafa* siRNA suppression, and no expansion of the cytoplasm was observed. Since lipid droplet formation is essential for adipocyte differentiation, MAFA may play a critical role in the process of adipocyte differentiation. The expression levels of peroxisome proliferator-activated receptor (PPAR γ 2) and CCAAT/enhancer-binding protein (C/EBP α) were recognized as being essential for the differentiation and function of 3T3-L1 cells. PPAR γ 2 plays a leading role in the synthesis and accumulation of lipid droplets in adipocytes, and C/EBP α is critical for the establishment of insulin sensitivity^[54]. At the molecular level, the mRNA expression levels of the *PPAR γ* gene or the *C/EBP* gene, which encode master adipogenic transcription factors, were markedly suppressed by *Mafa*-siRNA treatment, *i.e.*, by the suppression of MAFA expression. In conclusion,

adipocyte differentiation and formation is regulated by a network of multiple transcription factors, and Maf transcription factors are likely to be involved, in coordination with other transcription factors.

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Coronary atherosclerosis is already ongoing in pre-diabetic status: Insight from intravascular imaging modalities

Osamu Kurihara, Masamichi Takano, Yoshihiko Seino, Wataru Shimizu, Kyoichi Mizuno

Osamu Kurihara, Masamichi Takano, Yoshihiko Seino, Cardiovascular Center, Chiba-Hokusoh Hospital, Nippon Medical School, Chiba 270-1694, Japan

Wataru Shimizu, Division of Cardiology, Nippon Medical School, Tokyo 173-8605, Japan

Kyoichi Mizuno, Mitsukoshi Health and Welfare Foundation, Tokyo 173-8605, Japan

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Correspondence to: Masamichi Takano, MD, Cardiovascular Center, Chiba-Hokusoh Hospital, Nippon Medical School, 1715 Kamakari, Inzai, Chiba 270-1694, Japan. takanom@nms.ac.jp

Telephone: +81-476-991111

Fax: +81-476-991908

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for primary prevention of cardiovascular events. This guideline recommends aggressive lipid-lowering statin therapy for primary prevention in diabetes and other patients. The ultimate goal of patient management is to inhibit progression of systemic atherosclerosis and prevent fatal cardiovascular events such as acute coronary syndrome (ACS). Because disruption of atherosclerotic coronary plaques is a trigger of ACS, the high-risk atheroma is called a vulnerable plaque. Several types of novel diagnostic imaging technologies have been developed for identifying the characteristics of coronary atherosclerosis before the onset of ACS, especially vulnerable plaques. According to coronary angioscopic evaluation, atherosclerosis severity and plaque vulnerability were more advanced in prediabetic than in nondiabetic patients and comparable to that in diabetic patients. In addition, pharmacological intervention by statin therapy changed plaque color and complexity, and the dynamic changes in plaque features are considered plaque stabilization. In this article, we review the findings of atherosclerosis in prediabetes, detected by intravascular imaging modalities, and the therapeutic implications.

Key words: Diabetes; Prediabetes; Statin therapy; Coronary artery disease; Intravascular imaging modality

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Abstract

Diabetes mellitus is a powerful risk factor of coronary artery disease (CAD), leading to death and disability. In recent years, given the accumulating evidence that prediabetes is also related to increasing risk of CAD including cardiovascular events, a new guideline has been proposed for the treatment of blood cholesterol

Core tip: Coronary artery disease is the principal cause of death and disability in not only diabetes but also prediabetes patients. Aggressive statin therapy is an established method of primary prevention of cardiovascular disease events in diabetic patients. According to the findings of coronary imaging modalities detecting atherosclerotic lesions, statin therapy in prediabetes may be beneficial for reducing atherosclerotic cardiovascular risk.

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INTRODUCTION

Diabetes is categorized as a metabolic disease characterized by hyperglycemia arising from abnormal insulin secretion from the pancreas and/or lack (or absence) of insulin action. Diabetes causes damage, dysfunction, or failure of various organs involving heart and blood vessels^[1]. It is well known that diabetes promotes atherosclerotic disease of systemic and coronary arteries and increases the mortality rate from cardiovascular disease^[2,3]. However, myocardial ischemia owing to coronary artery disease (CAD) is occasionally absent from the typical symptoms in patients with diabetes^[4]. As a result, severe multivessel disease of the coronary arteries can manifest as silent myocardial ischemia before treatment is begun. A delayed recognition of CAD can worsen the prognosis in many diabetic patients^[5]. Moreover, a recent study showed that impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are also causes of adverse cardiovascular events^[6,7].

A goal of CAD management is to prevent cardiovascular diseases such as acute coronary syndrome (ACS). The principal pathogenesis of ACS is disruption of atheromatous coronary plaques and subsequent flow-limiting thrombus formation^[8,9]. Plaque disruption, the trigger of this serious event, is pathologically classified as a rupture, and shallower intimal injury is termed erosion. Additionally, previous pathological studies showed that the majority of disrupted plaques have a large lipid core under a thin fibrous cap, hence the term thin-cap fibroatheroma (TCFA). Not only plaque rupture but also superficial calcified nodules are possible origins of ACS^[10-12]. A vulnerable plaque is defined as a future high-risk plaque provoking ACS, and TCFA is representative of a vulnerable plaque. In recent years, novel intracoronary imaging modalities have been developed for detecting such vulnerable plaques.

Coronary angiography remains the gold standard for diagnosis of CAD and the following catheter intervention therapy. However, an angiogram represents a 2-dimensional silhouette of the coronary artery, and the angiogram does not supply certain information about the vessel wall components or the atherosclerotic plaque. Therefore, a coronary angiogram is not capable of detecting vulnerable plaques, including TCFA. Thus, supplemental CAD diagnostic modalities, including various intravascular imaging devices such as intravascular ultrasound (IVUS), coronary angioscopy (CAS), and optical coherence tomography (OCT), have been developed to discriminate each component of the plaque and to identify the presence of vulnerable plaques.

INTRACORONARY INVASIVE IMAGING MODALITIES

IVUS

IVUS is an intravascular imaging modality that supplies cross-sectional images of the coronary artery including the lumen and vessel wall. High-frequency (20-40 MHz) IVUS visualizes 3 layers of the vessel wall: the intima, media, and adventitia. IVUS allows *in vivo* qualitative measurements of the lumen and plaque area (and volume). Conventional grayscale IVUS images have major limitation of precise tissue characterization except calcification. Although a large plaque burden and microcalcifications, factors of plaque vulnerability, are detected by grayscale IVUS, this imaging system is not able to identify TCFA^[13]. Because of these limitations, 3 modalities using radiofrequency analysis, virtual histology IVUS (VH-IVUS; Volcano Therapeutics, Rancho Cordova, CA, United States)^[14], iMAP-IVUS (Boston Scientific, Santa Clara, CA, United States)^[15], and integrated backscatter IVUS (IB-IVUS; YD Co., Nara, Japan)^[16] are now available in clinical settings.

VH-IVUS takes into account detailed qualitative and quantitative assessment of the vessel wall components. The axial resolution of VH-IVUS is just about 150-250 μm . *Ex vivo* studies have shown that power spectrum-related parameters from raw backscattered ultrasound signals permit discrimination of plaque components^[17]. These parameters are used in a classification scheme to yield a tissue color map for each plaque characteristic as follows: dark green indicates fibrous, yellow-green indicates fibrofatty, red indicates necrotic core, and white indicates dense calcium. VH-derived TCFA was defined as at least 3 consecutive frames with a plaque burden of at least 40% and without overlying fibrous tissue^[18]. A recent prospective study using VH-IVUS, the PROSPER trial, has shown that the VH-derived TCFA with a minimal luminal area $\leq 4 \text{ mm}^2$ and a plaque burden $\geq 70\%$ was the highest-risk plaque type leading to adverse cardiovascular events^[19].

CAS

CAS using optic fibers is a technology that permits direct visualization of the lumen surface of the coronary artery and provides detailed information about plaque morphology and the presence of a thrombus with high resolution (50 μm). CAS clearly identifies irregularities of the lumen surface, such as ulceration, fissures, and tears. Disrupted plaques are involved in these plaques with irregularities (or complexity). In addition, CAS is an extremely sensitive detector of a thrombus. Angiographic stenosis of the lesion progresses despite healing of the silent plaque disruption in the nonculprit lesions^[20].

Based on angioscopic analysis, an atherosclerotic plaque is defined as a nonmobile, protruding structure that can be clearly delimited from the adjacent vessel wall. Although a normal vessel wall appears glistening white, plaques can be yellow or white according to the surface color. The color of the plaque is classified semiquantitatively: (1) grade 0 is white; (2) grade 1 is

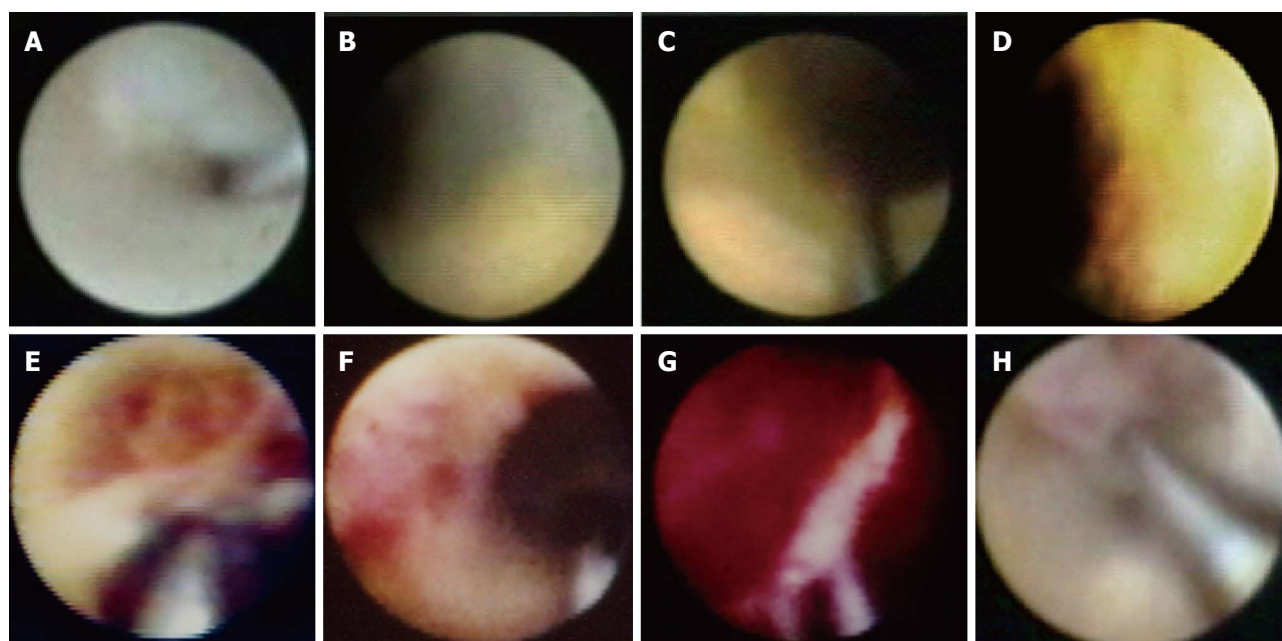


Figure 1 Classification of coronary angioscopic images. A: White plaque (yellow grade 0); B: Light yellow plaque (grade 1); C: Yellow plaque (grade 2); D: Intense yellow plaque (grade 3); E: Plaque rupture; F: Plaque erosion; G: Red thrombus; H: White thrombus.

light yellow; (3) grade 2 is medium yellow; and (4) grade 3 is intense yellow. The majority of yellow plaques contain lipid-rich tissue or a necrotic core according to comparative validation using OCT and IVUS. The grade of yellow plaques is affected by the thickness of the fibrous cap covering lipidic tissue. A high-intensity yellow plaque is identical to a TCFA. On the contrary, white plaques contain fibrous tissue or a thick fibrous cap covering the lipidic plaque^[21-24]. Yellow plaques are detected not only in the culprit lesion but also in the nonculprit lesions of ACS^[25-29]. Representative CAS images of plaques and thrombi are shown in Figure 1.

Prospective studies demonstrated that the incidence of ACS is higher in patients with intense yellow or multiple yellow plaques than in patients without yellow plaques^[30,31]. These findings indicate that intense yellow or multiple yellow plaques detected by CAS might be vulnerable and could cause future coronary events.

OCT

OCT imaging employs a near-infrared range light source, at approximately 1300 nm. OCT has a 10-fold higher image resolution (10-15 μm) than IVUS, and its image quality is superior to that of other imaging devices. In addition, OCT provides accurate tissue characterization of the plaque. Normal vessel walls appear as a 3-layer structure on OCT images as well as IVUS. The vascular media is seen as a dark band delineated by the internal elastic lamina and external elastic lamina. Fibrous plaques consist of homogeneous and low-attenuation areas. Lipid-rich plaques exhibit a high-attenuation mass with a diffuse border. A calcified plaque is presented as high-attenuation mass with a clear border^[32-36].

OCT is the only intravascular imaging technology

with high spatial resolution that can measure the fibrous cap thickness^[37,38].

CORONARY ATHEROSCLEROSIS INDUCED BY GLUCOSE METABOLISM DISORDER

Diabetes-associated coronary atherosclerosis as determined by imaging modalities

In diabetic patients, coronary angiograms characteristically reveal diffuse long lesions in multiple small vessels^[39,40]. In an IVUS study, plaques in diabetic patients were characterized by an increased amount of dense calcium, a necrotic core, and a high frequency of VH-TCFA^[41]. In addition, IVUS studies showed that the levels of hemoglobin A1c (HbA1c) was associated with atheroma volume and the severity of coronary atherosclerosis^[42,43]. CAS showed that diabetes was an independent predictor of plaque disruption in the nonculprit vessel^[44]. In an OCT study, patients with diabetes had large lipid plaque volumes and a high prevalence of calcified plaque and thrombus^[45].

Possible mechanism of hyperglycemia-induced coronary atherosclerosis

Free fatty acids and insulin resistance, which are elevated by hyperglycemia, stimulate molecular mechanisms and alter the function and structure of blood vessels, including increased oxidative stress and activation of protein kinase C and the receptor for advanced glycation end products. Consequently, hyperglycemia decreases the availability of nitric oxide, increases the production of endothelin, and activates transcription factors such as

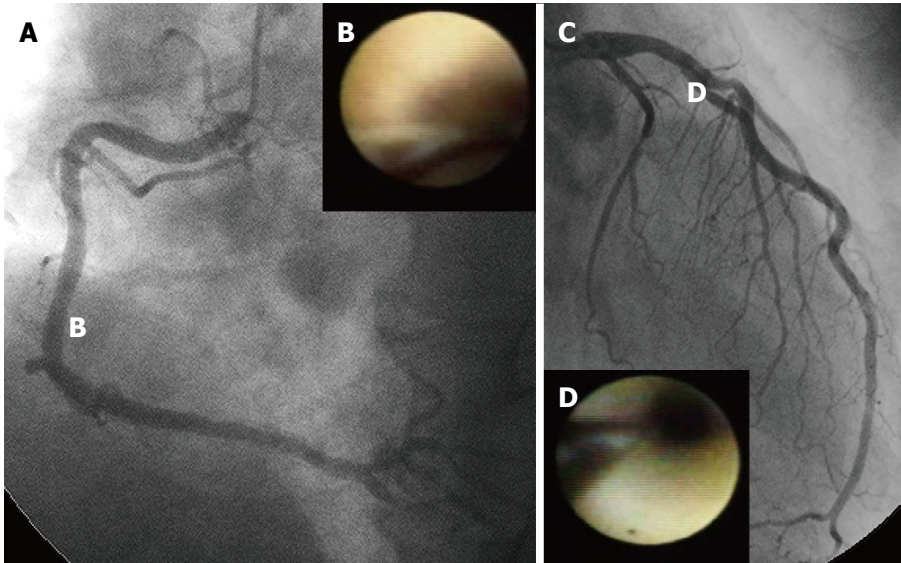


Figure 2 Representative images of nondiabetic patients. A and B: No angiographic stenosis was observed in the right coronary artery (A), whereas 1 yellow plaque was identified on angioscopy (B). The yellow intensity of these plaques was defined as grade 2; C: The left circumflex artery was too small to observe by coronary angioscopy, and a 75% stenosis was identified on angiography in the middle part of the left ascending artery; D: According to angioscopic findings, this lesion was evaluated as a grade 1 yellow plaque. In this case, the average number of yellow plaques per vessel was 1 (2 yellow plaques in 2 vessels), and the maximum yellow grade per coronary artery was 2.

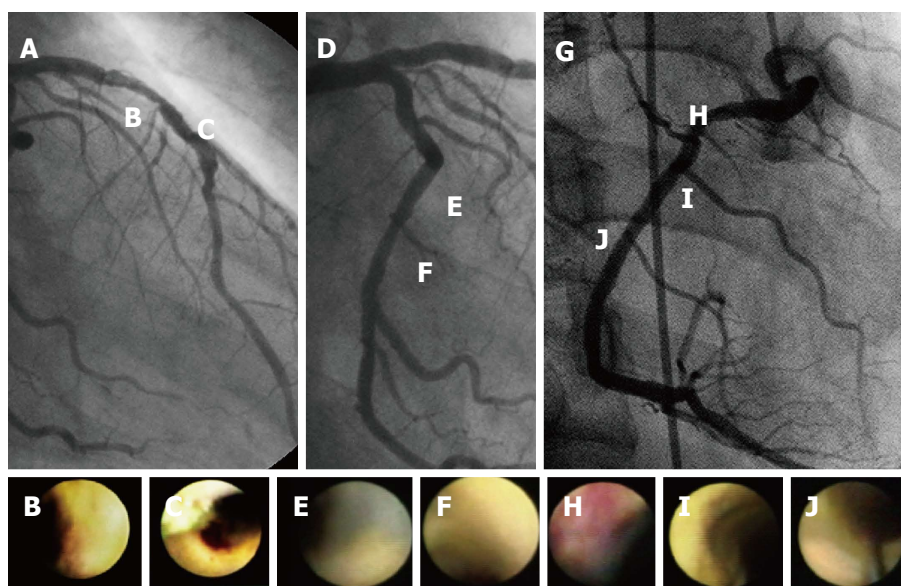


Figure 3 Representative images of prediabetes patients. A: A 50% stenosis and a 90% stenosis were identified on angiography in the middle part of the left ascending artery; B and C: According to angioscopic findings, both of these lesions were evaluated as grade 3 yellow plaques; D-F: No angiographic stenosis was observed in the left circumflex artery (D), whereas 2 yellow plaques were defined as grades 1 and 2, respectively (E and F); G-J: Significant stenosis was not observed in the right coronary artery (G), whereas an intramural red thrombus was observed at the proximal site (H), and 3 yellow plaques were identified on angioscopy (H-J). The yellow intensity of these plaques was defined as grades 1, 2, and 1, respectively. In this case, the average number of yellow plaques per vessel was 2.33 (7 yellow plaques in 3 vessels), and the maximum yellow grade per coronary artery was 3.

nuclear factor- κ B and activator protein-1. These factors bring about systemic vasoconstriction and inflammation and promote systemic atherosclerosis^[46-48]. A similar glucose metabolism disorder occurs in the prediabetic state^[49,50].

The American Diabetes Association defines prediabetes as IFG, IGT, and HbA1c values ranging 5.7%-6.4%^[1]. The patients with IFG and IGT should be informed

of their increased risk for diabetes as well as CAD. The HbA1c value is more commonly used to diagnose diabetes, and an HbA1c level of 5.7%-6.4% also indicates a relatively high risk for future diabetes and CAD^[1].

The low concordance in the relationships between IFG, IGT, and HbA1c, as well as the diagnoses of prediabetes using these parameters, accentuates the various dysfunctions of glucose metabolism. A dys-

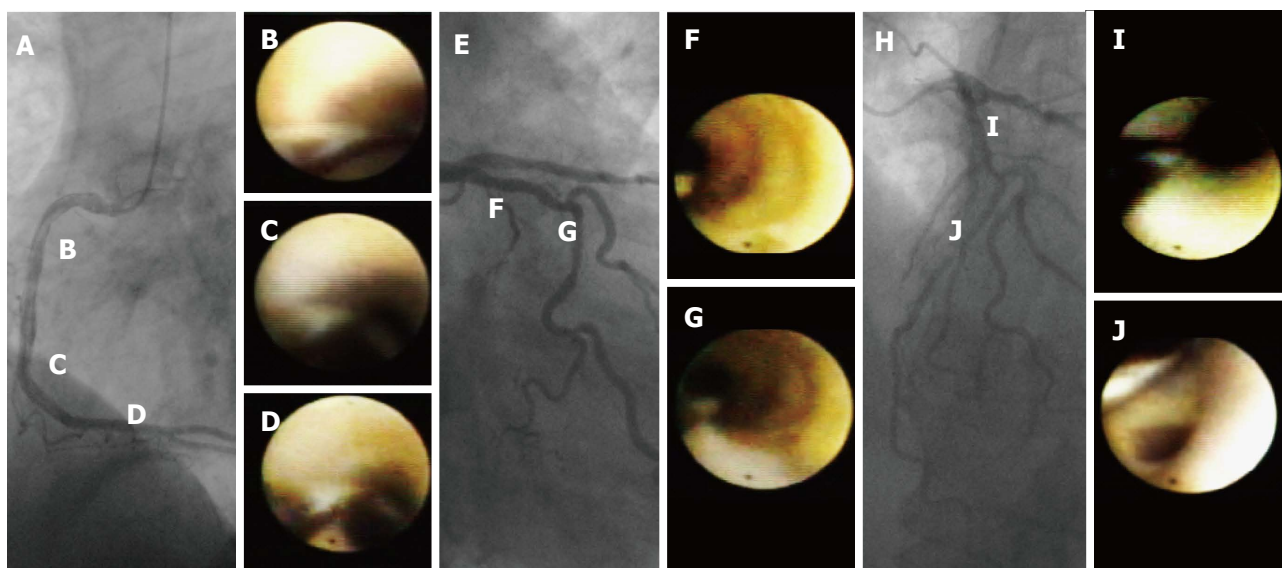


Figure 4 Representative images of diabetes patients. A-D: No angiographic stenosis was observed in the right coronary artery (A), whereas 3 yellow plaques were identified on angioscopy (B-D). The yellow intensity of these plaques was defined as grades 2, 1, and 2, respectively; E-G: Significant stenosis was not observed in the left circumflex artery (E), whereas 2 yellow plaques were identified on angioscopy (F and G). The yellow intensity of both of these plaques was evaluated as grade 3 (intense yellow); H: A 50% stenosis and a 90% stenosis were identified on angiography in the middle part of the left ascending artery; I and J: According to angioscopic findings, both these lesions were evaluated as light yellow plaques (grade 1). In this case, the average number of yellow plaques per vessel was 2.33 (7 yellow plaques in 3 vessels), and the maximum yellow grade per coronary artery was 3.

function in hepatic insulin resistance manifests as IFG, whereas muscle insulin resistance represents IGT^[51]. Although data for IFG and IGT are provided by the daily glucose snapshot, HbA1c levels after chronic exposure (over 60-90 d) to basal and postprandial hyperglycemia reflects a combination of IFG and IGT^[52].

Prediabetes-associated coronary atherosclerosis as determined by imaging modalities

An angiographic study revealed that atherosclerosis of coronary arteries developed not only in patients with diabetes but also in those with IGT^[53]. An IVUS study showed that even patients with a prediabetic status detected by IGT and IFG exhibited abundant lipid-rich plaques in their coronary arteries^[54]. Recently, we used CAS to identify yellow vulnerable plaques in the coronary arteries of patients with prediabetes and diabetes compared with controls. Representative images of patients without diabetes and with prediabetes and diabetes are shown in Figures 2-4. Our findings indicate that both the degree of coronary atherosclerosis and the plaque vulnerability were more advanced in patients with prediabetes than in those without diabetes, and were comparable to patients with diabetes. We showed that the number of yellow plaques (0.80 ± 0.64 vs 1.45 ± 0.81 vs 1.63 ± 0.99 ; $P = 0.011$) and yellow grade (1.44 ± 1.03 vs 2.00 ± 0.86 vs 2.30 ± 0.70 ; $P = 0.047$) in patients with prediabetes were greater than those in patients without diabetes, but similar to those in patients with diabetes (Figure 5)^[55].

Prevention of atherosclerotic cardiovascular diseases

Recently, the American College of Cardiology and the

American Heart Association proposed a new guideline for the treatment of hyperlipidemia to reduce the risk of cardiovascular events and recommended aggressive statin therapy for both primary and secondary prevention of atherosclerotic cardiovascular disease (ACVD) events in diabetes patients^[56]. Moreover, for patients with diabetes without preexisting CAD, the American Diabetes Association currently recommends starting pharmacological therapy at a low-density lipoprotein cholesterol (LDL-C) level of ≥ 130 mg/dL with a goal of < 100 mg/dL^[57]. In an angioscopic investigation, lowering LDL-C by statin induces reduction of color intensity in yellow plaques, and the phenomenon is regarded as its effect on plaque stabilization^[58].

Four major groups were identified that would benefit from statin therapy to reduce ACVD risk: (1) patients with clinical ACVD (secondary prevention); (2) patients with primary elevations of LDL-C ≥ 190 mg/dL (primary prevention); (3) patients 40-75 years of age who have diabetes and LDL-C 70-189 mg/dL (primary prevention); and (4) patients up to 75 years of age without diabetes and with an estimated 10-year ACVD risk $\geq 7.5\%$ and LDL-C 70-189 mg/dL (primary prevention). Selected patients with $< 5\%$ 10-year ACVD risk who are < 40 or > 75 years of age may also benefit from statin therapy^[56]. The 10-year ACVD risk was estimated from age, sex, race, blood cholesterol level, history of hypertension and diabetes, smoking habits, *etc.* In the group with 10-year ACVD risk $< 7.5\%$, a benefit of statin therapy was not completely established. Regarding prediabetes, we should pay attention to this group and consider the benefit of statin therapy.

Because coronary atherosclerosis is already present

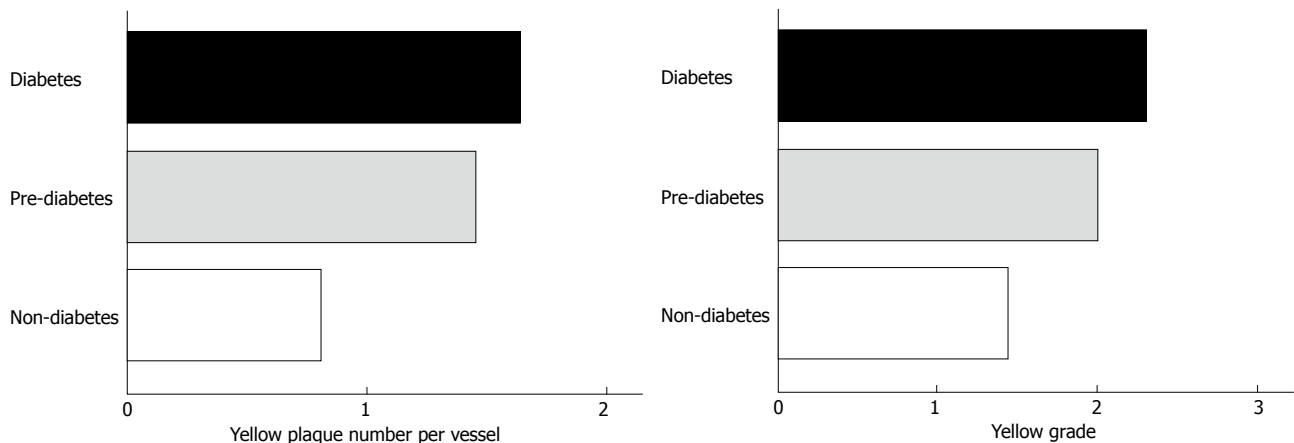


Figure 5 Comparisons of yellow plaque detected by coronary angiography among the 3 groups. A: Average number of yellow plaques per vessel; B: Average maximum yellow grade per coronary artery (MYG). The number of yellow plaques per vessel and maximum yellow grade per coronary artery in the prediabetic group were greater than those in the nondiabetic group ($P = 0.017$ and $P = 0.040$, respectively), whereas they were similar to those in the diabetic group ($P = 0.44$ and $P = 0.21$, respectively).

in prediabetes and our angiographic examination revealed that the level of coronary atherosclerosis with prediabetes is almost equal to that in patients with diabetes, even for patients with prediabetes, earlier pharmacological therapy should be recommended.

CONCLUSION

We should consider the risk of CAD in the prediabetic state with mild glucose metabolism disorder, and further clinical investigations are required to establish an exact risk stratification and prevent future cardiovascular events in patients with prediabetes.

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Literature review of type 2 diabetes mellitus among minority Muslim populations in Israel

Yulia Treister-Goltzman, Roni Peleg

Yulia Treister-Goltzman, Roni Peleg, the Department of Family Medicine and Sial Research Center for Family Practice and Primary Care, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva and Clalit Health Services, Southern District, Beer-Sheva 84105, Israel

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Correspondence to: Dr. Yulia Treister-Goltzman, MD, the Department of Family Medicine and Sial Research Center for Family Practice and Primary Care, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva and Clalit Health Services, Southern District, POB 653, Beer-Sheva 84105, Israel. yuliatr@walla.com

Telephone: +972-8-6477436

Fax: +972-8-6477636

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Bedouin populations of Israel. T2DM is a global health problem. The rapid rise in its prevalence in the Arab and Bedouin populations in Israel is responsible for their lower life expectancy compared to Israeli Jews. The increased prevalence of T2DM corresponds to increased rates of obesity in these populations. A major risk group is adult Arab women aged 55-64 years. In this group obesity reaches 70%. There are several genetic and nutritional explanations for this increase. We found high hospitalization rates for micro and macrovascular complications among diabetic patients of Arab and Bedouin origin. Despite the high prevalence of diabetes and its negative health implications, there is evidence that care and counseling relating to nutrition, physical activity and self-examination of the feet are unsatisfactory. Economic difficulties are frequently cited as the reason for inadequate medical care. Other proposed reasons include faith in traditional therapy and misconceptions about drugs and their side effects. In Israel, the quality indicators program is based on one of the world's leading information systems and deals with the management of chronic diseases such as diabetes. The program's baseline data pointed to health inequality between minority populations and the general population in several areas, including monitoring and control of diabetes. Based on these data, a pilot intervention program was planned, aimed at minority populations. This program led to a decrease in inequality and served as the basis for a broader, more comprehensive intervention that has entered the implementation stage. Interventions that were shown to be effective in other Arabic countries may serve as models for diabetes management in the Arab and Bedouin populations in Israel.

Key words: Type 2 diabetes mellitus; Pre-diabetes; Risk factors for diabetes; Muslims; Bedouins; Arabs; Ethnic differences

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Abstract

This review surveys the literature published on the characteristics and implications of pre-diabetes and type 2 diabetes mellitus (T2DM) for the Arab and

Core tip: Type 2 diabetes mellitus is a global health problem and its rapid rise in prevalence in the Arab and Bedouin populations in Israel is responsible for the lower life expectancy among Israeli Arabs compared to Israeli Jews. An important high-risk group is adult Arab women in the 55 to 64 year age range where obesity rates approach 70%. Our review found high hospitalization rates for micro and macrovascular complications among diabetic patients of Arab and Bedouin origin. There is evidence that care and counseling relating to nutrition, physical activity and self-examination of the feet are unsatisfactory in these populations. In Israel, data from the quality indicators program demonstrated inequality in health and served as the basis for an intervention program in minority populations to improve the monitoring and control of diabetics in these populations. Preliminary data indicated that this program has a significant potential to reduce health inequality between the Jewish and Arab populations.

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INTRODUCTION

Once considered a disease of western society, type 2 diabetes mellitus (T2DM) has now spread to every country in the world, with Asia accounting for 60% of the world's diabetic population^[1]. Obesity and T2DM have become a central medical problem among immigrants and minorities^[2].

There are three patterns of increase in diabetes prevalence: gradual, rapid and accelerated. The prevalence rates today are 4%-9% in Europeans and reach 14%-20% among Asian immigrants to India, Arabs, Chinese, individuals of African descent and Hispanics. Particularly high rates of diabetes, up to about 50%, are found in native populations in the United States, Canada, Australia and the Pacific region. Explanations for the increasing prevalence of diabetes in Europe include changes in lifestyle and obesity^[3]. Several hypotheses have been proposed over recent years to explain the rapid and accelerated rise in diabetes among developing nations. One of the explanations, known as the "thrifty phenotype" or "fetal origins of disease", assumes that malnutrition during pregnancy and infancy can lead to a process of adaptation and more "efficient" metabolic production that facilitates the anabolic processing of energy sources when the individual has an unrestricted intake of calories later on^[2,4]. Conversely, the "drifty genotype" hypothesis contends that the prevalence of thrifty genes is attributable to a genetic drift resulting from the disappearance of predative selection pressures^[5].

SETTING

According the Central Bureau of Statistics, the State of Israel had a population of 8102000 in March 2013, with close to 75% Jews, about 21% Arabs and about 4% others^[6].

Bedouins are one of the ethnic groups in Israeli society. They comprise 3.5% of the total population with about 280000 individuals, most living in the Negev region in southern Israel. They are Semitic tribes that originated in the Arabian Peninsula from where they began to disperse to the north to find areas worthy of grazing and living. They are Muslim Arabs that live in accordance with the unique customs of their society^[7].

The Bedouins in the Negev are at the bottom of the socioeconomic scale in Israel^[8]. Phenomena such as polygamy, in-marriage (marriage within one's own tribe or group as required by custom or law) and high birth rates (in 2009 the average Bedouin family numbered 6.8 children) are common in Bedouin society^[7].

These characteristics, intermingling with elements of modern life, have brought about a condition in which a significant proportion of their society is in a phase of "population in transition".

Today, about 61% of the Negev Bedouins live in permanent towns and 39% live in unauthorized villages. There is a large difference in living conditions between these two groups. The latter live in huts or tents without official supplies of water or electricity. Houses are heated in the winter primarily by burning wood over open fires. Cooking is done on gas stoves or open fires. The sanitation level is very low with no central sewerage or garbage removal. These conditions affect morbidity, adherence to treatment and access to healthcare services^[9].

Israel has a national health insurance law. In accordance with this law, the population receives medical care through non-profit medical organizations. The work principle of these medical organizations is based on the patient-oriented model based on primary care in the community by a team of doctors and nurses with specialist consultants, if required^[10]. Although the Arab and Bedouin populations of Israel live in the same geographical area as the Jews and have at their disposal the same broad basket of healthcare services, they are separate ethnic groups embracing a different lifestyle, nutritional habits and environmental exposures. Furthermore, in recent years, the Arab population of Israel has experienced a rapid change towards a westernized lifestyle.

We surveyed the literature on pre-diabetic states and T2DM and their consequences among the Arab and Bedouin populations of Israel. The search was conducted in the PubMed database using the search terms "nutrition and obesity", "diabetes" and "Arabs and Bedouins in Israel". Thirty six relevant articles were found, 30 in English and six in Hebrew.

ISRAELI ARABS

Genetic background, obesity and pre-diabetes in the Arab population of Israel

While the life expectancy of Israeli Arabs was lower

than Israeli Jews from 1975-2004, the gap decreased between 1975 and 1998. However, since 1998 the gap has increased again and the difference in 2004 was 3.2 years more for Israeli Jewish men and 4 years more for Israeli Jewish women. The main causes of death that lead to the gap in life expectancy are chronic diseases, especially ischemic heart disease and diabetes^[11]. The Arab community of Israel is characterized by a high rate of consanguinity. One study investigated the effect of consanguinity on multifactorial common adult morbidity, including T2DM. There was no significant difference in T2DM between patients with consanguinity and those without^[12]. Another study that investigated the existence of a direct genetic association that affects the development of diabetes demonstrated that distinct genetic backgrounds are responsible for the development of beta-cell dysfunction and insulin resistance among Arabs^[13]. Obesity comprises a central element in the development of T2DM and the risk of diabetes increases substantially with increased body mass index (BMI)^[14]. A study from central Israel showed that the mean BMI of 18-year-old Jews and Arabs is similar. This finding changes with age so that 52% of Arab women are classified as obese compared with 31% of Jewish women and 25% of Arab men compared to 23% of Jewish men. A central group pointed to in this study was Arab women aged 55-64 years where the rate of obesity reaches 70%^[16]. A study of randomly recruited healthy, overweight Arabs (BMI > 27) attending a primary healthcare clinic in Israel revealed that 27% of them had undiagnosed T2DM, 42% had impaired glucose tolerance (IGT), and only 31% had a normal OGCT. The metabolic syndrome was diagnosed in 48%^[17]. There is evidence from various populations that IGT and impaired fasting glucose (IFG) often are associated with different groups of patients^[18]. The study from Israel assessed insulin resistance and impaired pancreatic function among overweight Arab patients with IFG only, IGT only or IFG and IGT (combined glucose intolerance-CGT) compared to those with a normal response to glucose (NGT). Patients with IFG and CGT were more obese and had higher values of insulin resistance compared to those with IGT only or normal fasting glucose. There was no statistically significant difference in insulin resistance between patients with IGT only and those with NGT. Beta-cell function was depressed in patients with IGT only and CGT compared to those with IFG and NGT, while beta-cell function indices in patients with IFG were similar to those with NGT^[19].

DIABETES MELLITUS IN ISRAELI ARABS

Studies show that in recent decades the incidence rate of diabetes in the Israeli Arab population has increased by 9.1 per 1000 persons annually^[20]. A study of the urban Jewish and Arabs population from the central area of Israel found that the prevalence rates for adult-onset diabetes were 21% among Arabs and 12% among Jews.

The Arabs presented with diabetes at a younger age than the Jews and 25% of the Arab population was diagnosed with diabetes by age 57 compared to age 68 in the Jewish population^[21]. An alarmingly high prevalence of diabetes was found in Israeli Arab women over 50 years, reaching 50%^[22]. Another study found that the prevalence of diabetes among women younger than 65 years old was significantly higher compared to men. The mean age of diabetic women was 48.3 compared to 59.5 among men and women had a higher BMI (34.5 *vs* 30.04, respectively) at diagnosis. The age of diagnosis of diabetes correlated significantly with BMI^[14]. Despite the high prevalence of obesity, metabolic syndrome and overt diabetes among Arabs, there is evidence of inadequate care for diabetes in this population. More than a third of respondents reported that they did not receive any counseling on issues such as foot care or the effects of smoking on diabetes. Misconceptions, attributable to social norms, are common and more than a third forgo taking medications because they cannot afford them^[23]. Arab diabetics received less nutritional counseling (OR = 0.46), less counseling on physical activity (OR = 0.42) and less advice on self-testing of the feet than Jewish patients (OR = 0.55)^[24]. There is poor diabetes control and sub-optimal follow-up care among Arab patients with diabetes^[25].

The results of studies on the prevalence of obesity, pre-diabetes and diabetes type 2 in the Arab Israeli population are summarized in Table 1.

DIABETES AS A RISK FACTOR IN ISRAELI ARABS

In a five country observational study that determined the incidence of hypoglycemia during the holiday of Ramadan among Muslim subjects with T2DM treated with a sulphonylurea, the highest incidence of hypoglycemia was reported by patients from Israel (40%)^[26].

In a recent study among patients in hospitals that had predominantly Arab patients (more than 90%), the proportion of diabetics was 39%. There was a female preponderance among patients admitted with diabetes (52.9%), while only 45% of hospitalized patients without diabetes were women ($P = 0.0003$).

A difference was found in the reasons for hospitalization between patients with diabetes and those without. In the diabetic group, there were more hospitalizations (37% *vs* 27% respectively, $P < 0.001$) and urinary tract infections (7.7% *vs* 6.9%, respectively). The authors recommended that the prevention of cardiovascular disease and urinary tract infections among the diabetic population should be a priority, especially for Arab women over 40 who have a high risk for morbidity and a high rate of hospitalizations^[27].

A study that evaluated risk factors among Arab and Jewish patients who underwent rehabilitation for a first stroke revealed that a high percentage of Arab patients have hypertension and T2DM. The prevalence of diabetes

Table 1 Results of studies on the prevalence of obesity, pre-diabetes and diabetes type 2 in the Arab Israeli population

Ref.	Date	Subjects	Study results
Keinan-Boker <i>et al</i> ^[16]	2005	Representative sample of 3246 individuals from the general Israeli population	In the subgroup of older Arab women, aged 55-64 yr, obesity reached 70%
Abdul-Ghani <i>et al</i> ^[17]	2005	95 randomly recruited Arab subjects who were overweight and over the age of 40	27% had undiagnosed DM, 42% impaired fasting glucose or impaired glucose tolerance, 48% metabolic syndrome
Abdul-Ghani <i>et al</i> ^[14]	2005	7434 patients from an outpatient clinic in an Arab village	The prevalence of diabetes type 2 in Arab patients younger than the age of 65 was significantly higher among women than men. Diabetic women were younger than men at diagnosis (48 yr <i>vs</i> 59 yr) and had a higher BMI
Kalter-Leibovici <i>et al</i> ^[15]	2007	880 randomly selected Arab and Jewish patients	The prevalence of obesity was 52% in Arab women compared to 31% in Jewish women and 25% in Arab men compared to 23% in Jewish men
Idilbi <i>et al</i> ^[20]	2012	Review of official health statistics	The incidence rate of diabetes in the Israeli Arab population increased by 9.1 per 1000 persons annually. In contrast, it decreased among Jews
Kalter-Leibovici <i>et al</i> ^[21]	2012	1100 Arab and Jewish patients older than the age of 20	The prevalence of diabetes was 21% among Arabs and 12% among Jews. Arabs developed diabetes 11 years earlier than Jews

DM: Diabetes mellitus.

among Arabs was 51.4%, among non-immigrant Jews 38.5%, and among immigrant Jews 39.1% ($P < 0.001$)^[28]. In another study that evaluated ethnic disparities between patients with a first episode of primary intracerebral hemorrhage in northern Israel, the Arabs were found to be younger and to have a higher prevalence of diabetes^[29]. A national survey among 28 hospitals in Israel that assessed ethnic variations in acute ischemic stroke showed that the mean age of Arab patients was nine years younger than Jewish patients (63 ± 11 years *vs* 72 ± 12 years, respectively), Arabs were more likely to be obese (OR = 1.72) and to have diabetes (OR = 1.41)^[30]. A higher prevalence of diabetes among Arabs than Jews was also found in a study that compared ethnic differences in ischemic stroke in patients of working age (≤ 65 years)^[31].

In two studies that examined risk factors in hospitalized Arab and Jewish women with coronary heart disease who underwent cardiac catheterization, a higher prevalence of diabetes was found among the Arab women^[32,33].

These differences should be addressed when developing stroke and coronary artery disease preventative strategies, planning healthcare services and designing culturally relevant public education programs.

The results of studies on diabetes as a risk factor among Israeli Arabs appear in Table 2.

NUTRITION AND OBESITY AMONG BEDOUINS IN THE NEGEV

Similar to other Arab Israeli populations, the prevalence of obesity was also higher among Bedouins compared to Jews (27.9% *vs* 20%, respectively)^[34]. A study of Bedouin women of childbearing age found a high prevalence of obesity associated with nutritional deficits^[35]. In order to investigate possible dietary causes for the discrepancy in obesity rates between adult Jews and Bedouins, researchers from southern Israel compared eating patterns in the two populations. Bedouin men and women reported a lower intake of fat and protein and

a higher intake of carbohydrates than Jews^[34]. Another study demonstrated that the nutrition of Bedouin women who lead a semi-traditional lifestyle had a caloric value that was 50% higher than that of Jewish women. The mean BMI of the Bedouin women was 30^[36]. To evaluate the importance of modern food and drink in their daily diet, the nutrition of Bedouin women living in permanent towns and non-permanent settlements was compared. Residents in non-permanent settlements, where there are no means to preserve food, ate more traditional dairy products while those in permanent towns ate more meat. Both population groups based their two main meals on traditional food, but processed foods and drink were consumed as snacks. These processed products are calorie-rich and can be a factor in the rising rate of diabetes^[37].

T2DM AMONG BEDOUINS IN THE NEGEV

An epidemiological survey conducted among Bedouins about half a century ago reported that only a few patients had hypertension and diabetes and none had ischemic heart disease^[38,39]. Later, evidence accumulated that cardiovascular risk factors among the Bedouins were on the rise and that this increase was more pronounced among Bedouin living in settled settings compared to the traditional tribal groups. A study performed in 1990 demonstrated that among Bedouins who lived in permanent towns, 15% were obese and 23% were overweight compared to Bedouins who did not live in permanent towns, where there were no obese individuals and 23% were overweight. This difference was particularly apparent in the younger age group. No difference was found between the groups regarding fasting blood glucose^[40]. A study from 2005 found a difference in diabetes prevalence in urban compared to rural settlements (5.5% *vs* 3.9%, respectively, $P < 0.001$). In this study, diabetes control was less successful among Bedouin diabetes patients. Only 29.3% had their diabetes under control compared to 46.7% among non-Bedouin

Table 2 Results of studies on diabetes as a risk factor among Israeli Arabs and Bedouins

Ref.	Date	Subjects	Study results
Jabara <i>et al</i> ^[32]	2007	546 women (102 Arabs) after cardiac catheterization	Arab women had a higher prevalence rate for diabetes (61% <i>vs</i> 46% in Jews)
Salameh <i>et al</i> ^[33]	2008	40 Arab and 179 Jewish women hospitalized with coronary artery disease	More Arab patients had diabetes (73% <i>vs</i> 40%)
Telman <i>et al</i> ^[31]	2010	727 Arab and Jewish patients of working age (< 65 yr) with stroke	There was a higher prevalence of diabetes in the Arab patients
Telman <i>et al</i> ^[29]	2010	546 patients with a first episode of primary intracerebral hemorrhage	Diabetes was more frequent among the Arab patients.
Aravind <i>et al</i> ^[26]	2011	1378 Muslim patients from five countries who were treated with sulfonylurea during Ramadan	The highest percentage of hypoglycemia (40%) was reported in patients from Israel
Greenberg <i>et al</i> ^[28]	2011	2000 patients with a first stroke (237 Arabs)	A high percentage of Arabs had diabetes (51.4% <i>vs</i> 35.8% in Jews)
Gross <i>et al</i> ^[30]	2011	1540 patients with acute ischemic stroke, 169 Arabs	Arab patients were more likely to have diabetes (OR 1.41)
Chorny <i>et al</i> ^[46]	2011	523 diabetic patients (Jews and Bedouins) who were examined by an ophthalmologist	The prevalence of maculopathy and retinopathy was higher among the Bedouins (22% <i>vs</i> 13.4%)
Nseir <i>et al</i> ^[27]	2013	3784 patients from hospitals with predominantly Arab patients	39% of the hospitalized patients were diabetics. The diabetics had more hospitalizations due to atherosclerotic disease
Rabaei <i>et al</i> ^[45]	2014	220 patients admitted with diabetic ketoacidosis (19% Bedouins)	There was no difference in outcomes (in-hospital mortality, 30-d mortality) between Jews and Bedouins

diabetes patients^[41]. A study at the largest urban Bedouin outpatient clinic in 2002 revealed that the prevalence of diabetes was 7.3% among men and 9.9% among women. Women had significantly higher BMI levels than men but lower levels of HbA1c and microalbuminuria^[42]. Prescribed oral medicines were purchased by 69% of the women compared to 76% of the men. Insulin was purchased by 19% of the women compared to 15% of the men^[42]. The study from 2007 showed an age-adjusted prevalence rate for diabetes of 12% in the Bedouin urban population compared to 8% among Jews. The prevalence rate was especially notable among Bedouins in the 40-49 year age group where it was three times higher than in the Jewish population of the same age. The adherence rate to diabetes treatment was 27% among the Bedouins compared to 42% in the Jewish population. The Bedouin population was also less compliant with follow-up blood tests: 22% of the Bedouin patients had no HbA1C measurements over the course of the previous year, compared to 13% of the Jews. The rates of controlled diabetic patients were lower among the Bedouins than the Jews (29.5% *vs* 57%, respectively)^[43]. The results of studies on the prevalence of obesity and diabetes type 2 among Bedouins in the Negev are summarized in Table 3.

A recent study evaluated the reasons for non-treatment of cardiovascular disease and its risk factors in the Bedouin population. Structured interviews on knowledge and attitudes relating to chronic diseases and their treatment were conducted among patients with T2DM, hypertension and lipid metabolic disorders. Ninety-nine high and 101 low-adherent patients were interviewed. More patients in the low-adherence group believed that traditional folk treatment was an alternative to prescription drugs for the treatment of T2DM, hypertension and hyperlipidemia and 10% took traditional drugs only. Patients in the group that was classified as undertreated believed that adverse drug effects were more harmful than

the disease itself (65% *vs* 47%, respectively) and this was also the reason for the cessation of treatment among 47% who were classified as low-adherent^[44].

In a retrospective analysis of the clinical characteristics and outcomes of diabetic ketoacidosis in the Jewish and Bedouin populations that included patients with both type 1 and type 2 diabetes, no differences were found for in-hospital mortality, 30 d mortality or complication rates in Jewish and Bedouin patients^[45]. Damage to the eye as a result of microvascular injury is a common complication in diabetes patients. Among diabetic patients referred to ophthalmologists in southern Israel, significantly more diabetic complications (damage to the retina and the macula) were found among the Bedouins than among the Jews (22% *vs* 13.4%, respectively), although the Bedouin patients were younger than the Jews (average age 58.6 \pm 12 years *vs* 64 \pm 10.3 years). The predicting factors for diabetic eye complications among Bedouins were the duration of the diabetes, high levels of HbA1c, insulin treatment and smoking^[46]. The results of studies on diabetes as a risk factor among Israeli Arabs appear in Table 2.

THE PROGRAM FOR HEALTH QUALITY INDICATORS AND ITS EFFECT ON THE CONTROL OF DIABETES IN MINORITY POPULATIONS

Health quality indicators were introduced at the inception of the process of departmentalization of clinics and was part of the process of assessment of the clinics. The first indicator that was chosen in this process was the rate of influenza inoculation in the target population. Over the years the number of indicators increased so that today there are 70 indicators in 11 primary areas. A significant

Table 3 Results of studies on the prevalence of obesity and diabetes type 2 among Bedouins in the Negev

Ref.	Date	Subjects	Study results
Ben Assa ^[38]	1961	2000 examined Bedouins	10 diabetic patients
Fraser <i>et al</i> ^[40]	1990	Tribal and settled Bedouin males	Among settled Bedouins, 15% were obese and 35% were overweight. Among tribal Bedouins, none were obese and 23% were overweight
Abou-Rbiah <i>et al</i> ^[42]	2002	3115 patients from an urban Bedouin clinic	The prevalence of diabetes was 7.3% in males and 9.9% in females. The mean BMI was 30 in females and 29 in males
Cohen <i>et al</i> ^[41]	2005	Population of the Negev area	The prevalence of diabetes was 5.1% in Bedouins, 3.7% in non-Bedouins, 5.5% in urban Bedouins, 3.9% in rural Bedouins. Diabetes was well controlled in 29.3% of the Bedouins and 46.7% of the non-Bedouins
Tamir <i>et al</i> ^[43]	2007	28449 Bedouins, 14012 Jews, older than 20	The prevalence of diabetes was 12% in the Bedouins compared to 8% in the Jews)
Fraser <i>et al</i> ^[34]	2008	793 Jews and 169 Bedouins aged 35-64 yr	The non-compliance rate for treatment was 72.9% among diabetic Bedouins
Leshem <i>et al</i> ^[36]	2008	31 encampment Bedouin women	The obesity rate was 27.9% among Bedouins and 20% among Jews
Abu-Saad <i>et al</i> ^[35]	2012	683 pregnant Bedouin women	The mean BMI was 30.3
			42% were either overweight or obese (based on their pre-pregnancy BMI)

BMI: Body mass index.

proportion of these indicators relates to the implementation of preventive medicine and monitoring and control of patients with diabetes. The program's data pointed to a serious disparity in the monitoring and control of diabetes in the Arab sector as well as other Israeli sub-populations, including Ethiopian Jews, compared to the general population^[47,48]. In 2008, the Clalit Health Services, which serve 70% of the Israeli population, reached an organizational decision that the reduction of these disparities was a strategic goal, so a dedicated intervention program to address them was designed. Between 2008-2010, a "pilot" program was conducted aimed at 55 clinics serving 400000 clients from "difficult" populations, including Arab and Bedouin communities in the Negev. The program focused on seven quality indicators, including the monitoring and control of diabetes. The strategy included a concerted effort aimed at providing medical solutions to loci of inequality in health quality indicators. Language facilitators were introduced into the clinics and efforts were made to incorporate religious leaders into the program, including lectures by the local Kadi on the religious importance of maintaining a healthy body, the reading of prayers on the importance of preventive medicine and physical activity in mosques on Fridays, and discussions with the village sheikh to grant permission and consent to women to carry out physical activity in the form of walks. As a result of this intervention in key clinics, a reduction of 67% in health quality disparity (including measurements related to monitoring and control of diabetes) were achieved within less than two years. There was an increased risk at baseline of 8% in the key clinics in emergency room visits and hospitalizations, which was reduced to that of other population sectors as a result of the intervention. In light of the success of the pilot intervention, in a relatively short period of time a separate program was developed that was broader and more comprehensive. This program was designed to bring about a reduction in inequality among socioeconomic levels and different sectors of the

population. This program is now in the implementation stage^[48] and its results have not been reported to date.

CONCLUSION

T2DM is a global health problem and the rapid rise in its prevalence in Arab and Bedouin populations in Israel is a cause of the difference in life expectancy between Jews and Arabs. The increased prevalence of T2DM corresponds with increased obesity rates in these populations. The primary at-risk group is Arab women aged 55 to 64 years who have an obesity rate that approaches 70%. There are several genetic and nutritional explanations for the increase. In this review, we found evidence for high rates of hospitalizations and micro and macrovascular complications among diabetic patients of Arab and Bedouin origin. Despite the high prevalence of diabetes and its negative health implications, there is evidence of a lack of appropriate care and counseling about nutrition, physical activity and self-examination of feet. Financial difficulties are frequently cited as reasons for inadequate healthcare and belief in traditional therapy and misconceptions about drugs and their side effects are also significant factors.

Although these overall data are troubling, recent findings are more encouraging.

A quality indicators program in the community has been in existence in Israel for the last 15 years. It is based on some of the world's leading information systems with data regarding sociodemographic factors, drug therapy, healthcare services, laboratory and imaging data, and recording of chronic diseases. It consists of several domains, including preventive medicine and management of chronic diseases. The program has led to an improvement in the quality of medical care, including diabetes control.

Since the program's data indicated inequality in different health quality indicators, including indicators relating to the monitoring and control of diabetes,

between the general population and several population sectors including minorities, a pilot intervention program was conducted to reduce these inequalities in selected clinics from 2008-2010.

This intervention program has contributed to a narrowing of health-related gaps and has reduced inequalities between the Arab and Jewish populations as well as between socioeconomic levels. The program demonstrated that the healthcare system is capable of reducing health inequalities, even if they are the result of variables for which they are not directly responsible, such as disparities in income, educational level, culture differences and isolated residential areas. It appears that an evidence-based, dedicated intervention is the key to success. In the wake of this success, a broader intervention was planned and has now entered into the implementation phase. The results of this program which involves the entire minority population have not been reported yet.

Interventions that are based on empowerment for medical care, cultural elements presented in Arabic terms and concepts, nutritional habits and lifestyle were shown to be effective in other Arabic countries^[49] and can serve as models for diabetes management in the Arab and Bedouin populations in Israel.

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Retrospective Study

Obesity and cardiometabolic disease risk factors among US adolescents with disabilities

Sarah E Messiah, Denise C Vidot, Gabriel Somarriba, Kanathy Haney, Semra Aytur, Ruby A Natale, Jeffrey P Brosco, Kristopher L Arheart

Sarah E Messiah, Gabriel Somarriba, Division of Pediatric Clinical Research, Department of Pediatrics, University of Miami, Leonard M. Miller School of Medicine, Miami, FL 33101, United States

Sarah E Messiah, Denise C Vidot, Kanathy Haney, Kristopher L Arheart, Department of Public Health Sciences, University of Miami, Leonard M. Miller School of Medicine, Miami, FL 33101, United States

Semra Aytur, Department of Health Management and Policy, University of New Hampshire, Durham, NH 03824, United States

Ruby A Natale, Department of Pediatrics, Mailman Center for Childhood Development, University of Miami, Leonard M. Miller School of Medicine, Miami, FL 33101, United States

Jeffrey P Brosco, Mailman Center for Childhood Development, Department of Pediatrics, University of Miami Leonard M. Miller School of Medicine, Miami, FL 33101, United States

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Informed consent: Health information collected in the National Health and Nutrition Examination Survey is kept in strictest confidence. During the informed consent process, survey participants are assured that data collected will be used only for stated purposes and will not be disclosed or released to others without the consent of the individual or the establishment in accordance with section 308(d) of the Public Health Service Act (42 U.S.C. 242 m).

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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at smessiah@med.miami.edu. The presented data cannot be linked to individuals and risk of personal identification is minimal as such.

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Correspondence to: Sarah E Messiah, PhD, MPH, Division of Pediatric Clinical Research, Department of Pediatrics, University of Miami, Leonard M. Miller School of Medicine, Batchelor Children's Research Institute Room 541, NW 10th Avenue (D820), Miami, FL 33101, United States. smessiah@med.miami.edu

Telephone: +1-305-2431943

Fax: +1-305-2438475

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Abstract

AIM: To generate prevalence estimates of weight status and cardiometabolic disease risk factors among adolescents with and without disabilities.

METHODS: Analysis of the 1999-2010 National Health and Nutrition Examination Survey data was conducted among 12-18 years old with ($n = 256$) and without disabilities ($n = 5020$). Mean values of waist circumference, fasting glucose, high-density-lipoprotein cholesterol, triglycerides, systolic and diastolic blood pressure and metabolic syndrome (MetS, ≥ 3 risk factors present) were examined by the following standardized body mass index (BMI) categories for those with and without disabilities; overweight (BMI $\geq 85^{\text{th}}$ - $< 95^{\text{th}}$ percentile for age and sex), obesity (BMI $\geq 95^{\text{th}}$ percentile) and severe obesity (BMI $\geq 35 \text{ kg/m}^2$). Linear regression models were fit with each cardiometabolic disease risk factor independently as continuous outcomes to show relationships with disability status.

RESULTS: Adolescents with disabilities were significantly

more likely to be overweight (49.3%), obese (27.6%) and severely obese (12%) *vs* their peers without disabilities (33.1%, 17.5% and 3.6%, respectively, $P \leq 0.01$ for all). A higher proportion of overweight, obese and severely obese children with disabilities had abnormal SBP, fasting lipids and glucose as well as MetS (18.9% of overweight, 32.3% of obese, 55% of severely obese) *vs* their peers without disabilities (9.7%, 16.8%, 36.3%, respectively). US adolescents with disabilities are over three times as likely to have MetS (OR = 3.45, 95%CI: 1.08-10.99, $P = 0.03$) *vs* their peers with no disabilities.

CONCLUSION: Results show that adolescents with disabilities are disproportionately affected by obesity and poor cardiometabolic health *vs* their peers with no disabilities. Health care professionals should monitor the cardiometabolic health of adolescents with disabilities.

Key words: Adolescents; Children; Disability; Obesity; Cardiometabolic; Disease risk

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Core tip: Our results here show that US adolescents with disabilities are disproportionately affected by obesity and are over three times as likely to have the metabolic syndrome *vs* their peers with no disabilities. Half of all adolescents with disabilities are overweight, obese or severely obese. In addition to the metabolic syndrome, obese adolescents with disabilities are significantly more likely than their normal weight counterparts to have increased or abnormal systolic blood pressure, lipid and fasting glucose levels, placing them at risk for cardiovascular disease and/or type 2 diabetes. Health care professionals should monitor the cardiometabolic health of adolescents with disabilities.

Messiah SE, Vidot DC, Somarriba G, Haney K, Aytur S, Natale RA, Brosco JP, Arheart KL. Obesity and cardiometabolic disease risk factors among US adolescents with disabilities. *World J Diabetes* 2015; 6(1): 200-207 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i1/200.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i1.200>

INTRODUCTION

In 2011 an estimated 5.1% (2.3 million) of 5-15 years old and 5.6% (1.2 million) of 16-20 years old in the United States reported a disability (physical, sensory, and cognitive or developmental disabilities)^[1]. Even more troubling, obesity is 38% higher in children with disabilities and mobility limitations compared to their peers without disabilities^[2]. Similarly, 57% of adults who are disabled are obese compared to 35.7% of peers without disabilities^[3]. Healthy people 2020 reports that not only are individuals

with disabilities more likely to be overweight or obese, they are also less likely to engage in outdoor physical activities^[4,5], less likely to have social support to do so, and have worse overall health status *vs* their non-disabled counterparts^[6].

These above stated prevalence statistics are important because obesity is strongly linked to hypertension, hyperlipidemia, type 2 diabetes mellitus, respiratory and musculoskeletal problems, liver disease, psycho-social problems, low self-esteem, which all lead to increased healthcare costs^[7,8]. As such, it has been estimated that life expectancy will decrease due to obesity-related health issues alone^[9]. Many studies have shown that the current childhood obesity epidemic has resulted in poor cardiometabolic health consequences including the components of metabolic syndrome (MetS)-elevated blood pressure and glucose concentrations, hypertriglyceridemia, low high density lipoprotein (HDL) cholesterol concentrations, and central adiposity (elevated waist circumference) - and the syndrome itself (three or more of these components in the same individual)^[10-13]. Cardiometabolic disease risk factors present during the pediatric years predicts chronic diseases such as cancer, stroke, type 2 diabetes and cardiovascular disease in adults^[14,15]. While previous studies have documented that youth with the MetS are at high risk for cardiometabolic disease and atherosclerosis as adults, there are few population-based studies examining the prevalence of cardiometabolic risk among adolescents with disabilities despite their increased prevalence of obesity *vs* their peers without disabilities. Therefore, the purpose of the current analysis is to estimate the prevalence of cardiometabolic disease risk, including the MetS, among the United States adolescent population with and without developmental physical and/or learning disabilities by weight status (normal weight, overweight, obese, severely obese). It was hypothesized that obese adolescents with disabilities would be significantly more likely to have the metabolic syndrome *vs* obese adolescents without disabilities.

MATERIALS AND METHODS

Study population

Participant data from the National Health and Nutrition Examination Survey (NHANES) were analyzed. Six cycles of NHANES data (1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, and 2009-2010) were combined to ensure adequate sample size and statistical reliability^[16]. The NHANES sampling design to obtain a nationally representative sample of the United States population is described in detail elsewhere^[16].

Eligibility criteria

We selected all adolescents ages 12-18 years old from the combined 1999-2010 NHANES data who had the following variables available for analysis: waist circumference, body mass index (BMI), high density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure,

and fasting glucose and triglycerides. Because we chose to only analyze those who had data available on the cardiometabolic disease risk factors collected for their age group, the sample size was reduced from a total sample size of 10173 to 5276. There were no baseline significant differences between adolescents included in the sample ($n = 5276$) and those excluded ($n = 4897$) in terms of gender, ethnicity, education, income, or disability status. The mean age in the group included was 15.1 years compared to 14.9 years for those not included ($P = 0.01$).

Children were excluded from the analysis if they were known to have diabetes ($n = 51$), used medication that altered blood pressure, lipid metabolism, or blood glucose such as insulin, androgens, anabolic steroids, or adrenal corticosteroids ($n = 42$), or self-reported and/or tested positive *via* urine test as pregnant ($n = 117$).

Disability status

Individual physical functioning data were compiled from the NHANES Physical Function questionnaires^[17] to determine disability status. A participant was categorized as having a disability (yes/no) if they answered yes to any of the following questions: (1) “Do you/does child have an impairment or health problem that limits (your/his/her) ability to crawl, walk, run, or play?”; (2) “Is this an impairment or health problem that has lasted, or is expected to last 12 mo or longer?”; and (3) “Is (child) limited in the kind or amount of play activities he/she can do because of a physical, mental, or emotional problem?” Participants who did not report a disability were placed in the no disability category, which constituted the reference group for the analyses. Information on specific category of disability (autism, Down’s syndrome) is not available for NHANES participants under the age of 19.

Individual cardiometabolic disease risk factors

The criteria used to estimate the prevalence of abnormal or elevated (or low in the case of HDL cholesterol) individual cardiometabolic disease risk factors were modified to pediatric-specific criteria based on the National Cholesterol Education Program’s Adult Treatment Panel (ATP III) MetS definition for adults^[18]. The threshold values used in this study to define each pediatric-specific abnormal risk factor are described below.

Waist circumference: Abnormal waist circumference was defined as above the 90th percentile of the NHANES III (1988-1994) prevalence estimates adjusted for age, sex and ethnicity^[19].

Systolic and diastolic blood pressure: Blood pressure was considered to be abnormal if systolic and/or diastolic values were greater than standardized 90th percentile values adjusted for age and sex^[20].

HDL cholesterol: NHANES III values^[21] for cholesterol less than the 10th percentile were used to define abnormal or low HDL-cholesterol for the current study.

Triglyceride: NHANES III^[21] findings for triglyceride greater than the 90th percentile values adjusted for sex and ethnicity were used to define elevated levels in the current study.

Fasting glucose: A fasting glucose level of 100 mg/dL or higher was classified as abnormal^[22]. The fasting glucose-specific, 4-year weights were applied for analysis.

Metabolic syndrome

An adolescent met criteria for the MetS if they had ≥ 3 of the following risk factors: elevated waist circumference, triglycerides, fasting glucose, systolic and/or diastolic blood pressure, and low HDL cholesterol^[11-13].

BMI percentile categories

Comparison of abnormal cardiometabolic disease risk factors were examined by the following standardized BMI categories for those with and without disabilities; (1) normal weight = BMI < 85th percentile for age and sex; (2) overweight = BMI $\geq 85^{\text{th}}$ - < 95th percentile for age and sex; (3) obese = BMI $\geq 95^{\text{th}}$ percentile for age and sex^[23]; and (4) severely obese = absolute BMI ≥ 35 kg/m²^[24].

Measures and data collection

People who were selected and consented to participate in the NHANES completed an in-home survey collected *via* Computer Assisted Personal Interviewing (CAPI) procedures. Demographic, socioeconomic, dietary, and health-related information was collected during this process. After the in-home interview, participants were asked to undergo a physical exam at a Medical Examination Center (MEC).

All laboratory methods used at the MEC are reported in detail in the NHANES Laboratory/Medical Technologists Procedures Manual^[25,26]. Heights and circumferences were recorded to the nearest 0.1 cm.

Covariates

Demographic data including age in years, gender, ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic and Other) and education level were used in analysis as covariates. Mexican American and Other Hispanic categories were combined to create a “Hispanic” classification.

Statistical methods

All data were analyzed using SAS survey procedures (SAS version 9.3, SAS Institute, Cary, NC). Sample weights (created to generate estimates for an entire sampling frame) were readjusted to account for the combined survey cycles. Weighting takes into account the specific probabilities of selection for the individual domains that were over-sampled (for example, in the 1999-2000 and 2001-2002 surveys both Mexican Americans and blacks were over-sampled), as well as non-response and differences between the sample and the total population. The correct sampling weights must be used to produce unbiased estimates when multiple surveys/

Table 1 Demographic and anthropometric characteristics of those 12-18 years old with and without disabilities in the United States, 1999-2010 National Health and Nutrition Examination Surveys *n* (%)

	Overall <i>n</i> = 5276	Disability <i>n</i> = 256	No disability <i>n</i> = 5020	<i>P</i> -value ^a
Gender				0.31
Boys	2674 (50.8)	126 (2.5)	2548 (48.3)	
Girls	2602 (49.2)	130 (2.6)	2472 (46.6)	
Race/ethnicity				0.78
Non-hispanic white	1477 (61.1)	80 (64.3)	1397 (60.9)	
Non-hispanic black	1414 (13.5)	64 (12.1)	1350 (13.6)	
Hispanic	2146 (18.7)	97 (17.9)	2049 (18.7)	
Other	239 (6.7)	15 (5.7)	224 (6.8)	
Education level				0.22
Grade School	295 (5.2)	19 (6.1)	276 (5.1)	
Middle School	2247 (41.5)	101 (34.7)	2146 (41.9)	
High School	2264 (44.5)	117 (52.6)	2147 (44.1)	
High School graduate/GED	292 (6.4)	9 (3.5)	283 (6.6)	
More than High School	111 (2.3)	6 (2.9)	105 (2.3)	
Household income				0.17
< \$10000	236 (5.3)	22 (9.9)	214 (5.1)	
\$10000-\$19999	595 (13.0)	32 (17.1)	563 (12.7)	
\$20000-\$34999	692 (15.8)	32 (21.4)	660 (15.5)	
\$35000-\$54999	644 (20.2)	24 (14.0)	620 (20.5)	
\$55000-\$74999	375 (14.0)	16 (11.1)	359 (14.1)	
> \$75000	679 (31.7)	26 (26.4)	653 (32.0)	
Body mass index percentile group ^b				
Normal weight	3281 (66.1)	135 (50.7)	3146 (66.9)	< 0.001
Overweight	1995 (33.9)	121 (49.3)	1874 (33.1)	< 0.001
Obese	1099 (18.0)	67 (27.6)	1032 (17.5)	0.01
Severely obese	261 (4.2)	16 (5.8)	245 (4.1)	0.26
Body mass index	Mean (SE)	Mean (SE)	Mean (SE)	
Percentile	64.4 (0.6)	71.3 (2.4)	63.9 (0.6)	< 0.01
Z score	0.53 (0.02)	0.83 (0.1)	0.52 (0.0)	0.01
Age	15.1 (0.04)	15.1 (0.2)	15.1 (0.04)	0.68

^aRepresents mean difference between those with and without disabilities; ^bNormal weight = body mass index < 85th percentile for age and sex, overweight = body mass index \geq 85th - < 95th percentile for age and sex, obese = body mass index \geq 95th percentile for age and sex (Kuczmarski *et al*^[23], 2000) severely obese = absolute body mass index \geq 35 kg/m² (Kelly *et al*^[24], 2000).

years are combined.

Survey frequencies were used to summarize demographic descriptive characteristics of the sample, and the SAS survey means procedure was used to obtain descriptive characteristics of anthropometric measurements. A binary variable for disability status was created for comparison and analysis purposes. The prevalence of each cardiometabolic disease risk factor was estimated for all 4 BMI categories for those with and without disabilities.

Linear regression models were fit with each cardiometabolic disease risk factor independently as continuous outcomes to show relationships with disability status. Logistic regression models were fit with a cluster of \geq 3 abnormal cardiometabolic disease risk factor (MetS) as a binary outcome [$Y = \geq$ 3 abnormal factors; $N = \leq$ 3 abnormal factors. Adjustments were made in a step-wise procedure for Model (1) age, gender, ethnicity; Model (2) age, gender, ethnicity, education level; and Model (3) (Full Model)] age, gender, ethnicity, education level, and annual household income. Adjusted odds ratios were reported with corresponding 95% CIs.

Statistical analysis

The statistical review of the study was performed by senior author Dr. Kristopher Arheart, a biomedical

statistician and a leading expert on NHANES data and analysis. His approval of the methods are documented *via* his senior authorship inclusion on the manuscript.

RESULTS

Demographic characteristics of the sample ($n = 5276$, weighted $n = 15942916$) are presented in Table 1. Five percent (5.1%) of the sample ($n = 256$, weighted $n = 812061$) was classified as having a disability. There were no statistically significant differences in gender, ethnicity, education level, or annual household income between disabled and no-disability groups. Adolescents with disabilities were significantly less likely to be normal weight *vs* their peers with no disabilities (50.7% *vs* 66.9%, $P < 0.001$), and were significantly more likely to be overweight (49.3% *vs* 33.1%, $P < 0.001$) and obese (27.6% *vs* 17.5%, $P = 0.01$). Adolescents with disabilities had a significantly higher mean BMI percentile (71.3%ile), and Z-score (0.83) *vs* children without disabilities (64.4%ile; 0.53, respectively).

No significant differences between adolescents with and without disabilities were found for all cardiometabolic disease risk factors mean values among overweight, obese and severely obese sub-groups with the exception of

Table 2 Mean values of cardiometabolic disease risk factors among those 12-18 years old with and without disabilities in the United States by body mass index weight category^a, 1999-2010 National Health and Nutrition Examination Surveys

Cardiometabolic disease risk factors	Disability mean (SE)	No disability mean (SE)	P-value
Waist circumference, cm			
Normal weight	74.7 (0.61)	73.1 (0.18)	0.01
Overweight	96.5 (1.63)	94.7 (0.38)	0.31
Obese	105.1 (1.59)	102.2 (0.50)	0.10
Severely Obese	122.2 (3.30)	116.6 (0.71)	0.09
Systolic blood pressure, mmHg			
Normal weight	106.7 (1.22)	107.3 (0.26)	0.61
Overweight	114.2 (1.46)	112.2 (0.36)	0.19
Obese	115.7 (1.85)	113.7 (0.38)	0.31
Severely Obese	120.9 (3.49)	116.2 (0.65)	0.18
Diastolic blood pressure, mmHg			
Normal weight	60.8 (0.45)	61.2 (1.72)	0.82
Overweight	59.8 (0.59)	61.7 (1.45)	0.19
Obese	59.7 (0.67)	60.8 (2.17)	0.59
Severely obese	61.1 (1.46)	60.8 (3.75)	0.94
High density lipoprotein, mg/dL			
Normal weight	50.3 (1.23)	52.9 (0.29)	0.05
Overweight	44.9 (1.66)	45.9 (0.37)	0.53
Obese	41.0 (2.10)	43.9 (0.45)	0.19
Severely Obese	37.2 (3.07)	41.1 (0.92)	0.24
Triglycerides, mg/dL			
Normal weight	92.3 (10.77)	78.4 (1.14)	0.21
Overweight	105.4 (11.05)	100.3 (3.27)	0.63
Obese	115.9 (15.40)	113.2 (4.53)	0.85
Severely Obese	173.0 (23.62)	131.0 (12.76)	0.12
Glucose, mg/dL			
Normal weight	92.8 (1.32)	92.4 (0.37)	0.80
Overweight	96.7 (1.16)	94.0 (0.35)	0.03
Obese	96.8 (1.59)	95.2 (0.47)	0.32
Severely Obese	94.5 (1.10)	95.9 (1.00)	0.39

^aNormal weight = body mass index < 85th percentile for age and sex, overweight = body mass index $\geq 85^{\text{th}}$ - < 95th percentile for age and sex, obese = body mass index $\geq 95^{\text{th}}$ percentile for age and sex (Kuczmarski *et al*^[23], 2000) severely obese = absolute body mass index ≥ 35 kg/m² (Kelly *et al*^[24], 2000).

fasting glucose; among those who were overweight, mean values were significantly higher in those with disabilities (96.7 mg/dL) *vs* those without disabilities (94.0 mg/dL, $P = 0.03$). Normal weight adolescents with disabilities were significantly more likely to have an elevated waist circumference *vs* those children without disabilities (74.7 cm *vs* 73.1 cm, $P = 0.01$) (Table 2).

With the exception of diastolic blood pressure and triglycerides, overweight, obese and severely obese adolescents with and without disabilities were significantly more likely to have abnormal or elevated levels of waist circumference, systolic blood pressure, HDL cholesterol, triglycerides, fasting glucose, and MetS *vs* their normal weight counterparts. A higher proportion of overweight, obese and severely obese children with disabilities had abnormal SBP, fasting lipids and glucose as well as MetS (15.7% of overweight, 28.1% of obese, 61.3% of severely obese) *vs* their peers without disabilities (9.1%, 15.4%, 31.2%, respectively) (Table 3).

Adjusted logistic regression analysis showed that disabled adolescents are more than 3 times as likely as their nondisabled peers to have the MetS (AOR = 3.45, 95%CI: 1.08-11.0, $P = 0.04$). Females were significantly less likely to have MetS *vs* males (OR = 0.33, 95%CI: 0.21-0.53, $P < 0.001$) (Table 4).

DISCUSSION

Our results here show that US adolescents with disabilities are disproportionately affected by obesity and are over three times as likely to have the MetS *vs* their peers with no disabilities. Half of all adolescents with disabilities are overweight, obese or severely obese. In addition to the MetS, obese adolescents with disabilities are significantly more likely than their normal weight counterparts to have increased or abnormal systolic blood pressure, lipid and fasting glucose levels, placing them at risk for cardiovascular disease and/or type 2 diabetes.

The findings in this study are consistent with previous literature describing higher rates of obesity and obesity related conditions in adults with disabilities^[3]. Specifically, Froehlich-Grobe *et al*^[3] reported that the prevalence those with disabilities have a significantly higher prevalence of obesity and extreme obesity (41.6% and 9.3%, respectively) compared to individuals without disabilities (29.2% and 3.9%, respectively). Additionally, those with disabilities at all weight categories were significantly more likely to have cardiometabolic risk factors and overt disease risk present. Furthermore, when comparing level of physical activity among disabled and nondisabled adolescents the literature consistently shows that adolescents with disabilities are less

Table 3 Prevalence of abnormal cardiometabolic disease risk factors among those who are overweight, obese and severely obese and 12-18 years old with and without disabilities in the United States compared to those of normal weight^a, 1999-2010 National Health and Nutrition Examination Surveys *n* (%)

Cardiometabolic disease risk factors	Normal weight ^a	Overweight	<i>P</i> -value	Obese	<i>P</i> -value	Severely obese	<i>P</i> -value
Waist circumference, cm ^b							
Disability	0 (0)	33 (29.7)	< 0.0001	33 (53.0)	< 0.0001	15 (90.7)	< 0.0001
No disability	0 (0)	441 (23.8)	< 0.0001	432 (43.6)	< 0.0001	225 (94.6)	< 0.0001
Systolic blood pressure, mmHg ^c							
Disability	5 (4.2)	20 (17.6)	0.02	16 (24.2)	0.01	7 (37.4)	0.004
No disability	145 (3.7)	251 (12.8)	< 0.0001	169 (14.5)	< 0.0001	57 (21.5)	< 0.0001
Diastolic blood pressure, mmHg ^c							
Disability	15 (11.6)	17 (16.5)	0.49	8 (16.0)	0.69	2 (16.2)	0.86
No disability	367 (11.2)	194 (11.4)	0.83	121 (12.5)	0.35	45 (19.6)	0.0002
High density lipoprotein, mg/dL ^d							
Disability	23 (15.7)	42 (37.8)	< 0.001	30 (47.0)	0.001	11 (62.0)	0.006
No disability	370 (13.2)	583 (33.7)	< 0.0001	390 (41.0)	< 0.0001	113 (56.0)	< 0.0001
Triglycerides, mg/dL ^d							
Disability	13 (23.3)	21 (34.2)	0.82	16 (45.8)	0.06	7 (67.1)	0.007
No disability	224 (16.6)	271 (31.4)	< 0.0001	182 (40.8)	< 0.0001	46 (50.1)	< 0.0001
Glucose, mg/dL ^e							
Disability	9 (6.1)	13 (15.5)	0.03	8 (13.9)	0.5	0 (0)	-
No disability	232 (7.4)	171 (8.5)	0.35	107 (10.7)	0.02	26 (12.1)	0.05
Metabolic syndrome (≥ 3 risk factors)							
Disability	0 (0)	17 (15.7)	< 0.0001	17 (28.1)	< 0.0001	11 (61.3)	< 0.0001
No disability	8 (0.20)	153 (9.1)	< 0.0001	144 (15.4)	< 0.0001	71 (31.2)	< 0.0001

^aNormal weight = body mass index < 85th percentile for age and sex, overweight = body mass index $\geq 85^{\text{th}}$ -< 95th percentile for age and sex, obese = body mass index $\geq 95^{\text{th}}$ percentile for age and sex (Kuczmarski *et al.*^[23], 2000) severely obese = absolute body mass index ≥ 35 kg/m² (Kelly *et al.*^[24], 2000); ^b> 90th percentile for age and sex (Fernandez *et al.*^[18], 2004); ^c> 90th percentile for age and sex (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004); ^d> 90th percentile for age and sex for triglycerides, < 10th percentile for age and sex for HDL cholesterol (Hickman *et al.*^[20], 1998); ^e> 100 mg/dL (American Diabetes Association, 2006).

likely to participate in sports or regular physical activity and are thus exposed to more inactivity *via* screen time such as TV, computer and video games^[27].

Qualitative research has identified various barriers to facilitate participation in fitness and recreation programs and facilities among those with disabilities. These barriers include but are not limited to the built and natural environment, equipment, interpretation of guidelines, regulations, and laws, professional knowledge, education, and training issues; and facility- and community-level policies and procedures^[28]. Research conducted in urban areas suggests that three out of five individuals with disabilities do not have sidewalks between their residences and the nearest bus stop, and over 70% lack curb cuts and bus shelters^[29].

Thus, a Healthy People 2020 recommendation is to include those with disabilities in health promotion programs that include both healthy eating and active living components to help decrease their health risks^[1]. Inclusion of persons with disabilities in urban planning and transportation planning processes, and promoting the principles of Universal Design^[30] are also recognized as an important strategies. Similarly, the National Prevention Strategy, whose aim is to improve the health of each American at every stage of life and eliminate all health disparities, has formulated a plan that includes improving social inclusion of those with disabilities with mental and emotional well-being, healthy eating and active living with all citizens^[31]. The combination of healthy eating and active living programs have a positive health effect

on people with disabilities, including a decrease in weight and BMI, becoming more fit^[32], higher fruit and vegetable intake and self-reported activity levels, and decreased health risks.

We report that half of all United States adolescents with disabilities are either overweight, obese or severely obese, which has strong implications for adult health. Previous studies have documented the importance of childhood obesity as one of the strongest risk factors for adult obesity and cardiometabolic disease^[14,15]. We also found that adolescents with disabilities are at over triple the risk for the MetS *vs* their peers with no disabilities, which also has direct implications for their adult health. Previous studies have shown that if MetS is present in the childhood years, that individual has an almost 10 fold risk for cardiovascular disease, and 4 fold risk for type 2 diabetes as an adult^[7,14,15]. Our findings here suggest that adolescents with disabilities who are concomitantly challenged with unhealthy weight should be closely monitored for associated cardiometabolic risk to prevent chronic disease onset.

Limitations

A few study limitations should be noted. First, because NHANES is a cross-sectional design, causality cannot be inferred (*e.g.*, whether disability causes obesity or vice versa). Second, the prevalence of obesity in this subpopulation of NHANES data may be underestimated because those with the most severe disabilities may not be able to participate. Additionally, height and weight was

Table 4 Odds Ratios to predict the metabolic syndrome by selected covariates among those 12-18 years old with and without disabilities in the United States, 1999-2010 National Health and Nutrition Examination Survey

	OR (95%CI)	P-value
Disability status		
No disability (ref)	1	-
Disability	3.45 (1.08-10.99)	0.03
Age		
12 years old (ref)	1	-
> 12 years old	1.22 (1.03-1.44)	0.02
Sex		
Male (ref)	1	-
Female	0.33 (0.21-0.53)	< 0.0001
Ethnicity		
Non-Hispanic white (ref)	1	-
Race/Ethnicity	0.77 (0.59-1.00)	0.05
Education		
< High School (ref)	1	-
Education level	1.01 (0.97-1.05)	0.70
Household income		
> \$75000	1.02 (1.00-1.03)	0.05
Household Income		

not recorded in those participants who could not stand independently. However, our analysis only included those participants who had all cardiometabolic disease risk factors available, including BMI and waist circumference. Finally, information on specific category of disability (autism, Down's syndrome) was not available for NHANES participants under the age of 19.

Conclusion

Recently, the American Medical Association (AMA) officially labeled obesity as a disease “requiring a range of medical interventions to advance obesity treatment and prevention”^[33]. This statement has direct implications for our finding here that half of all US adolescents with disabilities are either overweight, obese or severely obese. As adolescents, those with disabilities are already more than three times as likely as their peers without disabilities to have the MetS. Future research efforts should focus on the etiology of the disproportionate prevalence of both obesity and cardiometabolic disease risk in those with developmental disabilities. Our findings suggest that overweight and obese adolescents with disabilities should be clinically monitored for elevated weight and concomitant cardiometabolic disease risk factors throughout their teenage years.

COMMENTS

Background

The prevalence of obesity is 38% higher in children with disabilities and mobility limitations compared to their peers without disabilities. Similarly, 57% of adults who are disabled are obese compared to 35.7% of peers without disabilities. Healthy People 2020 reports that not only are individuals with disabilities more likely to be overweight or obese, they are also less likely to engage in outdoor physical activities, less likely to have social support to do so, and have worse overall health status vs their non-disabled counterparts. There are few population-based studies examining the prevalence of cardiometabolic

risk among adolescents with disabilities despite their increased prevalence of obesity vs their peers without disabilities. Therefore, the purpose of the current analysis is to estimate the prevalence of cardiometabolic disease risk, including the metabolic syndrome, among the United States adolescent population with and without developmental physical and/or learning disabilities by weight status (normal weight, overweight, obese, severely obese).

Research frontiers

The purpose of the current analysis is to estimate the prevalence of cardiometabolic disease risk, including the metabolic syndrome, among the United States adolescent population with and without developmental physical and/or learning disabilities by weight status (normal weight, overweight, obese, severely obese).

Innovations and breakthroughs

The results here show that United States adolescents with disabilities are disproportionately affected by obesity and are over three times as likely to have the metabolic syndrome vs their peers with no disabilities. Half of all adolescents with disabilities are overweight, obese or severely obese. In addition to the metabolic syndrome, obese adolescents with disabilities are significantly more likely than their normal weight counterparts to have increased or abnormal systolic blood pressure, lipid and fasting glucose levels, placing them at risk for cardiovascular disease and/or type 2 diabetes.

Applications

The findings suggest that overweight and obese adolescents with disabilities should be clinically monitored for elevated weight and concomitant cardiometabolic disease risk factors throughout their teenage years.

Terminology

The metabolic syndrome is defined as having ≥ 3 of the following cardiometabolic disease risk factors present simultaneously - elevated blood pressure, elevated glucose concentrations, hypertriglyceridemia, low high density lipoprotein cholesterol concentrations, and central adiposity (elevated waist circumference).

Peer review

This is a very interesting and well written manuscript.

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Observational Study

Glycaemic control, treatment satisfaction and quality of life in type 2 diabetes patients in Greece: The PANORAMA study Greek results

Iraklis Avramopoulos, Alexandros Moulis, Nikos Nikas

Iraklis Avramopoulos, Hypertension Clinic, Hygeia Hospital, 15123 Maroussi, Athens, Greece
Alexandros Moulis, Nikos Nikas, Medical Department, AstraZeneca SA, 15125 Athens, Greece

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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at avramopoulos@medweb.gr. Consent from participants was not obtained but the presented data are anonymized and risk of identification is low.

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Correspondence to: Dr. Iraklis Avramopoulos, Hypertension Clinic, Hygeia Hospital, Er. Stavrou 4, 15123 Maroussi, Athens, Greece. avramopoulos@medweb.gr

Telephone: +30-21-06867060

Fax: +30-21-06867225

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European patients with type 2 diabetes mellitus (T2DM). We present the Greek population data of the study.

METHODS: An observational multicenter, cross-sectional study evaluating glycaemic control and a range of other clinical and biological measures as well as quality of life (QoL) and treatment satisfaction in 375 patients with T2DM enrolled by 25 primary care sites from Greece.

RESULTS: The mean age of the patients was 63.5 years and the male/female ratio 48.9%/51.1%. 79.7% of the patients exerted none or light physical activity, 82.4% were overweight or obese and 32.9% did not meet HbA1c target of less than 7.0% (53 mmol/mol). Patients reported high satisfaction to continue with treatment, high satisfaction with administered treatment and increased willingness to recommend treatment to others (mean Diabetes Treatment Satisfaction Questionnaire score 29.1 ± 5.6). However, 80% of the patients reported that their QoL would be better without diabetes. Finally, the most challenging parameter reported was the lack of freedom to eat and drink.

CONCLUSION: This analysis of the Greek Panorama study results showed that a considerable percentage of T2DM patients in Greece do not achieve glycaemic target levels, despite the favourably reported patient satisfaction from administered therapy. Additionally, the majority of primary care T2DM patients in Greece depict the negative effect of the disease in their QoL.

Key words: Quality of life; Treatment satisfaction; Type 2 diabetes mellitus

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Core tip: Diabetes is a common, chronic disease with serious complications. Despite the multiple antidiabetic

Abstract

AIM: To provide an update on glycaemic control in

treatment options and the clear treatment guidelines, a significant proportion of type 2 diabetes patients do not achieve the glycaemic goals. Few studies have examined the quality of life in these patients. PANORAMA was a Pan-European multinational study that provided an update of the glycaemic control and quality of life in patients with diabetes. The Greek results of this study showed that a significant proportion of Greek patients were not under glycaemic control despite the high satisfaction that they had from their treatment. A negative impact of the disease in quality of life was also noted.

Avramopoulos I, Moulis A, Nikas N. Glycaemic control, treatment satisfaction and quality of life in type 2 diabetes patients in Greece: The PANORAMA study Greek results. *World J Diabetes* 2015; 6(1): 208-216 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i1/208.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i1.208>

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic and complex metabolic disease characterized by hyperglycaemia, as a result of insulin resistance, impaired insulin secretion and excessive or abnormal glucagon release. It is well established that its prevalence increases globally especially in the developed countries, and this increased prevalence is associated with deleterious changes in lifestyle, unhealthy eating patterns and reduced physical activity^[1]. Epidemiological studies in the Greek population have shown that diabetes prevalence is also on the rise, increasing from 5.7% in 2001 to 10.4% in 2006^[2]. At the same time cardiovascular (CV) risk factors, tightly related to T2DM, such as obesity, hypertension and hypercholesterolemia demonstrated even a greater increase^[3]. Many effective pharmacological treatments for diabetes are now available that can be initiated after the behavioural modifications of exercise and diet. However, despite the progress in treatment strategies, many patients still face difficulties in achieving or maintaining HbA1c target levels. Moreover, diabetes is often accompanied by complications, stemming from various reasons including non-adherence to treatment and delayed adjustment of treatment regimen leading to progressive loss of β -cell function^[4,5]. These complications have a negative impact on patients' satisfaction with treatment as well as patients' quality of life (QoL)^[6-8]. Moreover, living with diabetes, reduces health related QoL which is often manifested as loss of functional ability, restrictions and barriers to everyday activities, limitations to work capacity and poor general health, while on the other hand may propagate psychological disorders such as anxiety and depression^[7,9-12]. Although the advantages and disadvantages of treatment intensification on glycaemic control and various clinical measures have been the focus of several recent investigations^[13-16], the data available

on patients' QoL and treatment satisfaction, especially in primary care, are still sparse. The Pan-European study PANORAMA has attempted to satisfy the need for a more up-to-date national and European data on glycaemic control from a pool of T2DM patients treated with diet, oral anti-diabetes drugs (OAD) and/or injectables. Given the alarming reports of increased prevalence of T2DM in Greece, the present study aimed at investigating the level of glycaemic control in T2DM patients and describing diabetes treatment satisfaction, QoL and fear of hypoglycaemic episodes in the Greek population of the PANORAMA study.

MATERIALS AND METHODS

Study design

The objectives, design and methodology of the study have recently been published by Bradley *et al.*^[17]. PANORAMA was an observational multicentre, multinational (Belgium, France, Germany, Greece, Italy, The Netherlands, Spain, Turkey and United Kingdom), cross-sectional study (NCT00916513), evaluating glycaemic control and a range of other clinical and biological measures as well as health-related QoL and treatment satisfaction in patients with T2DM. In Italy and Turkey, physicians were recruited both from hospitals and primary care practices due to country-specific healthcare systems, whereas the physicians from the other participating countries were recruited from the primary care setting only. The group of participating investigators in Greece ($n = 25$) included both diabetologists/endocrinologists ($n = 10$) and internists ($n = 15$).

Study population

Eligible patients enrolled in the study were aged ≥ 40 years, diagnosed with T2DM at least one year prior to study initiation and had at least 1-year of available medical records at the participating site. Patients were given dietary and exercise advice, and could have been treated with OADs with or without insulin as well as with GLP-1 receptor agonists, with treatment unchanged within the previous 3 mo. The study excluded patients with type 1 diabetes and/or history of diabetic ketoacidosis, secondary diabetes and pregnant women. Also, excluded from the study were patients treated with systemic corticosteroids other than replacement therapy, patients already participating in a clinical trial and patients unable to complete the questionnaires. The PANORAMA study accommodated two methods of enrolling patients, a randomized and a sequential method^[17]. In Greece, patient enrolment followed the sequential method, where each participating physician sequentially enrolled patients that attended the participating centre for a routine visit.

Study procedures

Once patients had signed the informed consent, data from their medical records were collected during a single study visit (index visit). These data included patient's socio-

demographic and anthropometric characteristics (age, gender, weight, height, educational level, socioeconomic status, alcohol consumption, smoking status, physical activity), biological measures [blood glucose, HbA1c levels, lipids: LDL-C, HDL-C, triglycerides (TG), total cholesterol (TC)] and disease-related variables (duration of diabetes, current and past diabetes treatment regimens, hypoglycaemic episodes, macrovascular and microvascular complications). The HbA1c levels of each patient were recorded by the physician at a single index visit using Bayer's A1CNow® device (certified test by the United States National Glycohemoglobin Standardization Program). This HbA1c measurement determined whether treatment goals had been achieved.

Patient reported outcomes

Patients' reported outcomes (PROs), using validated translations of standard and widely used assessment tools, were recorded *via* the DTSQ (Diabetes Treatment Satisfaction Questionnaire), ADDQoL (Audit of Diabetes Dependent QoL), worry subscale of HSF-II (Hypoglycaemia Fear Survey-II) and EQ5D (EuroQoL health utility questionnaire). Composite scores were calculated according to defined algorithms for each instrument.

DTSQ is a self-administered instrument that has demonstrated validity and reliability in diabetes populations and is recommended by the World Health Organization (WHO) and the International Diabetes Federation (IDF). The DTSQ assesses treatment satisfaction over the few weeks before its completion. The treatment satisfaction score is the sum of six of the items of the DTSQ for each respondent. Each of the treatment satisfaction scale item is scored from 6 to 0 with a higher score indicating greater satisfaction. The treatment satisfaction score can range between 36 (very satisfied) and 0 (very dissatisfied). The two additional items measuring perceived frequency of hypo- and hyperglycaemia are scored from 0 (none of the time) to 6 (most of the time)^[18,19].

ADDQoL is an individualized measure of the impact of diabetes on QoL. It is a self-administered questionnaire with 21 items. The first 19 items concern specific life domains such as social and work life and are scored on a 5-point impact scale, accompanied by a related importance rating scale for each domain used to assess the importance of each aspect of life for the individual's QoL. Weight impact scores can range from +3 (maximum positive impact of diabetes) to -9 (maximum negative impact of diabetes). The 2 remaining overview items are scored separately and include a single diabetes-specific QoL item measuring the impact of diabetes on QoL that is scored from +1 (maximum positive impact of diabetes) to -3 (maximum negative impact of diabetes) and a single item, present QoL, that is scored from +3 (excellent) to -3 (extremely bad) to measure overall QoL^[20,21].

The worry subscale of HSF-II consists of 18 items, rated by patients using a 5-point Likert scale ranging from 0 (never) to 4 (almost always). The 18 items are preceded

by the statement "Because my blood sugar could drop, I worried about ...". Scores on the "worry" subscale range from 0 to 72, with 0 representing "least worry"^[22,23].

Statistical analysis

Statistical analysis was performed using the SAS system (SAS for Windows v8.2) according to Statistical Analysis Plan prepared prior to database lock. Data were summarised by standard summary statistics. The continuous variables of age, duration of T2DM and HbA1c at index visit were expressed as mean \pm SD. Additionally, the categorical variables of demographics, disease characteristics, treatment regimens, physicians' perceptions for not reaching HbA1c goals and corrective actions taken, blood and lipid profiles and microvascular and macrovascular complications were expressed as frequencies.

RESULTS

Study population

The Pan-European data presenting the current level of glycaemic control and its associated factors in T2DM patients, as well as the data for the Spanish subgroup were published by Depablos-Velasco *et al.*^[24,25]. The PANORAMA study in Greece enrolled 375 patients. Their mean age was 63.5 ± 10.0 years with males and females proportionally represented (48.9% men *vs* 51.1 % women). Obesity was observed in 42.8% of the patients, while 21.9% were current smokers and 79.7% reported no or very light (less than once a week) physical activity. Demographic and other basic patients' characteristics are presented in Table 1.

Disease characteristics and comorbidities

Mean duration of T2DM in the Greek PANORAMA study population was 9.7 ± 8.8 years with 63.5% of patients presenting the disease for more than 5 years (Table 2). In total, 26.9% of patients suffered microvascular complications, with the most frequent being diabetic nephropathy and chronic diabetic polyneuropathy. In parallel, 24.0% of patients presented macrovascular disease. Coronary heart disease was the most prevalent complication (Table 3).

Diabetes management

Exercise and dietary advice only, was the treatment of 5.3% of the study population. Hence, the majority of the patients (94.7%) were under pharmacological treatment consisting of OADs only (65.1%), 2.7% received GLP-1 agonists, and 24.3% insulin with or without OADs. Regarding OADs, metformin was used by 73.3% patients, while fixed-dose combinations were administered to 12.3% of the patients. The most frequently administered oral hypoglycaemic agents are presented in Table 4.

Glycaemic control

The patients' mean HbA1c, recorded in the index visit of the PANORAMA study, was $6.7\% \pm 1.0\%$ (50 mmol/mol), while the 32.9% of the patients failed

Table 1 Demographic and anthropometric data in the Greek PANORAMA study population

	<i>n</i> (%)
Age (yr, mean \pm SD)	63.5 (\pm 10.0)
Gender (males)	183 (48.9)
Physical activity	
None	84 (22.5)
Light (less than 1 time/wk)	214 (57.2)
Intense (1 to 2 times/wk)	48 (12.8)
Intense (3 or more times/wk)	28 (7.5)
Body mass index	
Normal (18.5-25 kg/m ²)	66 (17.6)
Overweight (25-30 kg/m ²)	148 (39.6)
Obese (\geq 30 kg/m ²)	160 (42.8)
Smoking status	
Never smoker	205 (54.8)
Former smoker	87 (23.3)
Current smoker	82 (21.9)
Alcohol consumption (units per week)	
Males	2.1 (3.3)
Females	0.6 (1.6)

Table 2 Disease characteristics in Greek PANORAMA study population

	mean \pm SD
Average duration of type 2 diabetes in years (<i>n</i> = 375)	9.7 (\pm 8.8)
Duration of type 2 diabetes	<i>n</i> (%)
< 5 yr	137 (36.5)
\geq 5 yr	238 (63.5)
Years on insulin treatment, (<i>n</i> = 82, yr, mean \pm SD)	4.8 (\pm 7.2)

to meet HbA1c target levels presenting with HbA1c \geq 7.0% (53 mmol/mol) (Table 5). When physicians were asked about the reasons for not reaching HbA1c target, the most frequent answer was poor patient adherence to dietary and exercise recommendations (39.5%), while other common reasons were failure of current drug regimen, resistance or reluctance of the patient to intensify the medication regimen, poor patient adherence to self-monitoring of blood glucose levels, and reluctance of physician to intensify the regimen due to fear of hypoglycaemia. In order to achieve HbA1c target, reported actions taken by the physician included retraining of patients in diet/lifestyle recommendations that need to be adopted (educational approach) (42.7%) and intensification of dose of the current anti-hyperglycaemic medication (27.5%). The addition of another OAD agent was chosen as corrective action in 11.2% of the cases. Initiation of insulin treatment, with or without changing OAD medication, was recorded in a small percentage of cases (Tables 6 and 7).

Cardiovascular risk factors

More than half of the population did not attain LDL-cholesterol (LDL-C) target < 100 mg/dL (2.586 mmol/L) with 55.8% of the patients appearing with LDL-C \geq 100 mg/dL (2.586 mmol/L). Similarly, 40.4% of the

Table 3 Microvascular and macrovascular complications in Greek PANORAMA study population

	<i>n</i> (%)
Microvascular complications	
Any complication	101 (26.9)
Chronic diabetic polyneuropathy-Asymptomatic	33 (8.8)
Chronic diabetic polyneuropathy-Symptomatic	29 (7.7)
Autonomic neuropathy	6 (1.6)
Diabetic retinopathy	27 (7.2)
Diabetic nephropathy	49 (13.1)
Diabetic nephropathy-Microalbuminuria	32 (8.5)
Diabetic nephropathy-Proteinuria	12 (3.2)
Diabetic nephropathy-Renal insufficiency	9 (2.4)
Diabetic nephropathy-Dialysis	0 (0)
Macrovascular complications	
Any complication	91 (24.0)
Coronary heart disease	70 (18.7)
Cerebrovascular disease	10 (2.7)
Peripheral artery disease	21 (6.6)

Table 4 Diabetes treatment regimens in the PANORAMA study population

Treatment regimen	<i>n</i> (%)
No diet, no orals, no injectables (no available data)	10 (2.7)
Only diet and/or exercise	20 (5.3)
Only OADs	244 (65.1)
On oral plus insulin	63 (16.8)
Only on insulin	28 (7.5)
On GLP-1 analogues \pm insulin ¹	10 (2.7)
Oral hypoglycaemic agents	316 (84.3)
Sulphonylureas	121 (32.3)
Meglitinides/Glinides	12 (3.2)
Biguanides	275 (73.3)
Thiazolidinediones	41 (10.9)
DPP-4 inhibitors	94 (25.1)
Alpha glucosidase inhibitors	13 (3.5)
Fixed-dose combinations	46 (12.3)
Thiazolidinediones + metformin	3 (6.5)
DPP4 inhibitors + metformin	43 (93.5)

¹One patient receiving a GLP-1 analogue and insulin was classified in the latter category making the total number of patients 91 when the number should have been 92. OAD: Oral anti-diabetes drugs.

population appeared with triglyceride (TG) levels \geq 150 mg/dL (1.6935 mmol/L), while 24.3% of the population was off-target at \leq 40mg/dL (1.0344 mmol/L) for HDL-cholesterol (HDL-C). Additionally, the majority of patients did not also achieve blood pressure targets since 69.8% of the study's patients reported systolic/diastolic blood pressure \geq 130/80 mmHg (target \leq 130/80 mmHg) (Table 8).

Patient reported outcomes

DTSQ questionnaire: In the PANORAMA study, the Greek population's mean DTSQ score reported by the patients was 29.1 ± 5.6 . Patients reported high satisfaction grades in all domains of the questionnaire; satisfaction with treatment, convenience, flexibility and understanding of diabetes, willingness to recommend treatment to

Table 5 Glycaemic control in the Greek PANORAMA study population

Glycaemic control	n (%)
HbA1c value at index visit (mean \pm SD)	6.7 (\pm 1.0) (50 mmol/mol)
HbA1c value at index visit	
< 6.5% (47 mmol/mol)	179 (47.9)
\geq 6.5% (47 mmol/mol)	195 (52.1)
< 7.0% (53 mmol/mol)	251 (67.1)
\geq 7.0% (53 mmol/mol)	123 (32.9)

Table 6 Physicians' perceptions on reasons for not reaching HbA1c target

Reasons	n (%)
Therapeutic failure of current drug regimen	52 (13.9)
Poor patient adherence to diet and exercise	148 (39.5)
Poor patient adherence to self-monitoring of blood glucose levels	44 (11.7)
Poor patient adherence to recommendations	26 (6.9)
Resistance/reluctance of the patient to intensify his/her medication regimen	46 (12.3)
Reluctance of physician to intensify medication regimen	3 (0.8)
Reluctance of physician to intensify medication regimen-Fear of hypoglycaemia	44 (11.7)
Reluctance of physician to intensify medication regimen-Fear of unwanted side effects	14 (3.7)
Reluctance of physician to intensify medication regimen-Fear of interaction with other medications	6 (1.6)
Reluctance of physician to intensify medication regimen-Cost of treatment	9 (2.4)
Reluctance of physician to intensify medication regimen-Fear of additional weight gain	13 (3.5)

someone else and satisfaction to continue with current treatment. Unacceptably high or unacceptably low glucose levels were rarely reported. DTSQ scores were presented in Figure 1.

ADDQoL questionnaire: The mean ADDQoL questionnaire score reported by patients was -2.0 ± 1.9 . Overall, 79.5% of patients reported that their QoL would be better if they did not have diabetes. Following analysis of the individual questionnaire components, the most affected parameters of ADDQoL were freedom to eat and drink (Figure 2).

HFS questionnaire: The mean HFS questionnaire total score for the Greek PANORAMA population was 14.2 ± 14.7 . Taking into consideration that the score for the greatest fear equals to 72, the reported HFS score in the study represents an overall mild fear of hypoglycaemia. In particular, 15.3% of the patients were frequently afraid of having a hypoglycaemic episode while alone or during sleep where no one would be present to help (Figure 3).

DISCUSSION

The European PANORAMA study investigated the level of glycaemic control in Europe in addition to patients' treatment satisfaction and QoL^[17]. Here, the Greek

Table 7 Actions taken by the physicians to reach HbA1c target

Actions taken	n (%)
No specific actions	45 (12.0)
Educational approach	160 (42.7)
Increase dose of current medication	103 (27.5)
Addition of new oral antihyperglycaemic medication	
Sulphonylureas	8 (2.1)
Meglitinides/Glinides	4 (1.1)
Biguanides	5 (1.3)
Thiazolidinediones	3 (0.8)
DPP-4 inhibitors	13 (3.5)
Combination treatment	9 (2.4)
Start insulin therapy without changing oral diabetes medication	9 (2.4)
Start insulin therapy changing oral diabetes medication	14 (3.7)
Other action	9 (2.4)

Table 8 Blood pressure and lipid profile: Percentage of patients who meet AACE criteria for target blood pressure and lipid levels

	n (%)
Hypertension	
SBP/DBP < 130/80 mmHg	113 (30.2)
Triglycerides	
< 150 mg/dL (1.6935 mmol/L)	221 (59.6)
LDL-C	
< 100 mg/dL (2.586 mmol/L)	160 (44.2)
HDL-C	
> 40 mg/dL (1.0344 mmol/L)	278 (75.7)

AACE: American Association of Clinical Endocrinologists, AACE guidelines 2010^[35]; SBP/DBP: Systolic Blood Pressure/ Diastolic Blood Pressure; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol.

PANORAMA study results from primary care T2DM patients in Greece are discussed next. The study was performed in 2009-2010 and enrolled 375 subjects from 25 participating study centres.

Previously, other large, multinational European studies have attempted to assess the level of glycaemic control across Europe. The RECAP-DM study for example, that included Finland, France, Germany, Norway, Poland, Spain and United Kingdom, provided data on glycaemic control on T2DM patients who intensified their treatment by adding either a sulphonylurea or a thiazolidinedione to their standard metformin treatment. Approximately, 26% of European out-patients had adequate glycaemic control, *i.e.*, HbA1c < 6.5% (47 mmol/mol), after a mean of 2.6 years of combined oral antihyperglycaemic therapy. It was observed that glycaemic control modestly declined over time, even though more patients were being treated with insulin^[26]. Similarly, another earlier European, epidemiological survey on T2DM that provided data on glycaemic control was the CODE-2 study, of which many participating countries were also included in the PANORAMA study. In the CODE-2 study 69% of the patients did not attain the HbA1c target of less than 7%

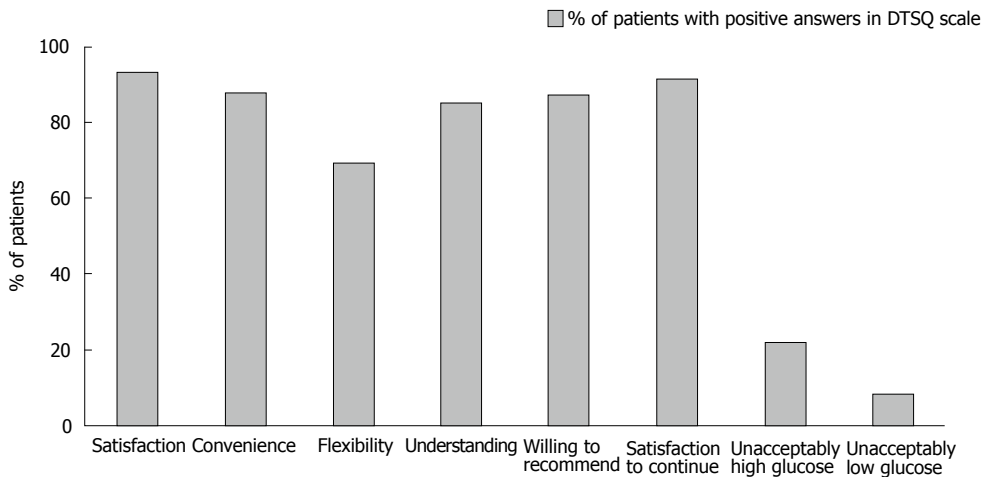


Figure 1 DTSQ questionnaire results (Diabetes Treatment Satisfaction Questionnaire - as assessed by patients) from the Greek PANORAMA study population. Graph presents the percentage of patients that provided positive answers following a series of questions of DTSQ's specific domains related to their treatment satisfaction (grades 4-6 in DTSQ scale).

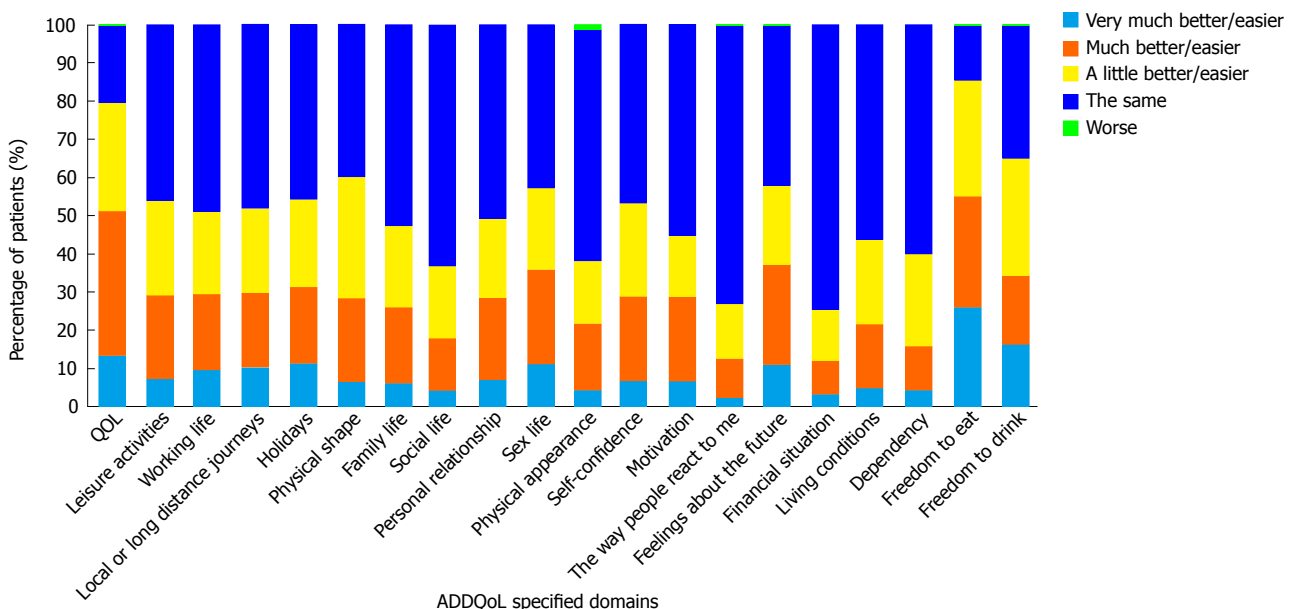


Figure 2 ADDQoL questionnaire results (Audit of Diabetes Dependent QoL) from the Greek PANORAMA study population. ADDQoL is an individualized measure of the impact of diabetes on QoL. Graph presents the distribution of answers reported by patients following the question: "If I did not have diabetes my QoL would be...".

(53 mmol/mol), as opposed to the 37.4% of the patients from the PANORAMA study and 32.9% of the Greek population from the PANORAMA study^[24,27]. The much better glycaemic control observed in PANORAMA and its Greek population in comparison to other studies can be attributed to the fact that patients were enrolled in the study only if medical records for at least the past 1 year existed in the study site. This could suggest that the study population was more closely followed.

Regarding CV risk factor control in T2DM patients from the Greek PANORAMA study, data showed that a large percentage of patients failed to meet the recommended target levels for LDL-C, triglycerides and especially blood pressure. This is in line with previous

studies conducted in Greece, showing that a considerable percentage of patients do not meet treatment goals for better CV risk control^[28,29]. CV risk factors continue to be the most critical determinants of mortality and morbidity in T2DM patients, and account for more than half of the observed mortality and morbidity in this population.

The issue of CV risk factor control emerges as a great challenge in the management and treatment of T2DM patients, especially when joint standards of medical care for patients with diabetes are considered. For example, the present data indicate inadequate control for LDL-C with 55.8% of patients not achieving LDL-C target < 100 mg/dL (2.586 mmol/L). This observation highlights the difficulty in regulating LDL-C and the status of

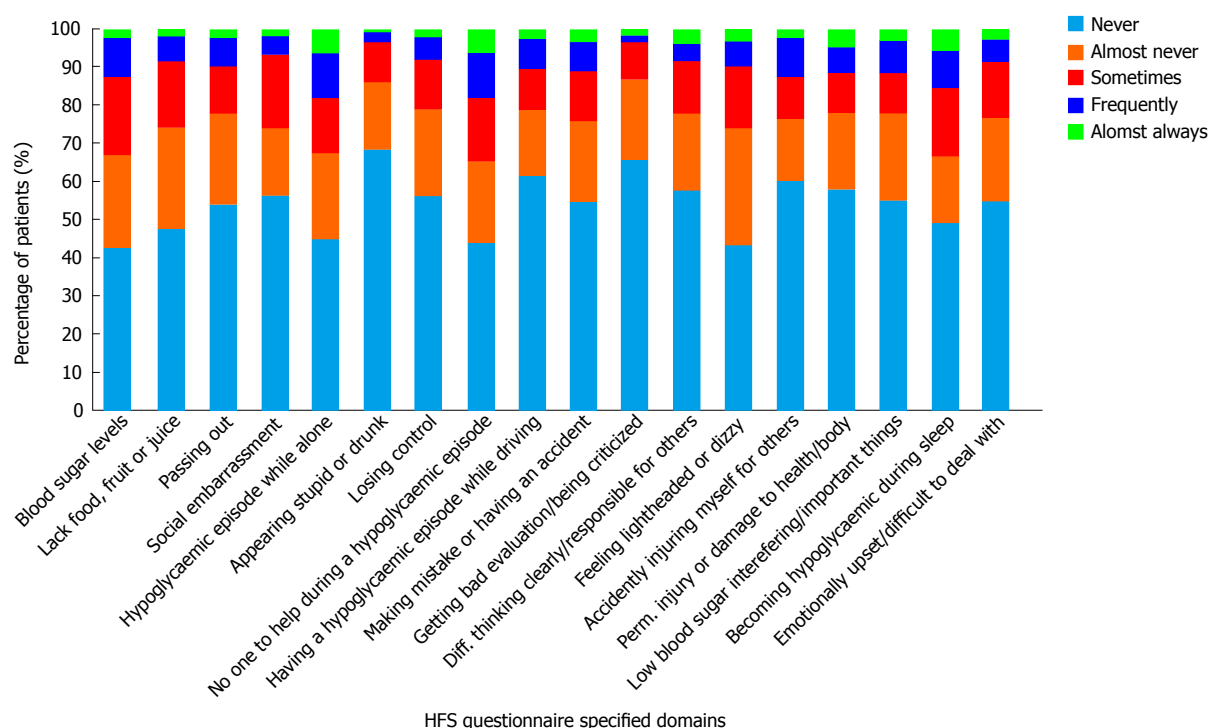


Figure 3 HFS questionnaire results (Hypoglycaemia Fear Survey-II) from the Greek PANORAMA study population. Graph presents the distribution of answers ("never", "almost never", "sometimes", "frequently" and "almost always") following questions in the 18 specific domains of the HFS questionnaire related to patients' fear of hypoglycaemia. Each of the 18 items was preceded by the statement "Because my blood sugar could drop, I worried about ...".

current available therapies and drugs.

The difficulty in total cardiovascular risk reduction observed in our results was also clearly shown in the total PANORAMA population recently published by de Pablos-Velasco *et al.*^[24], that reported that the joint triple target for HbA1c, blood lipids (total cholesterol) and blood pressure was achieved only in the 7.5% of the patients. This observation denotes an unmet medical need and that despite new improvements in pharmacotherapy, still a great deal of work is warranted for better T2DM disease management.

The majority of the Greek PANORAMA study population perceived positively their diabetes treatment. The high scores of the DTSQ questionnaire in the Greek PANORAMA study (29.1 ± 5.6 out of 36), are attributed to the high level of satisfaction reported in the sections concerning satisfaction with treatment, satisfaction to continue with treatment and willingness to recommend treatment to someone else.

DTSQ outcomes have been shown to correlate significantly with the duration of diabetes and the perceived glucose control by the patients, showing that the longer the diabetes duration and the less controlled glucose levels the more patients appear unsatisfied with their treatment^[19]. On the other hand, satisfaction also appears to be sensitive to treatment changes^[13,30] and differences between treatment groups^[31]. Furthermore, in a study about diabetes patients' perception of their disease, a clear correlation was demonstrated between patients' responses to the questionnaire, demographic characteristics, the health status and the type of their

anti-diabetic treatment^[32].

The overall ADDQoL questionnaire mean score in the present study suggests that diabetes exerts a negative impact on patients' perception of QoL. The QoL parameter identified to be most commonly, negatively affected by diabetes in the study population was freedom to eat as wish, a parameter valued as important or very important from 80.1% of the patients.

Use of the ADDQoL in people with type 1 or type 2 diabetes has shown, on average, almost universally, negative impact of diabetes on all domains^[13]. Significantly improved T2DM management has also been shown in non-insulin treated patients without complications in comparison to those insulin-treated with complications^[33]. The ADDQoL has also proven useful in detecting the negative impact of diabetes on QoL despite the high levels of treatment satisfaction, measured by the DTSQ^[34].

Lastly, the results of the use of the HSF questionnaire as a measure of the impact of hypoglycaemia in the patients' QoL, suggested a presence of a mild fear of hypoglycaemic episodes among the study population. History of hypoglycaemic episodes seems to also play an important role in shaping patients' perceptions on hypoglycaemic events^[35].

It was clear from the present data that patients were more often worried about having a hypoglycaemic episode while alone, at a time where no one would be available to help, or during sleep which by itself yields a negative impact on QoL.

The PANORAMA study has some inherent limitations such as the mixing of sampling techniques and

the cross-sectional design of the study that cannot determine the causal nature of the associations^[24]. In the present study, the A1CNow® (Bayer) was used to reduce the high variability of blood glucose measurements between centres. In addition, patient recruitment in Greece followed a sequential, rather than randomized manner, which was adopted in other European participating countries. This may raise concerns towards specific variables that could be affected by the lack of randomization at selection, such as duration of diabetes and diabetes-related problems or macro/microvascular complications, since the patients selected solely by their attendance to a participating centre may be more prone to clinic/hospital visits or diabetes related comorbidities and complications than others.

In conclusion, the Greek Panorama study data analysis demonstrated that a considerable part of the T2DM patient population does not achieve glycaemic target levels despite the coincident patient satisfaction by their administered antidiabetic treatment. Despite this high level of satisfaction, a mild fear of hypoglycaemia was detected and a considerable percentage of primary care patients, approximately 1 in 3, did not meet glycaemic goals. In parallel, the majority of the study's population reported that their QoL would be better without diabetes. Finally, since CV risk factors are proven to be inadequately controlled among T2DM patients in Greece, further intensification efforts regarding treatment and management are required to enable better management towards diminishing CV risk and to improve treatment of type 2 diabetes patients.

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COMMENTS

Background

Diabetes mellitus is a common disease with a rising prevalence worldwide. Its complications are divided to macrovascular disease (atherosclerosis) and microvascular disease which includes diabetic neuropathy, retinopathy and nephropathy. Glycaemic control is of paramount importance in patients with diabetes and it can be estimated by glycated haemoglobin (HbA1c) blood levels. According to treatment guidelines, for most patients with diabetes, a level of HbA1c lower than 7% is recommended. There are many antidiabetic medications of different categories that can be administered for blood glucose control as monotherapy or in combination in order to achieve the treatment targets.

Research frontiers

Despite the multiple available antidiabetic treatment options, a large proportion of patients with diabetes do not achieve the glycaemic goals. Furthermore, only a few studies have examined the impact of diabetes on the quality of life as well as the patients' perception of their treatment.

Innovations and breakthroughs

PANORAMA study was a multinational Pan-European study that provided an update on glycaemic control in European patients with type 2 diabetes mellitus. The authors present the results from the Greek population of the study which showed that a large proportion of patients with diabetes do not

achieve the glycaemic targets. The other cardiovascular risk factors as LDL cholesterol, triglycerides and blood pressure were also out of control in the majority of Greek patients that were enrolled in our study. Although the majority of the Greek PANORAMA study population perceived positively their diabetes treatment, most of them reported that their life has a negative impact from diabetes, especially because they do not have the freedom to eat and drink.

Applications

The primary care physician must know that most of the Greek patients with diabetes are not under control. More effort is needed to achieve glycaemic targets and cardiovascular risk factors control in these patients. Apart from treatment targets though, it must also be remembered that quality of life in these patients is reduced because of diabetes mellitus. More emphasis must be given on this issue by the physician.

Peer review

The control level of blood glucose seems to be better compared to Steno study.

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

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mTOR: Driving apoptosis and autophagy for neurocardiac complications of diabetes mellitus

Kenneth Maiese

Kenneth Maiese, Cellular and Molecular Signaling, Newark, NJ 07101, United States

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Correspondence to: Kenneth Maiese, MD, Cellular and Molecular Signaling, Newark, NJ 07101, United States. wntin75@yahoo.com

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stress is a significant determinant of cell fate during DM and leads to endoplasmic reticulum stress, mitochondrial dysfunction, apoptosis, and autophagy. Multiple strategies are being developed to combat the complications of DM, but it is the mechanistic target of rapamycin (mTOR) that is gaining interest in drug development circles especially for protective therapies that involve cytokines and growth factors such as erythropoietin. The pathways of mTOR linked to mTOR complex 1, mTOR complex 2, AMP activated protein kinase, and the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) complex can ultimately influence neuronal, cardiac, and vascular cell survival during oxidant stress in DM through a fine interplay between apoptosis and autophagy. Further understanding of these mTOR regulated pathways should foster novel strategies for the complications of DM that impact millions of individuals with death and disability.

Key words: Apoptosis; Autophagy; Cardiac disease; Diabetes mellitus; Erythropoietin; Metformin; Oxidative stress; Neurodegeneration; Mechanistic target of rapamycin; Vascular disease

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Abstract

The World Health Organization estimates that diabetes mellitus (DM) will become the seventh leading cause of death during the next two decades. DM affects approximately 350 million individuals worldwide and additional millions that remain undiagnosed are estimated to suffer from the complications of DM. Although the complications of DM can be seen throughout the body, the nervous, cardiac, and vascular systems can be significantly affected and lead to disorders that include cognitive loss, stroke, atherosclerosis, cardiac failure, and endothelial stem cell impairment. At the cellular level, oxidative

Core tip: The pathways of mechanistic target of rapamycin (mTOR) linked to mTOR complex 1, mTOR complex 2, AMP activated protein kinase, and tuberous sclerosis 1/tuberous sclerosis 2 complex can offer novel strategies for the complications of diabetes mellitus to prevent death and disability for the millions of individuals afflicted with this disorder.

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THE GROWING THREAT FROM DIABETES MELLITUS

The incidence of diabetes mellitus (DM) throughout the world is increasing at an exponential rate such that the World Health Organization predicts that DM will be the seventh leading cause of death by the year 2030^[1]. In 2013, greater than a million deaths were attributable to DM that is believed to affect 347 million individuals throughout the world. In the United States, 21 million individuals are diagnosed with DM and another 8 million individuals are estimated to suffer from DM but are currently undiagnosed^[2]. Reduced activity, increased body weight, and poor nutritional intake are considered significant factors that can lead to adult onset DM^[3,4]. Duration of obese-years rather than body mass index can become a significant risk for developing DM^[5].

DM is defined as being either non-insulin dependent (type 1) or insulin dependent (type 2)^[6,7]. Type 1 DM occurs in approximately 5%-10% of DM patients and is an autoimmune disorder with the presence of alleles of the human leukocyte antigen class II genes within the major histocompatibility complex. Destruction of pancreatic β -cells with inflammatory infiltration of the islets of Langerhans results in lost insulin production and regulation. About 90% of patients with type 1 DM have increased titers of autoantibodies (type 1A DM). The remaining 10% of type 1 DM individuals do not have serum autoantibodies and are considered to have maturity-onset diabetes of the young that can be a result of β -cell dysfunction with autosomal-dominant inheritance (type IB DM). Type 2 DM occurs in approximately 80%-90% of individuals with DM greater than the age of 40. Although approximately 10% of individuals with type 2 DM may have elevated serum autoantibodies similar to type 1 DM, type 2 DM represents a progressive deterioration of glucose tolerance with early β -cell compensation that has cell hyperplasia followed by a decrease in β -cell mass. Insulin resistance ensues as well as impairments in insulin secretion. Insulin resistance also may be a component of type 1 DM in some patients. Defective insulin secretion can result from impaired β -cell function, chronic exposure to free fatty acids and hyperglycemia, as well as the absence of inhibitory feedback through plasma glucagon levels.

CLINICAL IMPLICATIONS OF DM IN THE NERVOUS, CARDIAC, AND VASCULAR SYSTEMS

As a disease that affects all systems of the body, DM can lead to multiple clinical impairments especially in the nervous, cardiac, and vascular systems. DM results in cognitive loss not only through vascular disease and stroke^[8], but also during chronic neurodegenerative

disorders such as Alzheimer's disease^[9,10]. Insulin resistance similar to its occurrence in DM also has been reported in patients with Alzheimer's disease, suggesting that degenerative disorders such as Alzheimer's disease could be mediated in some patient populations by impaired cellular metabolism^[11]. DM also results in neuropsychiatric disorders^[12,13], retinal disease^[14-16], and peripheral nerve disorders^[17]. In the cardiac system, DM can lead to sympathetic nerve dysfunction^[18], cardiac fibrosis^[19,20], ischemic reperfusion injury^[21], cardiomyocyte injury^[22], and cardiac hypertrophy^[23]. DM also can significantly impact endothelial cells either in the brain or elsewhere in the body. Exposure to elevated glucose levels can result in endothelial cell senescence^[24], dysfunctional mobilization of endothelial progenitor cells from the bone marrow^[25], injury to the neuroglialvascular unit^[14], loss of angiogenesis^[26], and endothelial cell injury and loss^[27-33].

During DM, oxidative stress is an important driver of cell injury^[4,6,34-39]. In murine animal models of type 2 DM, oxidative stress can lead to elevated glutathione levels and increased lipid peroxidation^[23]. "Highly-oxidized glycated" low density lipoproteins that can occur in DM lead to oxidative and endoplasmic reticulum stress in human retinal capillary pericytes. Subsequently, mitochondrial dysfunction and cell death with apoptosis and autophagy ensues^[15]. Exposure of glucolipotoxicity caused by elevated plasma glucose and lipid levels to pancreatic β -cells promotes oxidative stress with cytochrome c release, caspase activation, and apoptosis^[40]. Advanced glycation end products (AGEs), entities that promote complications in DM^[41], lead to the release of reactive oxygen species (ROS) and caspase activation^[37]. In addition, high fat diets^[42] as well as free fatty acids have been shown to release ROS, lead to mitochondrial DNA damage, and impair pancreatic β -cell function^[43]. In cardiomyocytes^[20,22,44], neurons^[8,15,30,45,46], and endothelial cells^[14,25,27-29,47], exposure to elevated glucose levels foster oxidant stress mechanisms that can impair cellular function and lead to cell death. In clinical studies, patients with type 2 DM display serum markers of oxidative stress with ischemia-modified albumin^[48]. Interestingly, elevations in serum glucose can increase antioxidant enzyme levels in human endothelial cells, suggesting that some cells may initiate a reparative process against oxidative stress injury^[49]. Of note, chronic hyperglycemia is not necessary to lead to oxidative stress injury, since even brief periods of hyperglycemia generate ROS^[50]. Clinical correlates support these experimental studies to show that both acute glucose swings as well as chronic hyperglycemia can trigger oxidative stress mechanisms during type 2 DM^[51].

NOVEL STRATEGIES FOR DM WITH MECHANISTIC TARGET OF RAPAMYCIN

Numerous cellular pathways can lead to oxidative stress during DM. As a result, multiple therapeutic avenues are being pursued to develop therapy against

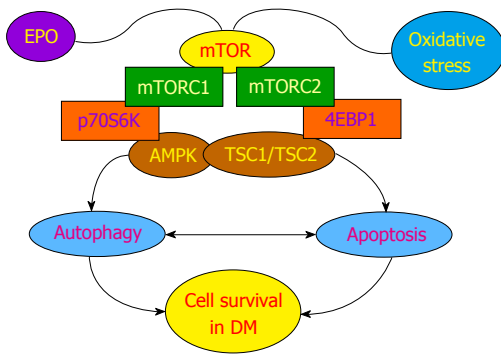


Figure 1 Cellular signaling of mechanistic target of rapamycin in diabetes mellitus. mTOR is a component of the protein complexes mTORC1 and mTORC2 with two important targets of p70S6K and 4EBP1 that promote mTOR kinase activity. mTOR signaling is controlled by AMPK that oversees the activity of TSC1/TSC2, an inhibitor of mTORC1. During periods of oxidative stress in DM, EPO uses mTOR to protect cell survival from programmed cell death injury. For example, EPO blocks cell injury in studies of diabetic retinal degeneration, maintains endothelial cell integrity during experimental models of DM, and regulates the detrimental effects of obesity in animal models. AMPK can have dual roles in cell survival. AMPK can limit oxidative stress that can lead to hypertension and reduce insulin resistance through autophagy that can have linked pathways to apoptosis. However, under other circumstances AMPK also can lead to neuroinflammation and cardiac dysfunction. Ultimately, a careful balance in the activities of autophagy and apoptosis is required through mTOR to foster cell survival during DM. mTOR: Mechanistic target of rapamycin; DM: Diabetes mellitus; AMPK: AMP activated protein kinase; EPO: Erythropoietin; p70S6K: p70 ribosomal S6 kinase; 4EBP1: 4E-binding protein 1; mTORC1: mTOR complex 1; TSC1/TSC2: Tuberous sclerosis 1/ tuberous sclerosis 2.

the complications of DM. These strategies include the recent focus upon sirtuins^[24,47,52-56], protein tyrosine phosphatases^[57,58], broad antioxidant therapies^[3,7,17,31,34,38,59], forkhead transcription factors^[56,60-63], and growth factors^[32,64-68].

In reference to growth factors, the cytokine and growth factor erythropoietin (EPO) serves as a provocative model for potential treatments for the complications of DM (Figure 1). EPO blocks cell injury in studies of diabetic retinal degeneration^[14], maintains endothelial cell integrity during experimental models of DM^[27,28], facilitates wound healing during DM^[65], reduces high glucose-induced oxidative stress in renal tubular cells^[69], maintains cellular mitochondrial function and energy metabolism^[32], and regulates the detrimental effects of obesity in animal models^[33]. Although EPO affects multiple cellular signal transduction pathways in the body^[70,71], of particular interest are the signal transduction pathways of the mechanistic target of rapamycin (mTOR) controlled by EPO that are intimately linked to cellular metabolism and DM^[72-76]. mTOR can influence neuronal, glial, and cell to cell activity^[77,78]. EPO uses mTOR to protect cells against oxygen-glucose deprivation^[79,80], limit cell injury during β -amyloid exposure^[81], control bone homeostasis^[82], improve cognitive function in models of sepsis-associated encephalopathy^[83], foster retinal progenitor cell survival during oxidant stress^[84], and promote the neuronal phenotype of adult neuronal precursor cells^[85].

mTOR, also known as the mammalian target of rapamycin and FK506-binding protein 12-rapamycin

complex-associated protein 1, is a 289-ku serine/threonine protein kinase. mTOR is encoded by a single gene *FRAP1*^[86-88] and is a component of the protein complexes mTOR complex 1 (mTORC1) and mTORC2 (Figure 1). Rapamycin, an agent that inhibits mTOR activity, blocks mTORC1 by preventing the phosphorylation of mTOR. In some cases with chronic administration, rapamycin also can inhibit mTORC2. mTORC1 is composed of raptor (regulatory-associated protein of mTOR), the proline rich Akt substrate 40 ku, deptor (DEP domain-containing mTOR interacting protein), and mLST8/G L (mammalian lethal with Sec13 protein 8, termed mLST8). Two important targets of mTORC1 through mLST8 that promote mTOR kinase activity are p70 ribosomal S6 kinase and the eukaryotic initiation factor 4E-binding protein 1^[89,90]. mTORC2 is composed of rictor (rapamycin-insensitive companion of mTOR), deptor, mLST8, the mammalian stress-activated protein kinase interacting protein (mSIN1), and the protein observed with rictor-1 (Protor-1)^[75,91].

In addition to phosphoinositide 3-kinase and protein kinase B (Akt)^[6,92], mTOR signaling also is governed by AMP activated protein kinase (AMPK)^[75,91]. AMPK can control the activity of the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2) complex that is an inhibitor of mTORC1. AMPK phosphorylates TSC2 as well as Raptor to block the activity of mTORC1 during energy stress^[93]. AMPK also controls TSC1/2 activity through RTP801 (REDD1/ product of the *Ddit4* gene). AMPK activity can increase REDD1 expression, such as in the presence of hypoxic environments, to suppress mTORC1 activity by releasing TSC2 from its inhibitory binding to protein 14-3-3^[94].

AMPK can have dual roles in cell survival (Figure 1). AMPK activation can suppress β -amyloid ($A\beta$) production^[95], regulate tau phosphorylation^[96], limit oxidative stress that can lead to hypertension^[97], increase cell survival during hypoxia^[98], and promote autophagy that may resolve memory impairment^[99]. However, in other experimental models, AMPK activity has been suggested to influence neuroinflammation^[100], lead to aberrant $A\beta$ stress^[96] and $A\beta$ toxicity^[101], result in cardiac dysfunction^[102], and result in the hypertrophy of cardiac tissues^[103]. In regards to cellular metabolism with AMPK^[104], AMPK can reduce insulin resistance and diminish oxidative stress mediated through the programmed cell death pathway of autophagy^[105], reduce myocardial ischemia in experimental models of diabetes^[21], be necessary for proper metabolic function of cells^[106], and block adipocyte differentiation, lipid accumulation, and obesity^[107]. Loss of AMPK may lead to insulin resistance^[108].

TARGETING APOPTOSIS AND AUTOPHAGY WITH mTOR FOR DM

For the development of new strategies against DM with mTOR, a careful balance in the activity of the programmed

cell death pathways of apoptosis and autophagy must be considered. Both apoptosis^[4,7,17,32,38,109] and autophagy^[6,74,110,111] can influence cell survival during oxidative stress^[112]. In regards to cellular metabolic pathways, activation of mTOR that blocks apoptotic pathways may limit insulin resistance and vascular thrombosis in patients with metabolic syndrome^[113]. Increased activity of mTOR also may prevent the development of atherosclerosis^[114]. Furthermore, mTOR activation through glucagon-like peptide-1 agonists has recently been reported to protect pancreatic β -cells from cholesterol mediated apoptotic cell injury^[115], promote pancreatic β -cell proliferation^[116], and prevent neural apoptotic cell loss during DM through the epidermal growth factor receptor^[117].

In other studies with DM, it is the induction of autophagy with requisite mTOR inhibition that is suggested to foster cellular protection. For example, metformin, an agent used to control hyperglycemia in DM, inhibits mTOR activity and promotes autophagy. Metformin can offer protection against endothelial cell senescence^[24], limit androgen up-regulation during prostate cancer through mTOR inhibition^[118], prevent cell loss during hypoxia through increased AMPK activity^[98], and protect against neuronal cell apoptosis^[119]. Metformin through pathways that activate AMPK also prevents cardiomyopathy in experimental models of DM^[120], fosters cardiomyocyte cell survival^[121], and reduces cortical infarction in stroke models^[122].

Additional work suggests that autophagy irrespective of the contribution of mTOR may be protective during DM. Autophagy haploinsufficiency in murine animal models of obesity leads to increased insulin resistance with elevated lipids and inflammation^[123], suggesting that loss of autophagy may foster the progression from obesity to DM. Autophagy also may be required to remove misfolded proteins and eliminate non-functioning mitochondria to prevent β -cell dysfunction and the onset of DM^[124]. In addition, exercise in mice has been shown to initiate autophagy and regulate glucose homeostasis^[125]. These results may be associated with observations that autophagy has been reported to improve insulin sensitivity during high fat diets in mice^[105].

Yet, in other experimental models, autophagy may not be beneficial even though it can be less of a prominent modulator of cell survival than apoptosis in some experimental models^[126]. Autophagy during high glucose exposure has been shown to impair endothelial progenitor cells, lead to mitochondrial oxidative stress, and prevent the formation of new blood vessels^[127]. Increased autophagy also has been associated with significant loss of cardiac and liver tissue in diabetic rats during attempts to achieve glycemic control through diet modification^[128]. During periods of elevated glucose that occur in DM, AGEs have been shown to lead to the induction of autophagy and vascular smooth muscle proliferation that can result in atherosclerosis^[129] as well as cardiomyopathy^[44].

FUTURE CONSIDERATIONS

DM is a significant and growing disorder throughout the world that leads to increased disability and death through multiple complications in the nervous, cardiac, and vascular systems. Current therapies for these complications are limited. As a result, novel therapeutic strategies are required to address the cellular mechanisms of oxidant stress and cell injury that can mediate complications of DM. Given the recent discovery that cytoprotective strategies against oxidative stress, *i.e.*, EPO, employ mTOR, the mTOR signaling pathways that include AMPK and TSC1/TSC2 have become increasingly recognized as a potential targets for the treatment of the complications of DM. However, future work will need to concentrate upon the complex relationship that the programmed cell death pathways of apoptosis and autophagy hold over cellular survival and longevity to attain both efficacy and safety for mTOR targeted strategies.

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Technology and diabetes self-management: An integrative review

Caralise W Hunt

Caralise W Hunt, School of Nursing, Auburn University, Auburn, AL 36849, United States

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Correspondence to: Caralise W Hunt, PhD, RN, Assistant Professor, School of Nursing, Auburn University, 219 Miller Hall, Auburn, AL 36849, United States. huntcar@auburn.edu

Telephone: +1-334-8446763

Fax: +1-334-8445654

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Abstract

Technology can be used to supplement healthcare provider diabetes care by providing both educational and motivational support. Education can be provided using technology allowing patients to learn new practices and routines related to diabetes management. Technology can support daily diabetes self-management activities including blood glucose monitoring, exercising, healthy eating, taking medication, monitoring for complications, and problem-solving. This article describes an integrative review conducted to evaluate the types of technology being used to facilitate diabetes self-management and

the effect of that technology on self-management and diabetes outcomes for adults living with type 2 diabetes mellitus. A literature review was conducted by searching Medline, PubMed, and Psych INFO databases using the search terms: diabetes self-management, technology, type 2 diabetes, smartphones, cell phones, and diabetes mellitus covering the years from 2008-2013. Articles relying on secondary data (editorials, systematic reviews) and articles describing study protocol only were excluded. Fourteen studies including qualitative, quasi-experimental, and randomized controlled trial designs were identified and included in the review. The review found that technological interventions had positive impacts on diabetes outcomes including improvements in hemoglobin A1C levels, diabetes self-management behaviors, and diabetes self-efficacy. Results indicate that technological interventions can benefit people living with diabetes when used in conjunction with diabetes care delivered by healthcare providers.

Key words: Diabetes mellitus; Diabetes self-management; Type 2 diabetes mellitus; Technology; Integrative review

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Core tip: Technology may be used to support diabetes self-management. Both mobile phone and internet-based technological interventions have been found to support self-management behaviors of people living with diabetes. Technology can extend the reach of diabetes self-management to patient's communities and homes, provide for individualized care, and provide just-in-time information.

Hunt CW. Technology and diabetes self-management: An integrative review. *World J Diabetes* 2015; 6(2): 225-233 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i2/225.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i2.225>

INTRODUCTION

Using technology to facilitate diabetes self-management is not a new idea, but as patients become more technologically savvy, devices become more available, and new technologies emerge, the variety of technological self-management strategies increases. Recent reports indicate that 90% of Americans have cellular phones and 58% of American adults have a smartphone. Among racial groups, Caucasians and African Americans have equal percentages of ownership (90%), while Hispanic Americans have a 92% ownership percentage^[1].

Technology can be used to supplement healthcare provider diabetes care by providing both educational and motivational support. Technology can extend the reach of diabetes education and support when primary care resources are insufficient or patient resources and access to care are limited^[2]. Patients may have difficulty scheduling and attending diabetes education classes or meeting regularly with a diabetes educator due to time, financial, or other constraints^[3]. Education can be provided using technological resources so that patients learn new practices and routines related to diabetes management. Technology can support the daily diabetes self-management activities of blood glucose monitoring, exercising, healthy eating, taking medication, monitoring for complications, and problem-solving. Visual feedback of clinical information, including these self-management activities, improves patients' ability to see how diabetes is affected by their behaviors and promotes decision-making and problem-solving. Monitoring of self-management behaviors can be motivational and allows for more frequent contact between patients and healthcare providers. This can lead to necessary changes in self-management behaviors and treatment plans^[4].

The purpose of this integrative review is to evaluate the types of technology being used to facilitate diabetes self-management and the effect of that technology on self-management and diabetes outcomes for adults living with type 2 diabetes mellitus. The paper identifies technological methods for self-management, outcomes from use of technology in self-management, and future recommendations for the development of technology in diabetes self-management.

RESEARCH

Articles were identified by searching Medline, PubMed, and Psych INFO databases using the search terms: diabetes self-management, technology, type 2 diabetes, smartphones, cell phones, and diabetes mellitus covering the years from 2008-2013. Articles relying on secondary data (editorials, systematic reviews) and articles describing study protocol only were excluded. Fourteen studies including qualitative, quasi-experimental, and randomized controlled trial designs were identified and included in the review. A summary of the reviewed articles is provided in Table 1.

MOBILE PHONES

Evidence suggests that mobile health applications may be used to deliver health services and self-management tools and overcome barriers to provider access^[5]. Mobile phones can offer alternatives to in-person diabetes intervention delivery and support^[6]. Mobile phones provide patients the ability to process and communicate data in real time. A meta-analysis of 22 intervention studies found that mobile phone interventions led to statistically significant improvements in glycemic control and self-management^[7]. A Cochrane review of computer-based diabetes self-management interventions found a small beneficial effect on blood glucose control with a larger effect noted in mobile phone interventions. Reviewers concluded that mobile phone interventions may be more effective due to convenience, increased contact with the intervention, and cues and feedback provided through the phone^[8].

Six of the studies examined in this review utilized a mobile phone intervention. A pilot study qualitatively evaluated a disease management program utilizing mobile phones and gaming systems for individuals living with type 2 diabetes^[9]. Participants uploaded blood glucose readings using a smartphone, received charts with blood glucose daily, weekly, and monthly trends, emailed or text-messaged healthcare providers with questions, and received reminders and messages about self-management. Qualitatively, participants reported that connecting with a healthcare provider through email was beneficial, but an initial face-to-face meeting made the email communication more meaningful. Uploading data from glucose meters to visualize trends was also beneficial for most participants. They felt that the graphs enabled them to see how their exercise and eating patterns had affected blood glucose. Participants also felt that the intervention program promoted their own general health awareness. Most participants did indicate frustration with the smartphones due to difficulty using the phone. One participant recommended using phones with which patients are already familiar for future studies. Overall, the study results indicate that mobile phones can be effective in assisting people with diabetes self-management. Personal contact with healthcare providers should be included in technological interventions and participants should be included in the decision of which type of technology to be used^[9].

A second study piloted an intervention to test the feasibility of an automated, two - way text messaging system to promote blood glucose monitoring in teenagers and young adults with diabetes^[10]. Participants were randomized to receive messages *via* cell phone text messaging or email for a three month time period. Reminders were sent to check blood glucose and if no response occurred, a second reminder was sent. After the blood glucose value was submitted, a positive feedback message was sent. If the value was out of range, a warning to take action and recheck blood glucose was sent. Of the 40 participants who enrolled in

Table 1 Summary of reviewed articles

Ref.	Technology	Study purpose	Brief description of intervention	Major study results
Arsand <i>et al</i> ^[13]	Mobile phones	Qualitative evaluation of a mobile system for monitoring of blood glucose, nutrition habits, and physical activity as motivation for increasing and benefitting from these self-management behaviors in patients with type 2 diabetes	Participants assisted with development and testing of the mobile phone application. The application included blood glucose monitoring, a step counter that downloaded to the phone, and software for recording food habits and providing feedback on how users performed in relation to their own personal goals	Participant feedback demonstrated good usability of the system and several participants made adjustments in blood glucose, food habits, and/or physical activity based on the tracked self-management behaviors
Avdal <i>et al</i> ^[15]	Internet-based education program	Evaluation of the effect of providing internet-based diabetes education to individuals with type 2 diabetes	Participants in the intervention group viewed individualized diabetes education, asked questions of researchers, and monitored daily blood glucose levels using the internet-based system. Control group participants received education from a diabetes nurse in a clinic setting	After six months, HA1C levels in the intervention group significantly decreased and rates of health check attendance significantly increased. No differences in HA1C or health check attendance were noted for the control group
Fisher <i>et al</i> ^[16]	Internet-based diabetes self-management improvement program	Comparison of effectiveness of internet-based, CASM, CAPS, and computer-administered minimal support interventions	Participants in the CASM group received an internet-based diabetes self-management improvement program that included education, goal-setting, feedback from healthcare providers, and periodic phone calls to monitor progress. CAPS participants received the same plus a 60 min in person intervention to discuss problem-solving therapy related to diabetes distress. The minimal support intervention included computer-delivered health risk appraisal and diabetes information and phone calls from healthcare providers to answer questions about the information	Significant decreases were noted for diabetes distress and significant improvements in healthy eating, physical activity, and medication adherence in all three conditions, with no significant between-group differences
Glasgow <i>et al</i> ^[12]	Internet based DSMP	Comparison of an internet-based DSMP, internet-based DSMP with additional support, and enhanced usual care on healthy eating, physical activity, medication-taking, HA1C, body mass index, lipids, blood pressure, and psychosocial factors	Participants were randomized to one of the three groups. The internet-based DSMP participants used a website to select individual goals related to medication adherence, physical activity, and food choices, record progress, create action plans, identify barriers to self-management, and choose problem-solving strategies. Participants in the internet-based DSMP with additional support group received the above and two follow-up phone calls and three 120 min group sessions with other study participants	Internet conditions improved health behaviors significantly compared to usual care over the 12-mo period. No significant differences were noted between the two internet-based groups. All conditions improved moderately on biological and psychosocial outcomes
Hanauer <i>et al</i> ^[10]	Mobile phones	Pilot study for feasibility of a fully automated, two-way text messaging system to encourage increased blood glucose monitoring	Participants were randomized to receive electronic reminders to check blood glucose levels <i>via</i> mobile phone text messaging or email reminders. Participants determined the frequency and timing of reminders. Reminders were sent to check blood glucose. After entering the value, users received motivational feedback and, if the value was out of range, a warning to take appropriate action was sent. Participants could also receive daily diabetes facts to the mobile phone or email	Compared to the email group, participants in the mobile phone group received more reminders and responded with blood glucose results significantly more often. During the first month, mobile phone group participants submitted twice as many blood glucose values as email users
Lim <i>et al</i> ^[12]	Mobile phones	Improve glycemic control without hypoglycemia in elderly people living with type 2 diabetes using patient-specific messages and reminders delivered to mobile phones	All participants received diabetes education and then were randomly assigned to intervention, routine care, or SMBG groups. Participants in the intervention group received glucometers with a public switched telephone network-connected cradle that automatically transferred blood glucose results to a hospital-based server. Once the data was transferred to the server, an automated system generated and sent patient-specific messages by mobile phone. Routine care participants did not receive an intervention and were told to follow-up with their current medical care. SMBG participants were told to measure blood glucose at least eight times per week	After 6 mo of follow-up, HA1C was significantly decreased from 7.8 to 7.4 in the intervention group and from 7.9 to 7.7 in the SMBG group, compared with 7.9 to 7.8 in the control group. The proportion of patients with HA1C < 7% without hypoglycemia was 30.6% in the intervention group, 23.4% in the SMBG group, and 14.0% in the control group

Lorig <i>et al</i> ^[127]	Internet-based self-management program	Evaluation of effect of an internet-based DSMP on HA1C, diabetes symptoms, exercise, self-efficacy, and patient activation	Participants were randomized to the internet-based program, the internet-based program with e-mail reinforcement, or a usual care control group. The internet-based program consisted of six asynchronous educational sessions, weekly learning activities, discussion boards, and individualized action plans for self-management. Participants in the reinforcement group received the intervention followed by an online discussion group	At 6 mo, HA1C, patient activation, and self-efficacy were significantly improved for program participants compared with usual care control subjects. There were no changes in other health or behavioral indicators. The subgroup with initial HA1C > 7% demonstrated greater significant improvement in HA1C. The reinforcement intervention showed no additional improvements over the intervention alone
Lyles <i>et al</i> ^[10]	Mobile phones and gaming system	Qualitative evaluation of a disease management program utilizing mobile phones and gaming systems for individuals living with type 2 diabetes	Participants received a smartphone to upload blood glucose values and email or text message with a healthcare provider and a gaming system to gain access to a shared medical record that provided summaries of clinical information related to diabetes	Participants expressed frustration with using cell phones and gaming system, but liked collaborating with a healthcare provider on uploaded glucoses and receiving automatic feedback on blood glucose trends
Noh <i>et al</i> ^[18]	Internet-based information system for computers and mobile phones	Evaluation of the effect of a computer and mobile phone accessible internet-based system on blood glucose control	A web-based information system for mobile phone users and a website for Internet users provided diabetes education. Participants in this group were compared to a control group receiving conventional diabetes education	HA1C and postprandial glucose levels were significantly decreased in the intervention group, but not in the control group. There was a significant relationship between the change in HA1C and the frequency of web-based system access
Nundy <i>et al</i> ^[13]	Mobile phones	Qualitative exploration of mechanisms by which a text-message based diabetes program affected self-management among African-Americans living with type 2 diabetes	Participants completed a 4-wk text messaged based diabetes program in which they received text message reminders about diabetes self-management	Themes that emerged from the study included self-awareness and control of diabetes, reinforcement of success in managing diabetes, acceptance and awareness of seriousness of diabetes, and caring and support
Pacaud <i>et al</i> ^[19]	Internet-based system to provide education for newly diagnosed people with type 2 diabetes	Comparison of three varied media educational systems on diabetes knowledge, self-efficacy, and diabetes self-management activities	Participants were randomly assigned to either the web interactive group who received electronic education and virtual appointments using both synchronous and asynchronous communication, the web static group who received electronic education and virtual appointments using asynchronous communication, or the control group who received face-to-face education and synchronous and asynchronous communication	All three groups had similar improvements in diabetes knowledge, self-efficacy, and diabetes self-care activities. Independent of which group subjects were randomized to, findings were significant when examining correlation between website usage and outcomes: a higher total use was significantly associated with a higher diabetes knowledge score, a higher total diabetes self-efficacy score, and lower HA1C by final study visit
Quinn <i>et al</i> ^[14]	Mobile phones	Evaluation of a diabetes coaching system that used mobile phones and patient-provider portals for individualized treatment and communication	Participants were randomly assigned to one of three treatment conditions or a usual care control group. Participants utilized a patient-coaching system consisting of a mobile diabetes management software application that allowed them to enter diabetes self-care data including blood glucose values, carbohydrate intake, and medications into mobile phones and receive automated, real-time educational, behavioral, and motivational messages related to entered data. The intervention also included a web portal consisting of a secure messaging center for patient and provider communication, personal health record, a learning library, and logbook to review entered data	The mobile phone-based intervention significantly improved HA1C compared to the usual care group. No differences were observed between groups for diabetes distress, depression, diabetes symptoms, blood pressure, or lipid levels
Song <i>et al</i> ^[3]	Internet-based DSMP	Evaluation of the efficacy of an internet-based diabetes self-management education program for newly diagnosed patients with type 2 diabetes as an alternative to group lectures	Participants in the intervention group participated in an internet-based diabetes self-management program and control group participants attended three hours of group lectures provided by healthcare professionals specializing in diabetes care	HA1C and diabetes care knowledge improved significantly in the intervention group at six weeks and diabetes care behaviors improved significantly at six weeks and three months. Diabetes

Tang <i>et al</i> ^[20]	Internet-based system	Evaluation of an online disease management system supporting patients with uncontrolled type 2 diabetes	Multicomponent intervention that included: wirelessly uploaded home glucometer readings with graphical feedback; patient-specific diabetes summary status report; nutrition and exercise logs; insulin record; online messaging with healthcare providers; self-management advice and medication management; and personalized text and video educational messages	care knowledge and diabetes care behaviors improved significantly in the control group at six weeks, but HA1C did not significantly change at six weeks or three months Participants in the intervention group had significantly reduced HA1C levels at 6 mo compared to a usual care group. At 12 mo, the differences were not significant
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SMBG: Self-monitored blood glucose; CASM: Computer-assisted self-management; CAPS: Computer-assisted self-management plus diabetes distress specific problem-solving; DSMP: Diabetes self-management program; HA1C: Hemoglobin A1C.

the study, 22 were randomized to the cell phone group and 18 to the email group; however, only 18 of the cell phone group and 11 in the email group actually used the system. Participants in the cell phone group requested more reminders and submitted more blood glucose values than those in the email group, but over time both groups significantly decreased the number of requested reminders and submitted blood glucose values^[10].

In another study, researchers developed a mobile phone application in conjunction with 12 people living with type 2 diabetes to assist with self-management^[11]. Participants participated in focus groups and feasibility testing during development of the application. Qualitative interviews were conducted at study conclusion. The application included blood glucose monitoring, step counter, software for recording food habits, and feedback based on personal goals established prior to application use. Results of the study were positive. The majority of participants utilized the blood glucose sensor system one or more times per day and had a slight decrease in average blood glucose over the study period. As a group, participants had a reduced intake of carbohydrate-rich foods by the end of the study compared with the beginning of the study. Some participants found daily entry of consumed foods to be a tedious task. The step counter automatically transferred number of steps to the mobile phone once per day. Overall, participants increased their number of daily steps from study beginning to end. Participants especially liked the tips and feedback related to personal goals^[11].

A study designed to improve glycemic control without hypoglycemia in elderly people living with type 2 diabetes utilized mobile phones for the intervention^[12]. Participants received a glucometer that downloaded to a hospital-based server and based on the data, patient-specific messages were generated and sent to their mobile phone. Text messages included instructions about changes to medications based on blood glucose values and reminders to check blood glucose as instructed. The intervention group had significantly lower hemoglobin A1C (HA1C) values compared to two control groups at

six months follow-up. Participants in the intervention group did have higher rates of hypoglycemia than participants in the two control conditions, but the difference was not statistically significant^[12].

Support for diabetes management has been provided through text messaging using mobile phones. A sample of 18 African American people living with type 2 diabetes completed a 4 wk text message diabetes program^[13]. Participants were required to receive a daily medication reminder, question about medication adherence, question about foot care, and appointment reminders for diabetes-related visits. Participants could also receive additional diabetes management text reminders if desired. A certified diabetes educator (CDE) phoned participants weekly to obtain feedback on the experience and make adjustments to the personalized text message. The CDE did not provide any education, counselling, or clinical support for participants. Qualitative interviews revealed that the text message program reinforced the importance of self-management, increased awareness of diabetes, and improved feelings of control over diabetes^[13].

A mobile diabetes intervention study examined the effect of mobile phones and patient and provider portals for individualized patient treatment and communication on HA1C levels^[14]. Participants with type 2 diabetes enrolled in the one year study and utilized a patient-coaching system consisting of a mobile diabetes management software application that allowed them to enter diabetes self-care data including blood glucose values, carbohydrate intake, and medications. After entering this data into mobile phones, participants received automated, real-time educational, behavioral, and motivational messages related to entered data. The intervention also included a web portal consisting of a secure messaging center for patient and provider communication, personal health record, a learning library, and logbook to review entered data. Researchers found a statistically significant improvement in HA1C levels in the intervention group compared to a usual care control group. The study did not evaluate how the mobile intervention affected behavior leading to

blood glucose changes. How the intervention affected medication adherence, physical activity, quantity and quality of patient-provider communication, and treatment intensification are important variables which should be considered in future studies^[14].

Of the six mobile phone intervention studies, three were qualitative and three were randomized controlled trials. Participants in the qualitative studies generally reported positive outcomes from using the mobile phone intervention. Participants appreciate the personalized feedback and education received from the intervention^[11]. Participants in randomized controlled trials using a mobile phone intervention noted improvements in HA1C levels^[12,14]. Overall, mobile phone interventions had small sample sizes making generalization of study findings difficult.

INTERNET-BASED

Internet diabetes interventions provide opportunities to offer diabetes education, support, and motivation for self-management behaviors^[2]. Web-based learning provides easy access without time or location restrictions and allows users to work at their own pace^[3].

A randomized controlled trial evaluated the effects of web-based diabetes education on HA1C levels and health check attendance^[15]. Participants in both the experimental and control groups had completed basic diabetes education prior to this study. After six months of individualized patient education delivered over the web, the experimental group had significant decreases in HA1C and significantly higher health check attendance rates compared to the control group who received education from a diabetes nurse in a polyclinic setting^[15]. No information was provided regarding amount of time participants spent accessing diabetes education either over the web or in the polyclinic.

A second randomized controlled trial aimed at reducing distress and enhancing effective management of type 2 diabetes compared three interventions to reduce diabetes distress and improve self-management^[16]. The study enrolled 392 participants who were randomly assigned to computer-assisted self-management, computer-assisted self-management plus diabetes distress-specific problem-solving, or a computer-administrated minimal support intervention. Computer-assisted self-management included a web-based diabetes self-management improvement program that allows patients to select goals for medication adherence, diet, or exercise and monitor those goals. Participants in this group also had access to a forum to ask questions of diabetes experts and received phone calls from an interventionist to monitor progress and problems. The second group received this same computer-assisted self-management plus problem-solving therapy specifically for diabetes distress. The third intervention group received a computer-delivered health risk appraisal and diabetes information regarding healthy

living, diet, and physical activity. Significant decreases in diabetes distress, emotional burden, and regimen distress occurred in all three groups with no significant between-group differences. The study did not include a usual care control group so the effect of attention alone could not be measured^[16].

Similarly, a three-arm randomized controlled trial compared computer-assisted diabetes self-management, computer-assisted diabetes self-management plus human support, and enhanced usual care^[2]. Participants in the computer-assisted diabetes self-management program (DSMP) selected achievable goals in the areas of medication adherence, physical activity, and food choices. They were able to view displays of their biophysical data, record progress toward goals, participate in a moderated forum, and view diabetes self-management information. Participants in this group also received periodic motivational calls. The computer-assisted plus human support group received the same computer intervention and received follow-up calls from an interventionist and opportunities to attend group educational sessions. The internet interventions significantly improved health behaviors including eating habits and adherence to medications compared to usual care over the 12 mo study period. All three conditions moderately improved self-efficacy, problem-solving, and HA1C^[2].

A six month randomized trial evaluated the effect of an internet-based DSMP on HA1C, diabetes symptoms, exercise, self-efficacy, and patient activation^[17]. Participants were randomized to the intervention, intervention plus email reinforcement, or usual care. The internet-based DSMP consisted of six weekly sessions that participants could view asynchronously anytime during the week. The site also contained a learning center where participants could respond to a posed question and develop an action plan for dealing with diabetes-related problems. A discussion center included interactive threaded discussion boards viewable by all participants where comments, questions, and discussions could be posted. Lastly, a help section was included that allowed participants to email program administrators. The study had a large sample size with 732 completing the six months study and 645 completing the six months completion questionnaire. Following the 18 mo reinforcement period, 528 participants completed questionnaires. At six months, HA1C, self-efficacy, and patient activation were significantly improved for intervention group participants compared to usual care participants. The subgroup with a pre-study HA1C greater than seven demonstrated stronger improvement in HA1C. No significant changes were noted for diabetes symptoms and exercise. Reinforcement did not affect study outcomes. Those in the intervention plus email reinforcement had no significant improvements compared to the intervention group. Researchers recommend follow-up to determine if the type of reinforcement was not beneficial or if it was not properly utilized^[17].

A randomized controlled trial was conducted to

evaluate the effect of a web-based comprehensive information system on blood glucose control^[18]. The system was available using a computer or cellular phone. The system provided real-time information about diet, dining out, hypoglycemia, sick day management, stress management, and diabetes management. HA1C and postprandial blood glucose levels were significantly decreased in the intervention group, but not the control group after six months. There was a significant relationship between the HA1C change and frequency of website access with greater decreases in HA1C associated with higher website usage. The most frequently accessed information using cellular phones was the dining out section which may have contributed to improved postprandial glucose levels. Participants accessed the website more often using the cellular phone than the computer^[18].

A randomized controlled trial found that electronic presentation of diabetes education was as effective as traditional face-to-face education in newly diagnosed patients with type 2 diabetes^[19]. A total of 68 participants were randomly assigned to one of three educational models. The control group received structured diabetes education in a traditional classroom setting. This group also had direct verbal communication with health care providers. The second group had access to electronic educational materials and tools and used asynchronous communication through email for interactions with providers. The third group had access to electronic education materials and tools, used asynchronous and synchronous communication with providers and patients, and used an electronic blood glucose journal and other functions. All three groups had similar improvements in diabetes knowledge, self-efficacy, diabetes self-care activities, and HA1C with no significant between-group differences^[19].

A quasi-experimental design study was conducted to compare a web-based diabetes education program with a traditional classroom diabetes education program for newly diagnosed adults with type 2 diabetes^[3]. The web-based program included six education modules covering diabetes basics, dietary management, exercise, medications, stress management, and foot care. The website also included a password-protected space where participants could enter glucose levels and see a display of those levels, calculate caloric content of meals consumed, record activities, and measure daily stress levels. Participants in the control group attended one hour lectures every week for three consecutive weeks in a group setting consisting of 30 to 40 participants that were taught by a diabetes care specialist nurse, dietician, and physician. Diabetes knowledge, care behaviors, and glycemic control were compared for the intervention and control groups at baseline, six weeks, and three months. Diabetes care knowledge significantly increased in both intervention and control groups from baseline to six weeks, but not from six weeks to three months. Diabetes care behaviors

significantly increased in both groups from baseline to six weeks and also significantly increased from six weeks to three months in the intervention group. HA1C levels for the intervention group significantly decreased from baseline to six weeks, but not from six weeks to three months. No differences in HA1C were found in the control group. Limitations of this study include the small sample size (31 participants) and the lack of random group assignment. Participants in the intervention group were required to have the ability to use the internet which prevented random assignment. The improvement in diabetes care behaviors and HA1C in the intervention group offers promise for using web-based diabetes education as a substitute for group education^[3].

A randomized controlled trial evaluated an online diabetes management system for patients with uncontrolled type 2 diabetes^[20]. A usual care control group was compared to an intervention group that utilized an online disease management program that included wireless uploading of glucose readings, individualized diabetes summary status reports, nutrition and exercise logs, insulin records, online messaging with the health care team, advice and medication management from a nurse care manager and dietician, and personalized educational information. Participants in the intervention group had significantly lower HA1C levels at 6 mo compared to the control group, but at 12 mo, the difference was no longer significant. As in other studies, participants who utilized the online system more often achieved greater benefits^[20].

Internet interventions include education, goal-setting, tracking of behaviors, patient feedback and support. Of the eight internet studies reviewed, seven were randomized controlled trials and the remaining study had a quasi-experimental design. All studies that measured changes in HA1C levels noted improvements and all improvements were significant with one exception^[2]. In two of the studies, short-term improvements were noted in HA1C, but not at the second, long-term follow-up^[3,20]. Several studies noted improvements in outcomes in both intervention and control groups^[2,3,16,19].

CONCLUSION

Previous reviews found mixed results with some noting significant improvements in HA1C and self-management behaviors^[8]. This review found mainly positive results though some interventions had no effect or only short term improvements. It is important to note that greater usage of technological interventions, both mobile and internet-based, was associated with greater improvements in outcomes^[18,20]. One of the reviewed studies included a web-based intervention that could be accessed using the computer or mobile phone. Researchers found that participants in the mobile phone group accessed the site more often than those using the computer^[18]. Mobile phone interventions are an important source of diabetes self-management

to pursue as their convenience may increase access of information and support for people living with diabetes.

Due to time constraints of both patients and healthcare providers, web-based education and monitoring may be beneficial and can be used to complement healthcare provider visits^[15]. Increased access, whether in-person or electronic, to diabetes education and healthcare providers can improve diabetes knowledge and self-efficacy^[19]. The increased use of diabetes-related mobile applications indicates that people living with diabetes are interested in using these methods to improve self-management and diabetes outcomes. The use of applications to provide education and real-time feedback needs to be developed^[5].

RECOMMENDATIONS

While technology can be effective for promoting diabetes education, support, and self-management, patients report a need for personal contact with health care providers in addition to technology^[9,13]. In the study by Nundy *et al*^[13], automated text messages were sent, but participants stated they preferred to think of them as coming from the certified diabetes educator (CDE) who enrolled them in the study. They also appreciated the weekly calls from the CDE to obtain feedback on the experience and make adjustments to text messaging as needed. Some participants felt the text messaging intervention would not be effective for them without a person to monitor and provide clinical support^[13]. A website that provides diabetes education, monitoring, and support through communication with a healthcare provider may be most effective^[15]. Web-based interventions can be used in conjunction with healthcare provider education and support and as a follow-up to healthcare provider interventions^[21].

Researchers and healthcare providers should include participants in the development of technological interventions and in the decision of which technology to use^[9]. Patient needs must be explored to determine the best method for individual needs realizing that not all patients will be amenable to technological interventions^[21]. A previous review of mobile diabetes applications found that current applications are lacking personalized education and decision support features are not being included. Additionally, inclusion of peer support features through mobile applications are largely underused and could be beneficial for people living with diabetes^[22].

IMPLICATIONS FOR PRACTICE

Healthcare providers should actively select and adapt technological self-management methods to extend the reach of diabetes self-management to patient's communities and homes, provide for individualized care, and provide just-in-time information. People living with diabetes who have limited access to care due to lack of transportation, physical restrictions, or other limitations

could benefit from technological interventions that bring care to them^[21]. Additionally, with limited primary care resources, technology can provide cost-effective ongoing diabetes self-management education and support^[2].

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Type 2 diabetes after gestational diabetes: The influence of changing diagnostic criteria

Eoin Noctor, Fidelma P Dunne

Eoin Noctor, Steno Diabetes Center, DK-2820 Gentofte, Denmark

Fidelma P Dunne, Galway Diabetes Research Centre, National University of Ireland, Dublin 2, Ireland

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Correspondence to: Eoin Noctor, Chief Physician, Steno Diabetes Center, Niels Steensens Vej 2-8, DK-2820 Gentofte, Denmark. eoge@steno.dk

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in the literature with regard to the proportion of women with a history of GDM who go on to develop diabetes. Heterogeneity between cohorts with regard to diagnostic criteria used, duration of follow-up, and the characteristics of the study population limit the ability to make meaningful comparisons across studies. As the new International Association for Diabetes in Pregnancy Study Group criteria are increasingly adopted worldwide, the prevalence of GDM is set to increase by two-to three-fold. Here, we review the literature to examine the evolution of diagnostic criteria for GDM, the implications of changing criteria on the proportion of women with previous GDM progressing to diabetes, and how the use of different diagnostic criteria may influence the development of appropriate follow-up strategies.

Key words: Gestational diabetes; Pregnancy; Type 2 diabetes; Impaired glucose tolerance; Diagnostic oral glucose tolerance test criteria

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Core tip: Gestational diabetes (GDM) is associated with a greatly increased future risk of type 2 diabetes, but there are many different GDM diagnostic criteria in clinical use. Criteria with lower glucose thresholds increase GDM prevalence, and therefore the number of women requiring follow-up to detect progression to diabetes. However, lower diagnostic thresholds are also likely to decrease the proportion that progress to diabetes. Heterogeneity across studies with regard to diagnostic criteria, demographics, and duration of follow-up, limit direct comparison. As the International Association of Diabetes in Pregnancy Study Groups criteria enter widespread use, follow-up of these women will be an important issue.

Abstract

A previous diagnosis of gestational diabetes (GDM) carries a lifetime risk of progression to type 2 diabetes of up to 60%. Identification of those women at higher risk of progression to diabetes allows the timely introduction of measures to delay or prevent diabetes onset. However, there is a large degree of variability

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INTRODUCTION

Background

Gestational diabetes (GDM) has long been recognised clinically. First described in pregnancy in 1824 in Germany^[1], Joslin^[2] described in 1916 a case of diabetes which presented in pregnancy, resolved with delivery, and recurred later in life. In the 1940s and 1950s, Hoet *et al.*^[3] recognised the association of this type of diabetes with adverse perinatal outcome, and characterised the relationship between glucose tolerance during pregnancy, and in the post-partum period. However, despite the long-recognised association, no standardised criteria for diagnosis were devised until 1964. In Boston City Hospital, O'Sullivan *et al.*^[4] carried out 3-h 100 g oral glucose tolerance tests on 752 patients at different stages of pregnancy. Women with 2 out of 4 values that were greater than 2 standard deviations (rounded to the nearest 5 mg/dL) above the mean glucose levels determined in this cohort were classified as having GDM. These criteria (with some modification) have continued in clinical use over the following four decades.

Evolution of diagnostic criteria for GDM

The major feature of these criteria was that they defined a cohort of women with a greatly increased future risk of progression to type 2 diabetes, demonstrating a lifetime risk of up to 60%^[5]. The National Diabetes Data Group (NDDG) criteria, proposed in 1979^[6] (Table 1), converted the O'Sullivan/Mahan criteria from whole blood to plasma values (see Figure 1 for timeline). The Carpenter-Coustan criteria^[7], proposed in 1982, also converted the O'Sullivan/Mahan criteria to plasma values, but in addition, took a change in enzymatic methods into account. They soon entered widespread clinical use, and were subsequently validated for prediction of adverse perinatal outcome^[8-12]. Essentially, therefore, all 3 sets of criteria were intended to define a similar population.

Studies directly comparing the prevalence of GDM by either NDDG or Carpenter-Coustan criteria show, however, significant differences, with an approximately 50% relative increase in GDM prevalence if the Carpenter-Coustan criteria are used^[9,11-13]. In addition, in 2001, the American Diabetes Association (ADA), having previously endorsed the Carpenter-Coustan criteria, also allowed for the use of a 75 g, 2-h oral glucose tolerance test (OGTT) to make a diagnosis of GDM, using the same one- and two-hour cut-offs as the three-hour 100 g OGTT. The post-load glucose levels are estimated as being 0.9 mmol/L lower at one hour, and 0.5 mmol/L lower at two hours with the lower

glucose load^[14], therefore these criteria will identify a different group of women. Indeed, only weak diagnostic agreement has been noted between the two glucose loads^[15] (Cohen kappa index 0.18; although some this difference may also be attributable to day-to-day glycaemic variability).

The World Health Organisation (WHO) also recommended alternative criteria for the diagnosis of gestational diabetes beginning in 1980 (the 1965 WHO report did not comment on this issue). These thresholds were the same as those for non-pregnant adults. Initially, the WHO recommended a fasting glucose threshold of 8 mmol/L (see Table 1). These recommendations were revised again in 1985^[16] (fasting glucose threshold lowered to 7.8 mmol/L, recommendation to treat impaired glucose tolerance added) and 1999^[17] (fasting glucose threshold reduced to 7.0 mmol/L) (see Table 1). Although these thresholds were not chosen on the basis of predicting adverse pregnancy outcome, a subsequent large ($n = 4998$) prospective cohort study did show that these thresholds predicted increased risk for macrosomia (RR = 1.45, 95%CI: 1.06-1.95) and preeclampsia (1.94, 95%CI: 1.22-3.03), even when women with values diagnostic of diabetes in the nonpregnant adult^[18].

The European Association for the Study of Diabetes also proposed new GDM criteria in 1996^[19], using a fasting value of 6.0 mmol/L and a two-hour post 75 g glucose load value of 9.0 mmol/L, based on the distribution of glucose values on 75 g OGTTs on over 1000 European women. A subsequent retrospective cohort study supported this 2-h value in prediction of adverse perinatal outcome^[20]. However, subsequent analysis of women in this cohort, with 2-h values below the 2-h threshold of 9.0 mmol/L (not treated for GDM), demonstrated a linear relationship between 2-h glucose and pregnancy outcome, with no clear threshold value^[21].

In addition to these major criteria, multiple different diagnostic criteria are in use worldwide, some related to older criteria, some derived on the basis of local data. Therefore, the situation still exists where different centres in the same country, or even the same region, may employ different criteria for GDM diagnosis.

GDM criteria to predict adverse perinatal outcome

However, none of the available criteria had been designed specifically to predict adverse pregnancy outcome. To look at this issue, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) convened a consensus conference in 2008 to review the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study findings (published and unpublished), along with other relevant studies. This consensus conference had two major outcomes^[22]. Firstly, women meeting the cut-off values for diagnosis of diabetes in the non-pregnant adult (Table 1) would now fall into the new category of "overt diabetes" rather than GDM. The rationale for this was that

Table 1 Comparison of thresholds for criteria for gestational diabetes diagnosis

Criteria	Glucose load	Fasting glucose mmol/L (mg/dL)	1-h glucose mmol/L (mg/dL)	2-h glucose mmol/L (mg/dL)	3-h glucose mmol/L (mg/dL)	No. of criteria required
O'Sullivan <i>et al</i> ^[4]	100 g	5 (90)	9.2 (165)	8.1 (145)	6.9 (125)	≥ 2
NDDG	100 g	5.8 (105)	10.6 (190)	9.2 (165)	8.1 (145)	≥ 2
WHO 1980	75 g	8 (144)	N/A	8 (144)	N/A	≥ 1
Carpenter and Coustan	100 g	5.3 (95)	10 (180)	8.6 (155)	7.8 (140)	≥ 2
ADA	75 g or 100 g	5.3 (95)	10 (180)	8.6 (155)	7.8 (140)	≥ 2
WHO 1985	75 g	7.8 (140)	N/A	7.8 (140)	N/A	≥ 1
EASD	75 g	6 (108)	N/A	9 (162)	N/A	≥ 1
WHO 1999	75 g	7 (126)	N/A	7.8 (140)	N/A	≥ 1
IADPSG GDM	75 g	5.1 (92)	10 (180)	8.5 (153)	N/A	≥ 1
IADPSG overt diabetes	*None/75 g	7 (126)	N/A	11.1 (200)	N/A	≥ 1

*This diagnosis can also be made on a random glucose sample, a fasting glucose sample, or on an HbA1c value [if 6.5% (48 mmol/mol or over)]. NDDG: National Diabetes Data Group; WHO: World Health Organisation; EASD: European Association for the Study of Diabetes; ADA: American Diabetes Association; IADPSG: International Association of Diabetes and Pregnancy Study Groups; GDM: Gestational diabetes; N/A: Not applicable.

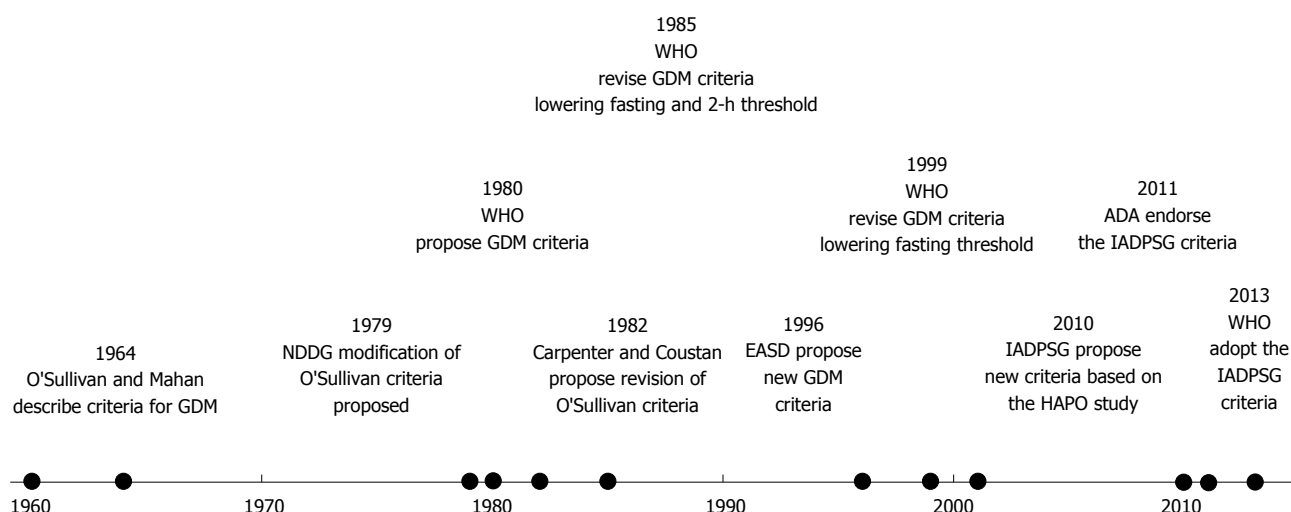


Figure 1 Timeline of evolution of criteria used to diagnose gestational diabetes from 1964-present. NDDG: National Diabetes Data Group; WHO: World Health Organisation; EASD: European Association for the Study of Diabetes; ADA: American Diabetes Association; IADPSG: International Association of Diabetes and Pregnancy Study Groups; GDM: Gestational diabetes; HAPO: Hyperglycemia and pregnancy outcomes study.

this group were felt to be distinct clinically and biochemically from women with milder degrees of hyperglycaemia. Secondly, the data from the 2008 HAPO study^[23] was reviewed. This large (over 25000 participants screened), multicentre study showed that glucose levels at all time points on the 2-h 75 g OGTT were associated with adverse pregnancy outcomes (large for gestational age, macrosomia, cord c-peptide concentration greater than the 90th centile). In the absence of a clear threshold effect, and having considered various cutpoints, the IADPSG consensus committee ultimately decided to set new values for GDM diagnosis at the mean glucose values for which the odds ratio for adverse pregnancy outcome was 1.75. This lowered the fasting and 1-h values compared to previous values, while raising the 2-h value slightly. However, the major change was allowing a diagnosis to be made on just a single abnormal value, a change likely to greatly increase the prevalence of gestational diabetes. On applying these

criteria retrospectively to the HAPO cohort, 17.8% (range 9.3%-25.5%) met the criteria for diagnosis^[24].

These consensus criteria were published in March 2010, and began to enter clinical use shortly afterwards. At the time of writing, in addition to the IADPSG endorsing the criteria, the ADA^[25] and WHO^[26] have also endorsed the criteria. However, the American College of Obstetricians and Gynaecologists (ACOG) have not adopted the new criteria, and still recommend a 100 g OGTT using the Carpenter-Coustan criteria, for diagnosis, a position endorsed by a National Institute of Health Consensus Conference in March 2013^[27].

With this in mind, we will review the impact of changing criteria for GDM diagnosis with regard to the prevalence/cumulative incidence of abnormal glucose tolerance/diabetes post GDM, risk factors for progression to diabetes, and follow-up of women with previous GDM. This is a clinically relevant problem for 2 major reasons - firstly, prevention or delay of type 2 diabetes in women with previous GDM is a possibility,

as demonstrated by a subgroup analysis of the diabetes prevention program^[28], and the Troglitazone In the Prevention Of Diabetes^[29] and Pioglitazone In the Prevention Of Diabetes^[30] studies. Secondly, undetected type 2 diabetes developing prior to a subsequent pregnancy carries the risk of congenital malformation and an increased risk of pregnancy complications.

HETEROGENEITY OF STUDIED COHORTS

Many studies have assessed the risk of progression to type 2 diabetes post gestational diabetes.

A major issue with all studies in this area however, is their marked heterogeneity. This is seen in several ways: (1) As discussed, the diagnostic criteria in clinical use for GDM diagnosis over the last four decades are numerous. This leads to the identification of cohorts who may not be directly comparable in terms of the severity of glucose intolerance; (2) Both the criteria and method used to diagnose diabetes and/or abnormal glucose tolerance in women who have previously had GDM varies significantly; (3) The ethnic mix of the cohorts is extremely heterogeneous with some composed entirely of a single ethnicity, and others showing very mixed composition; and (4) Duration of follow-up varies between studies, from 6 wk to almost 30 years^[31].

In summary, meaningful comparison of the actual cumulative incidence or prevalence across studies is not possible. It is clear, however, that regardless of the criteria used, GDM signifies a high risk of future progression to type 2 diabetes.

RISK FACTORS FOR FUTURE PROGRESSION

Despite the heterogeneity of the cohorts, many studies identify similar factors predicting progression to diabetes/abnormal glucose tolerance. We will consider the most commonly associated risk factors here.

Pre-pregnancy factors

Given that most studies identify women with GDM at the time of diagnosis, most studies assess pre-pregnancy risk factors retrospectively. Therefore, information on this is limited. The exception to retrospective recall of pre-pregnancy factors is the large long-term longitudinal cohort population-based studies, such as the Nurse Health Study^[32], which have detailed information preceding the index pregnancy. However, these also use self-reported GDM as an outcome measure. Although the diagnosis has been validated in a subset by medical record review, the precise criteria used by the healthcare provider are uncertain, and therefore lie outside the scope of this review. Of pre-pregnancy variables assessed, weight or BMI is the most common measure, and is commonly associated with increased

risk of progression to abnormal glucose tolerance or diabetes^[33-38], although the relationship is not particularly strong. Polycystic ovary syndrome has also been reported in a one retrospective study to be associated with later progression to abnormal glucose tolerance^[39] on multivariable analysis, although this study used two different sets of criteria to diagnose GDM.

Index pregnancy-related factors

Pregnancy glucose values: Higher glucose values during pregnancy, as reflected by the index pregnancy OGTT, are consistently associated with increased later progression to diabetes. This is measured in various ways (number of abnormal values, area under the curve), but most commonly the values for plasma glucose at fasting, one hour, two hours (and three hours if applicable) are used. Fasting glucose shows the strongest association, being the most commonly identified risk factor associated with later abnormal glucose tolerance and diabetes^[31,40-45]. Studies that have not identified fasting glucose as a factor associated with later progression to abnormal glucose tolerance tend to have either not measured it^[46], not included it in the statistical models^[47], or have excluded women with the highest fasting glucose levels from follow-up^[48,49]. One large Australian study found fasting glucose was not associated with later abnormal glucose tolerance and diabetes despite its inclusion in the model^[50]. One-hour^[48,50,51] and two-hour glucose levels^[37,40,51,52] are also associated with later glucose abnormalities, although less consistently, and to varying degrees. Also, higher haemoglobin (HbA1c) during pregnancy, although much less frequently studied, has been found to be associated with future risk of progression to diabetes^[52,53].

More detailed characterisation of glycaemic response to a glucose load such as measures of insulin secretion^[43], when undertaken, are also associated with later progression to abnormal glucose tolerance and diabetes. These measures, of course, are generally not available routinely clinically. Insulin use during pregnancy has also frequently been shown to be associated with increased risk of future progression to diabetes/abnormal glucose tolerance^[36,46,54-56], presumably as a marker of higher glucose levels in pregnancy, even taking into account likely differences in prescribing practice between centres.

Body weight/body mass index: Body weight [or body mass index (BMI)] during the index pregnancy is commonly reported in studies of GDM cohorts, occasionally with waist circumference or body fat measurements. Studies are inconsistent as to whether weight or BMI persist as a risk factor when adjusted for other risk factors using multivariate analysis. Studies that have not found an association between pregnancy weight and BMI tend to examine women who have progressed to abnormal glucose tolerance in the early post-partum period. BMI during pregnancy may be

associated with abnormal glucose tolerance at this stage, but is not independently associated when antepartum glucose levels (indicating severity of hyperglycaemia) are included in the model^[41]. Most studies that do show an association between pregnancy BMI and later abnormal glucose tolerance, independent of antepartum glucose measurements, involve longer-term follow-up post delivery^[43,57,58], although this is not a universal finding^[59].

Gestational age at diagnosis: Gestational age at diagnosis is another commonly reported association^[37,38,41,42,44,60,61]. However, many of the studies also specify a screening protocol that involves screening higher-risk women in early pregnancy, causing a significant bias. Women diagnosed with GDM in early pregnancy, before insulin resistance begins to rise^[62,63], are likely to have a greater degree of hyperglycaemia, and therefore an increased likelihood of progression to abnormal glucose tolerance/diabetes. However, gestational age at diagnosis remains a risk factor, even when measures of glycaemia from the index pregnancy are included in the model, in many of these studies^[41,42,44,60,61].

Ethnicity: There are few studies specifically examining the effects of ethnicity, although these that do have generally found an increased prevalence among those women of ethnicity other than white European origin^[47,64-68]. Other studies have found no association^[40,69]. The reasons for this are unclear. However, many studies have examined ethnically homogenous cohorts, who are often already at high risk of GDM. The prevalence of GDM is higher among ethnic groups who are not of white European origin, while the prevalence of GDM increases at a lower BMI^[70] in the Asian populations studied. In addition, adoption of the IADPSG criteria may cause a disproportionate rise in GDM prevalence among Asian populations^[71], which will be of relevance when determining the future risk of abnormal glucose tolerance or diabetes in these populations. In addition to the studies outlined above examining this question, comparison between studies does suggest a higher proportion of women of non-white European ethnicity progress to abnormal glucose tolerance^[68]. However, meaningful comparison between studies is generally not possible due to the heterogeneity of the studies on the points listed above.

Family history of diabetes: This is uncommonly associated with progression to abnormal glucose tolerance/diabetes among women with GDM after measures of glycaemia are taken into account. Several studies examining family history have found no effect^[49,72,73]. Although some studies have shown an independent effect^[39,47,59,74], it appears to be small, and the association is often not seen when analysed as part of a multivariate model^[38,58,75,76]. Therefore, family history does not appear to play a major independent role in predicting future risk of diabetes or abnormal glucose tolerance.

Other factors: Age at diagnosis of GDM^[44,52,54,76] has

been associated with future abnormal glucose tolerance or diabetes also, but is inconsistent, with other studies showing no association^[57,77,78], and again, is rarely significant^[54] when other variables are taken into account. Parity, most commonly classified as a binary variable (multiparous or nulliparous) has been identified^[53,55,79] as potentially associated with higher risk of progression later, but this finding is inconsistent^[41,78]. Potential gene associations have also been identified, but currently appear to add little to clinically assessing individual risk^[80]. Autoantibody testing also been examined^[81], and appears to be associated with risk of progression to type 1 rather than type 2 diabetes.

Risk factors post-pregnancy

Breastfeeding: Breastfeeding among women with GDM is associated with improved glycaemic indices in the early post-partum period^[47,82]. Its role in prevention of later progression to abnormal glucose tolerance is at present unclear, although long-term follow-up of the Study of Women, Infant Feeding and type 2 diabetes mellitus after GDM (SWIFT) pregnancy cohort will address this issue.

Body weight/BMI: Weight (or associated measures) after the index pregnancy has been shown to be correlated in a number of studies^[33,56,59,83-86] with progression to diabetes or abnormal glucose tolerance. This correlation appears more robust than that seen with pregnancy weight/BMI, which often loses significance in multivariable models (see above). Also, weight gain since the index pregnancy has been associated with metabolic deterioration^[43]. Studies not demonstrating BMI as a predictive factor may take high-risk cohorts, for example, entirely composed of participants with postpartum impaired glucose tolerance^[87], or are carried out in the early post-partum period^[41,69,88]. Interestingly, Wang *et al*^[84] showed that both waist circumference and body fat performed better than BMI in predicting type 2 diabetes in a Chinese cohort, while Jang demonstrated that waist circumference showed a stronger association than BMI in a Korean cohort^[37]. This may help to explain why some Asian cohorts^[38,87] have not demonstrated an association between BMI and future abnormal glucose tolerance or diabetes, despite longer-term follow-up.

Others: The type of contraceptive - specifically the progesterone-only oral contraceptive - is thought to confer a higher risk^[89]. Subsequent GDM is also associated with greater risk of progression to diabetes/abnormal glucose tolerance^[83]. Age at follow-up is commonly reported. Although an association with later abnormal glucose tolerance has been noted^[40,44,90-92], and despite the increasing prevalence of type 2 diabetes with advancing age in the general population, this is not a universal finding^[38,93], particularly in multivariate analysis^[94]. This may be due to the relatively small difference in ages within the cohorts of women involved in these studies, compared to the population as a whole.

Despite the heterogeneity of the studies for the reasons above, including diagnostic criteria used, there is consistency among most studies in the factors associated with a greater risk of diabetes after the index pregnancy in women with GDM. As can be seen, measures of glycaemia during the index pregnancy are not only the strongest predictor, but also frequently attenuate or remove the predictive ability of other traditional risk factors for type 2 diabetes. Thus, the most important risk factor for future abnormal glucose tolerance or diabetes in these women is simply a previous diagnosis of GDM, taking into account the degree of hyperglycaemia at diagnosis.

PREVALENCE OF DIABETES POST-GDM

The prevalence of progression from GDM to abnormal glucose or type 2 diabetes varies greatly. The lifetime cumulative incidence of diabetes among women with GDM is frequently cited at up to 60%, but this summary figure does not illustrate the many underlying different factors (*e.g.*, time since delivery, cohort demographics, and criteria for diagnosis of GDM and postpartum diabetes).

Duration of follow-up

With regard to timing, many studies have documented short-term follow-up only (*i.e.*, to the first post-partum test). Prevalence rates for diabetes at this time point differ, and are generally less than 10%, but depending on the cohort studied, and criteria used, may be significantly higher - Metzger *et al.*^[40] showed a prevalence of 38% up to one year post-partum in women meeting NDDG criteria^[40]. These women are likely to be different from those developing diabetes at a later post-partum interval, and are more likely to have had pre-existing type 2 diabetes. It is therefore unlikely that any of the criteria in use for GDM diagnosis would fail to detect these women.

Beyond the post-partum period, prevalence or cumulative incidence figures continue to show great variation. Figures may be as low as 3% (up to 3 years post-partum from a Swedish cohort, using area under the glucose curve measures from the OGTT for diagnosis^[85]), and as high as 62% (at up to 6.5 years in a cohort from Trinidad meeting the 1980 WHO criteria^[64]). Follow-up of O'Sullivan's original cohort at 16 years showed a cumulative incidence by life-table analysis of 60%^[85]. A systematic review from 2002^[31] attempted to control for the marked heterogeneity in time among studies, by plotting actuarial projections of cumulative incidence of cohorts at up to 28 years follow-up, and concluded that most cohorts progressed to diabetes at a similar rate in the first 5 years post index pregnancy, and then levelled off by 10 years with few cases after this (however, this calculation included NDDG-diagnosed women only).

Cohort features

Cohort selection also plays a vital role in determining later progression to abnormal glucose tolerance/diabetes, and makes comparison difficult. Selection of women who are known to have normal glucose tolerance in the early post-partum period^[48], or restricting follow-up to those who did not require insulin for glycaemic control in pregnancy^[34], would be expected to reduce the proportion progressing to abnormal glucose tolerance or diabetes, removing those women with the highest glucose levels during pregnancy. Ethnicity, as outlined above, appears also to be a risk factor for progression, with non-white populations demonstrating increased risk, although comparison across studies is difficult.

Criteria used

There is little evidence to directly compare future progression to diabetes or abnormal glucose tolerance among the different criteria in use. Studies directly comparing progression in women meeting the NDDG vs Carpenter-Coustan criteria^[78] showed little difference in prevalence of diabetes at a median of 6 years post-partum (25.5% vs 25.3%) or at 3 mo post-partum (4.0% vs 3.2%)^[95].

However, the WHO criteria (Table 1) would be expected to show a smaller proportion of women progressing to diabetes/abnormal glucose tolerance, given the increased number of women identified with GDM compared to the NDDG and Carpenter-Coustan criteria. However, again, direct comparison across studies is difficult. In any given population, therefore, lower diagnostic thresholds will lead to a greater prevalence of GDM. Conversely, criteria using higher thresholds to define GDM will identify fewer women with GDM, but these women will, on average, have higher glucose levels. Therefore, the proportion progressing to abnormal glucose tolerance/diabetes will be higher, despite the lower GDM prevalence.

Also, the criteria used to diagnose type 2 diabetes and abnormal glucose tolerance postpartum may differ - older cohorts in particular, using the NDDG or older WHO criteria would be expected to show a lower prevalence at follow-up due to higher thresholds for diagnosis of diabetes in the nonpregnant adult.

RELEVANCE OF IADPSG GDM CRITERIA

The new IADPSG criteria pose an important clinical question with regard to intensity of follow-up. With potentially up to one in four pregnancies in some centres meeting the new criteria for GDM diagnosis^[24], lifelong follow-up of these women will have important clinical and resource implications. However, the optimal mode and timing of a follow-up strategy remains unclear. More women with milder degrees of hyperglycaemia are now classified as GDM. Accordingly, the proportion progressing to abnormal glucose tolerance should decrease. There are as of yet no prospective figures

on progression to type 2 diabetes or abnormal glucose tolerance post-partum in women with IADPSG-defined GDM. The ATLANTIC-DIP study retrospectively classified women using IADPSG criteria after a universal screening programme, and found that 19% had abnormal glucose tolerance at early post-partum follow-up^[47]. Capula *et al.*^[39] looked at a mixed (approximately 60% diagnosed by IADPSG criteria) cohort, and found 4% had diabetes, and a further 32% abnormal glucose tolerance at 6-12 wk post-partum, although conclusions on the relative contribution of each set of criteria are not possible. Overall, it appears certain that more women will need to be tested to identify those women progressing to abnormal glucose tolerance and diabetes.

Some clues as to how women diagnosed with GDM by IADPSG criteria may behave on follow-up may be seen in several papers which follow women meeting just a single abnormal value on the pregnancy OGTT using the older criteria. Retnakaran *et al.*^[95,96], using NDDG criteria for GDM diagnosis, examined early post-partum outcomes among women along the spectrum of glucose tolerance: from normal glucose tolerance, to abnormal glucose challenge test (GCT) with normal OGTT, a single abnormal value on OGTT, and GDM. This demonstrated a graded relationship in abnormal glucose tolerance; from 3.2% in the normal glucose tolerance (NGT) group, 10.2% in the GCT abnormal, OGTT normal group, 16.5% in the GCT abnormal, single abnormal value on OGTT group, to 32.8% in the GDM group. Indeed, detailed characterisation of these groups^[97] demonstrates the similarity between women with a single abnormal value at 1-h post glucose load (as opposed to later abnormal values) and women with GDM, as measured by AUC curve on OGTT and beta-cell dysfunction at 3 mo postpartum.

Thus we can see that a cohort of women with a single abnormal value only, albeit using higher cut-offs than the new IADPSG values, still have a clinically important increased risk of abnormal glucose tolerance. Other prospective studies examining similar cohorts, although at a longer follow-up interval, have drawn similar conclusions; Stuebe *et al.*^[98], using the stricter Carpenter-Coustan criteria, showed a higher HbA1c in women with a single abnormal value at 3-year follow-up, vs both women with GDM, and those with NGT in pregnancy. Vambergue *et al.*^[76] (using Carpenter-Coustan criteria) showed a similar graded relationship for progression to type 2 diabetes at almost 7 years follow-up, with 6% of women with a single abnormal value progressing to diabetes, as compared with 18% in the those meeting GDM criteria (less than 1% of those with no abnormal values had progressed to diabetes). Carr *et al.*^[99] (using Carpenter-Coustan criteria), in a large retrospective cohort study, found a HR of 2.0 for diabetes diagnosis among women with a single abnormal value on OGTT vs those who did not.

Therefore, all degrees of glucose abnormalities

in pregnancy, even those not meeting older GDM criteria, are associated with an increased risk of later glucose abnormalities. This may have important implications for those women with lesser degrees of hyperglycaemia who will now be classified as having GDM by IADPSG criteria.

RELEVANCE OF OVERT DIABETES

Women meeting criteria for diabetes diagnosis in the non-pregnant adult are now classified as separate category by the IADPSG criteria and represent the highest-risk GDM cohort, having an increased risk of congenital abnormalities and diabetes complications, and are likely to have had undiagnosed type 2 diabetes preceding the index pregnancy^[22]. The future risk of these women is unclear at present. A retrospective audit of 254 women meeting criteria for overt diabetes demonstrated that 41% had normal glucose tolerance at 6-8 wk postpartum (although testing was carried out at 24-28 wk rather than at the booking visit, and diagnoses based on a 2-h value of ≥ 11.1 mmol/L were not confirmed with HbA1c or FPG measurements)^[100]. Further prospective follow-up comparing women meeting both sets of IADPSG criteria will therefore be useful in further refining risk in this population.

POST-PARTUM FOLLOW-UP STRATEGIES

Current recommendations for follow-up of women with gestational diabetes vary from region to region. The ADA recommend an early post-partum OGTT (in line with ACOG guidelines) and follow-up with HbA1c, fasting plasma glucose (FPG) or 75 g OGTT thereafter, on a 1-3 yearly basis^[101]. The International Diabetes Federation^[102] recommend an early post-partum OGTT, and thereafter vary recommendations on whether a further pregnancy is planned, (OGTT prior to conception) and whether the woman is high-risk (annual OGTT) or low-risk (FPG every 2-3 years), the criteria for which are not defined. The British National Institute for Health and Care Excellence guidelines^[103] recommend FPG alone in the early post-partum period, and an OGTT at follow-up only if a further pregnancy is planned. Several studies have examined the use of HbA1c and FPG^[104-107] for both early and medium term follow-up in women with previous GDM, in order to avoid the inconvenience associated with the OGTT. Sensitivity for the detection of abnormal glucose tolerance after delivery varies widely according to the thresholds chosen, ranging from 23%-65% (specificity 68%-96%) for HbA1c values, increasing to a sensitivity of 82%-93% (specificity 84%-92%) when combined with FPG values. Further prospective study will be needed to examine the potential use of these approaches. This will be particularly important if IADPSG criteria are used, as the optimum frequency and mode of testing for such a large cohort of women with previous GDM is unknown.

CONCLUSION

Marked heterogeneity across studies of women with previous GDM with regard to the diagnostic criteria used, duration of follow-up, and cohort demographics limits the ability to compare findings across studies. However, regardless of which criteria are used, a history of GDM confers a large excess risk of progression to type 2 diabetes in later life, and the risk factors predicting progression remain similar across cohorts. The new IADPSG criteria increase the prevalence of GDM by 2-3 fold, and lifelong follow-up of these women has significant clinical and resource implications. Therefore, further prospective studies are necessary to determine the longer-term risk of progression to diabetes in those diagnosed using the new criteria, and also to determine the optimal method and frequency follow-up needed.

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Diabetic cardiac autonomic neuropathy: Do we have any treatment perspectives?

Victoria A Serhiyenko, Alexandr A Serhiyenko

Victoria A Serhiyenko, Alexandr A Serhiyenko, Department of Endocrinology, National Medical University named after Danylo Galytski, 79017 Lviv, Ukraine

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Correspondence to: Dr. Victoria A Serhiyenko, Department of Endocrinology, National Medical University named after Danylo Galytski, 69 Pekarska Str., 79017 Lviv, Ukraine. serhiyenko@inbox.ru

Telephone: +380-322-769496

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Abstract

Cardiac autonomic neuropathy (CAN) is a serious and common complication of diabetes mellitus (DM). Despite its relationship to an increased risk of cardiovascular mortality and its association with multiple symptoms and impairments, the significance of CAN has not been fully appreciated. CAN among DM patients is characterized review the latest evidence and own data regarding the treatment and the treatment perspectives for diabetic CAN. Lifestyle modification, intensive glycemic control might prevent development or progression of CAN.

Pathogenetic treatment of CAN includes: balanced diet and physical activity; optimization of glycemic control; treatment of dyslipoproteinemia; correction of metabolic abnormalities in myocardium; prevention and treatment of thrombosis; use of aldose reductase inhibitors; dihomog- γ -linolenic acid (DGLA), acetyl-L-carnitine, antioxidants, first of all α -lipoic acid (α -LA), use of long-chain ω -3 and ω -6 polyunsaturated fatty acids (ω -3 and ω -6 PUFAs), vasodilators, fat-soluble vitamin B₁, aminoguanidine; substitutive therapy of growth factors, in severe cases-treatment of orthostatic hypotension. The promising methods include research and use of tools that increase blood flow through the vasa vasorum, including prostacyclin analogues, thromboxane A₂ blockers and drugs that contribute into strengthening and/or normalization of Na⁺, K⁺-ATPase (phosphodiesterase inhibitor), α -LA, DGLA, ω -3 PUFAs, and the simultaneous prescription of α -LA, ω -3 PUFA and DGLA.

Key words: Diabetes mellitus; Cardiac autonomic neuropathy; Postural hypotension; treatment

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Core tip: Cardiac autonomic neuropathy (CAN) is a serious complication of diabetes mellitus, that is strongly associated with increased risk of cardiovascular mortality. CAN manifests in a spectrum of things, ranging from resting tachycardia and fixed heard rate to development of "silent" myocardial infarction. Although it is common complication, the significance of CAN has not been fully appreciated and there are no unified treatment algorithms for today. In this review we have analyzed the effectiveness of lifestyle modification, prescription of α -lipoic acid, aldose reductase inhibitors; γ -linoleic acid, acetyl-L-carnitine, antioxidants, long-chain ω -3 polyunsaturated fatty acids, vasodilators, vitamin B₁ and some other substances.

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INTRODUCTION

Diabetes mellitus (DM) is a global epidemic affecting at least 8.3% of the population and 371 million people worldwide with a significant proportion (50%) remaining undiagnosed. It is estimated that almost one of six people are currently at risk of developing diabetes-related complications^[1,2].

The majority of patients with long-term course of DM [mainly type 2 diabetes (T2DM)] are diagnosed with coronary heart disease (CHD) due to coronary vessels arterial sclerotic disease. Often the course of CHD is complicated by combination of hypertension, specific kidney arterial involvement, eyes and lower limbs affection. Metabolic alterations in the myocardium are combined with early coronary atherosclerosis. All these changes in heart occur out of prolonged duration of DM among middle age and elderly patients [coronary vessels affection, myocardium changes, diabetic cardiac autonomic neuropathy (CAN) and arterial sclerotic disease] are associated with the term "diabetic heart or diabetic cardiomyopathy". Conditionally, there are two main forms of heart disease in case of DM: diabetic cardiomyopathy (non-coronary genesis); ischemic heart disease. There is a metabolic stage (actual cardiomyopathy); metabolic-ischemic stage-ischemic heart disease; myocardial infarction (MI); dystrophic coronary cardiosclerosis; CAN^[3-5].

Cardiac autonomic neuropathy among T2DM patients, is characterized by lesion of nerve fibers in the sympathetic and parasympathetic divisions of the autonomic nervous system, is diagnosed unsatisfactorily and may be accompanied by severe postural hypotension, decreased tolerance to the physical loadings, and cause the cardiac arrhythmias, ischemia of coronary vessels, "silent" MI, sudden death syndrome^[6-9]. The aim of this study is to review the latest evidence and own data about the treatment perspectives of patients with DM and CAN.

THERAPEUTIC APPROACHES FOR CAN

Based on the CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy^[10], CAN is defined as the impairment of cardiovascular autonomic control among patients with established DM following the exclusion of other causes.

CAN in T2DM, which is characterized by lesion of nerve fibers in parasympathetic and sympathetic nervous systems, is one of the leading causes of heart arrhythmias and an independent risk factor for cardiovascular mortality among these patients^[11,12]. CAN, especially at the early stages, can be subclinical

and thus as the disease progresses, it becomes clinically evident.

Therefore, the problem of effective treatment of CAN is particularly relevant. Pathogenetic treatment of CAN includes: balanced diet and physical activity; optimization of glycemic control; treatment of dyslipoproteinemia (DLP); correction of metabolic abnormalities in myocardium; prevention and treatment of thrombosis; use of aldose reductase inhibitors (ARI); γ -linolenic acid, acetyl-L-carnitine, antioxidants, first of all α -lipoic acid (α -LA), use of long-chain ω -3 and ω -6 polyunsaturated fatty acids (ω -3 and ω -6 PUFAs), vasodilators, fat-soluble vitamin B₁, aminoguanidine; substitutive therapy of growth factors and others^[13-17].

It is obvious that the foreground should be therapy aimed at reducing insulin resistance (IR), correction of hyperglycemia, prevention and treatment of cardiomyopathy, symptomatic treatment of concomitant diseases and syndromes (hypertension, coronary artery disease, heart failure and arrhythmias)^[18,19]. In this regard it is necessary to perform the following preventive and remedial therapy.

Lifestyle modification

Nutrition and physical activity. Correction of obesity. Limit salt intake to 2-4 g/d. Limit smoking, alcohol, foods that contain caffeine. It has been established that compliance with recommended lifestyle modifications (exercise, weight loss, etc.) help improve insulin sensitivity level. Sedentary lifestyle (less than 1000 kcal/wk) is accompanied by the risk of mortality three times higher than when living an active lifestyle. Dosed physical activity reduces hyperinsulinemia and encourages the tendency to normalize lipid metabolism in addition to body weight decrease. Physical activity is associated with higher heart rate variability (HRV) and lower heart rate, therefore may be a predictor of positive changes in HRV indices^[20]. Obtaining the necessary amount of energy combined with physiologic food ration forms the dietary principles. The traditional Mediterranean diet (Greece and Southern Italy) is associated with longevity and/or low mortality due to cardiovascular disease (CVD) complications, decrease the incidence of T2DM, low frequency of wide range of chronic diseases, including rheumatoid arthritis, Parkinson's disease and others^[21-23].

Intensive glycemic control

Compensation state of T2DM is recognized as a primary goal in the prevention of development and/or progression of CVD^[2]. IR is a defining feature in most cases of T2DM and plays a key role in the pathogenesis of myocardial alternations. Obviously, pharmacological agents that are used in the treatment of diabetes should have positive qualities for correction of functional and structural disorders of the cardiovascular system^[3,11,12].

Theoretically, pharmacological agents that improve insulin sensitivity [metformin, thiazolidinediones (TZD)]

appear to be the most appropriate in this regard. It is established that metformin has a positive effect on glucose metabolism; Ca^{2+} concentration in cardiomyocytes, but metformin, unlike TZD, does not show any positive effect on optimization of glucose metabolism in the myocardium^[4,24]. TZD stimulate receptor transcription factors, activated by peroxisome proliferator activated receptor- γ (PPAR- γ), which improves insulin sensitivity and reduces the level of circulating free fatty acids (FFA). It is likely that TZD, despite the absence of the myocardium PPAR- γ type receptors, improve the functional state of the myocardium by reducing the content of FFA. However, the use of TZD among patients with CVD is limited due to the possibility of fluid retention and/or development of edema^[25,26].

Insulin and/or insulin secretagogues: Theoretically, their use may improve glucose metabolism in the myocardium and reduce the content of FFA, however, the assignment of these pharmacological agents is not conducive to the prevention of CVD in the experiment^[4]. Inhibition of PPAR- α expression, which stimulates glucose metabolism and inhibit the metabolism of FFAs, prevents the development of CVD in the experiment, and activation causes the formation of severe cardiomyopathy. Reduction of fat contents in nutrition among animals with increased expression of PPAR- α is accompanied by myocardium lesions warning, confirming the pathophysiological significance of activation of FFA metabolism. Similarly, the use of PPAR- γ agonist medications encourages the activation of glucose metabolism, inhibition of FFA metabolism, prevention of CVD^[4].

Glucagon-like peptide-1 medication: Glucagon-like peptide-1 is one of the two leading "incretins" in the body-hormones that stimulate postprandial secretion and improve insulin sensitivity. The experiment established that the use of glucagon-like peptide-1 (GLP-1) improves the functional state of left ventricular (LV) hemodynamic parameters^[27]. However, GLP-1 medication can not be used in pharmacological therapy of CVD as under influence of dipeptidyl peptidase-4 (DPP-4), GLP-1 is rapidly destroyed (effective half-life is only 1-2 min). Exenatide is 53% GLP-1 homologous and functions as a partial GLP-1 agonist receptor. Alternative to GLP-1 is the use of antagonists of DPP-4 (sitagliptin). However, the exenatide effectiveness as well as antagonists and DPP-4 in suspension/prevention of CVD in T2DM is not clear^[27].

Treatment of dyslipoproteinemia

For DLP pharmacotherapy using statins, fibrates, bile acid sequestrants, nicotinic acid and its derivatives, products of long-chain ω -3 and ω -6 PUFA, or as an alternative-their combination with cholesterol absorption inhibitors^[28].

Statins: Statins (along with lifestyle changes) should be prescribed to patients with T2DM aged over 40

where there is at least one of the risk factors for CVD (regardless of basic lipid levels); prescription of statins among patients with T2DM aged under 40 years without diagnosed CVD should be considered when low density lipoprotein (LDL) cholesterol level exceeds 2.6 mmol/L^[29,30]. Achievement of LDL level in the blood < 1.8 mmol/L or reduction by 30%-40% compared with initial level (in case of failure to achieve value targets in the course of the prescription of the maximum tolerable dose statin) is suitable for patients at high risk of CVD, particularly patients with T2DM. However, statins are often ineffective when used for treatment of atherogenic DLP as pharmacological agents to achieve reduction in triglycerides (TG) and increase high density lipoprotein (HDL) cholesterol; statin use (even at high doses) only partially solves the problem of the risk of CVD^[31-33].

Fibrates: Fibrates limit the availability of substrates for the synthesis of TG in the liver, encourage lipoprotein lipase effects, increase LDL receptor/ligand interaction, stimulate cholesterol secretion with bile; stimulate reverse cholesterol transport, that is accompanied by reduction of TG and very LDL (VLDL) cholesterol levels, and improve insulin sensitivity. Possible mechanisms that help fibrates improve insulin sensitivity are: fibrate binding to receptors that activate PPAR- β enhances fatty acids oxidation in the liver and, consequently, causes increase of insulin sensitivity; fibrates are involved in the regulation of adipokine expression [adiponectin, leptin, tumor necrosis- α (TNF- α), resistin, etc.], accompanied by the increase of insulin sensitivity^[34].

Bile acid sequestrants: Bile acid sequestrants are safe lipid-lowering medicaments, however often causing gastrointestinal adverse reactions. The second generation bile acid sequestrants, including colesevelam binds bile acids with higher affinity and better tolerance. It is used as a supplement to diet therapy and physical activity to reduce the concentration of LDL cholesterol among patients with primary DLP, during monotherapy and/or in combination therapy with statins and to improve glycemic control among patients with T2DM. In addition, it is important that the bile acid sequestrants reduce the concentration of glucose and HbA_{1c} in the blood (approximately 0.9%)^[35] and thus may be useful in the treatment of hypercholesterolemia among patients with T2DM.

Niacin: Niacin is the most efficient pharmacological agent for raising HDL cholesterol level and, to a lesser extent, to reduce the concentration of TG and LDL cholesterol. It is reported that the therapeutic effect of prolonged forms of niacin on lipid profile occurs with the medicament intake in the dose range 0.5-2.0 g. A common reason for not using niacin, which significantly affects patient's susception and accurate application is the problem of "flushing". Current approach to this issue

is the use of combined prolonged form of niacin with laropiprant, an inhibitor of prostaglandin D₂^[36,37].

Long-chain ω -3 PUFAs: The use of long-chain ω -3 PUFAs due to their effects on glucose homeostasis and IR (IR reduction in muscle > adipose tissue >> liver; presumably inhibit insulin secretion and delay the development of T2DM); influence on the state of lipid metabolism (decrease TG concentrations, presumably increase the concentration of HDL cholesterol, improve lipid profile among patients with T2DM and DLP); moderately reduce blood pressure (BP); improve endothelial function; reduce the inflammation and improve antioxidant protection^[38-41].

Ezetimibe: Ezetimibe is used as a nutrition and exercise supplement to reduce the concentration of LDL cholesterol, total cholesterol (TC), and treatment of homozygous familial hypercholesterolemia. Despite some reservations, ezetimibe remains the medicine of first choice among other pharmacological agents in the absence of target specific level of LDL cholesterol using statin monotherapy^[42].

Combined treatment: Therapy of first choice for T2DM in case of lipid profile correction is usage of statins to achieve specific target of LDL cholesterol level < 2.6 mmol/L for primary prevention and < 1.8 mmol/L for secondary prevention of CVD. Failure to get this target is the indication to combine statins with other lipid-lowering agents of other pharmacological groups. A number of international guidelines as a compulsory component of CVD risk monitoring recommend to control apolipoprotein B level on the first-priority basis. However, no results in multicentred, randomized, double-blind, placebo-controlled clinical trials makes it a therapeutic dilemma, since it is unclear whether the intensification of statin therapy or combination of statins with fibrates and/or nicotinic acid will give the desired results^[42,43].

Correction of metabolic abnormalities in the myocardium

Correction of metabolic abnormalities in the myocardium is the basis of pharmacotherapy that aims at optimization of the energy metabolism of the myocardium. Pharmacological impact system includes the following main aspects: use of metabolism regulators; energy-saving solutions; activators of endogenic high-energy compounds and O₂ transportation; inhibitors of metabolic acidosis; membran protection: inhibition of lipid peroxidation membranes of cardiomyocytes; stabilization of lysosomal membranes, neutralization of membranotropic action of humoral agents of lysosomal proteases and others. Medicaments that enhance cell energy state (means of potential energy supply survival of ischemic myocardium). Deterioration of intracellular reserves of carbohydrates needs to be replenished by use of glycolysis activation

measures. The use of macroergic phosphates (ATP, etc.) as a direct energy source is problematic, as the therapeutic effect of ATP in case of ischaemia, probably has less to do with disposing of its macroergic bonds but more with involving products of catabolism of ATP into energy metabolism of cardiomyocytes^[4,44,45].

Modulators of metabolism: Insulin resistance affects myocardial function by reducing glucose transportation and oxidation of carbohydrates; enhancing the use of FFA; inhibition of Ca²⁺ transportation in the sarcolemma; violation of the structure and function of regulatory contractile proteins of myofibrils. In case of DM the reduction of myocardial energy formation leads to inhibition of glucose oxidation and preferential oxidation of fatty acids in the myocardium and skeletal muscle, which increases sensitivity to myocardial ischemia and leads to significant disturbances of Ca²⁺ homeostasis, deterioration of diastolic and systolic myocardial function. The presence of coronary artery disease (CAD) among patients with diabetes worsens the disease and significantly increases cardiovascular mortality. It is considered that even the initial stages of glycemic profile violations may influence the myocardial metabolism and contribute to the development of cardiomyopathy^[4,44,45]. It is important that myocardial dysfunction is a suppositive stage of chronic hyperglycemia elaboration. Thus, dysfunction of cells metabolism, rather than systemic hyperglycemia is the reason for the elaboration of cardiac malfunction^[4,46,47].

Metabolic medicaments: Optimization of myocardial energy metabolism is based on increased myocardial glucose oxidation, which enhances cardiac function and protects myocardial fibers from ischemic and reperfusion injuries. Myocardial use of glucose in case of chronic disease may be improved due to intake of the medicines, that can improve fatty acids metabolism and inhibit their oxidation. New therapeutic approach has been implemented after advent of trimetazidine- the first representative of a new class of metabolic agents- inhibitors of 3-ketoacyl coenzyme A thiolase. Trimetazidine reduces oxidation of fatty acids; stimulates glucose intake; restores the link between glycolysis and carbohydrate oxidation, which leads to the formation of ATP, reducing O₂ consumption; redirects fatty acids towards phospholipids; increases cell tolerance to ischemic and reperfusion injuries; increases the oxidation of glucose, the activity of Na⁺, K⁺-ATPase and Ca²⁺-pumps in the sarcoplasmic reticulum. Anti-ischemic properties of trimetazidine do not depend on changes in hemodynamics and are associated with a distinct recovery of mechanical function after ischemia, which makes it recognized as cardyo-cytoprotective agent. Trimetazidine prescription improves glucose metabolism; reduces endothelin-1 among patients with diabetic cardiomyopathy, that is taken to have effect on the vascular endothelium; accompanied by a significant

positive changes in ejection fraction (EF) parameters among patients with heart failure; improves quality of life parameters and NYHA functional class^[48,49]. Another pharmacological agent that facilitates the inhibition of metabolism of fatty acids is perhexiline. Perhexiline prescription to patients with heart failure significantly contributes to the improvement of EF, VO₂max and quality of life. Unfortunately, the clinical use of this medicament is limited because of the risk of hepatotoxicity and peripheral neuropathy^[50]. Ranolazine is the third antianginal pharmacological agent with a potential of metabolism modulator. However, the following factors do not allow to implement its use: the degree of inhibition of fatty acids metabolism is limited by physiological indicators; ranolazine prescription associates with the possibility of corrected QT interval prolongation^[51].

Limitation of extracellular Ca²⁺ into the cell:

Blockers of Ca²⁺-channels show a protective effect on myocardium in case of ischemia. In terms of correction of cell power the most pathogenetically efficient option is the use of Ca²⁺ blockers, however they only eliminate secondary dysfunction links of oxidative phosphorylation in mitochondria. Prescription of β -adrenergic receptor blockers for T2DM with CAD and CAN has significant pathogenetic grounds as high sympathetic activity that is followed by CAN, accelerates the development of CVD and significantly affects prognosis. In addition, several studies demonstrated the ability of β -blockers to reduce the incidence of "silent" myocardial ischemia episodes and improve prognosis among these patients. However, adrenergic receptors β -blockers negatively affect the performance of glycemic profile, increase the risk of hypoglycemia, showing a negative effect on blood lipid profile and can provoke acute heart failure. The above described events occur with prescription of non-selective β -blockers. Selective β -adrenergic receptor blockers, including metoprolol, are free of side effects, including the effectiveness of metoprolol in the treatment of CVD demonstrated in numerous controlled studies. Metoprolol has cardioprotective properties; improves prognosis among patients with CAD; has a fair tolerance in case of prolonged use. Cardioselective β -blockers can also balance the effects of autonomic dysfunction in particular by resisting sympathetic stimulation they can restore parasympathetic-sympathetic balance. However, traditional antianginal agents that affect hemodynamic parameters (β -blockers, Ca²⁺ antagonists, etc.), have lower tolerance among elderly due to the high risk of the interaction of pharmacological agents with a significant incidence of side effects^[3,4,45,46].

Medicaments that contain micro- and macro-elements, primarily Mg²⁺:

One of the risk factors that can decrease insulin sensitivity is hypomagnesaemia. It is suggested that Mg²⁺ deficiency plays a significant role in increasing the risk of diabetic macro- and microvascular complications and, especially, risk of

CAD^[4,16,17].

Thrombosis prevention and treatment

Platelets obtained from patients with T2DM and tested *in vitro* are characterized by a real ability to aggregate under the influence of ADP, adrenaline, collagen, arachidonic acid, and thrombin. Aggregation of platelets is significantly increased in the second, irreversible phase, which depends on the transformation of arachidonic acid into labile prostacyclin and thromboxane. Thus, the possibility of ADP receptors of platelet membranes blocking is a pathogenetically justified measure. Prescription of antiplatelet agents, namely acetylsalicylic acid (ASA), clopidogrel and others can help prevent blood clots, stenocardia and development of MI. The active clopidogrel metabolite irreversibly binds to ADP receptor on the platelet membrane, which leads to inhibition of adenylate cyclase; inhibition of ADP-dependent secretion of platelet granules; inhibition of ADP-dependent process of binding fibrinogen receptor to the platelet membrane; does not affect the expression of receptors directly; blocks myointimal proliferation in case of vascular damage; unlike ASA does not affect the activity of cyclooxygenase. Effect of clopidogrel and ASA synergy is demonstrated in the study of platelet *ex vivo*. However, clopidogrel is more effective pharmacological agent within the frames of the combined risk of MI, stroke, and the syndrome of "sudden death" reduction^[52-55].

ARI

ARI inhibit the glucose polyol way metabolism, prevent the reduction of the redox potentials. Analysis of the double-blind, placebo-controlled study established that tolrestat contributes to the improvement of independent tests results and vibration sensitivity among patients with symmetric diabetic peripheral neuropathy (DPN). Zenarestat prescription for 12 mo was accompanied by a dose-dependent changes in the spissitude of nerve tissue, increased the velocity of nerve impulses, improved myocardial systolic function. Zoporestat, ranirestat-medicaments of a new generation of ARI group showed sufficient efficacy in experimental studies^[56-59].

Replacement therapy with help of myoinositol

Several individual clinical trials were conducted for the study of myoinositol efficacy in the treatment of diabetic neuropathy. The results are quite positive, but the future clinical double-blind, placebo-controlled trials are needed^[60-62].

Aminoguanidine

Aminoguanidine improves capacity of nerve velocity, increases blood flow, inhibits the formation of advanced glycation endproducts, delays the emergence and development of albuminuria. Analysis of controlled trials confirmed quite aminoguanidine high efficiency among patients with diabetic neuropathy, but the development of a number of side effects terminated their application.

The use of aminoguanidine derivatives is accompanied by clinical efficacy and lack of adverse side effects^[6,8,11]. The results are promising, but need further clinical double-blind, placebo-controlled studies.

Neurotrophic therapy

Inhibition of nerve growth factor (NGF) expression and its receptors suppresses NGF axonal retrograding transport and reduces the activity of small demyelinated neurons and their neuropeptides, including substance P and gene-linked calcitonin peptide. The use of recombinant human NGF normalizes neuropeptide concentration and prevents the development of sensory neuropathy in the experiment. However, the results of clinical placebo-controlled studies deny the positive impact of recombinant human NGF among patients with diabetic neuropathy^[6,8].

Antineural autoimmunity human immunoglobulin for intravenous use

Intravenous human immunoglobulin prescription is recommended for patients with DPN, which have signs of antineural autoimmunity symptoms. The side effects include headache, and the main danger could be the development of an anaphylactic reaction, however, it affects mainly patients with deficiency of immunoglobulin A^[6,8].

Endoneural perfusion inhibition with the development of hypoxia

Experimental and clinical studies have shown benefit in the efficiency of vasodilators when used for improvement of nerve flow velocity, but there is not enough information about the impact of vasodilators on the course of DPN during clinical double-blind placebo-controlled studies. The research results of characteristics that impact the angiotensin-converting-enzyme inhibitors on heart rate variability parameters among diabetic patients with CAN appeared to show diametrically opposed results. In particular, prescription of quinapril for 3 mo was accompanied by statistically significant increased parasympathetic activity, and the use of trandolapril for 12 mo did not affect the performance of autonomic myocardial function. However, most of these pharmacological agents have no proven clinical and electrophysiological positive effects and have certain limitations and contraindications^[4-6,11].

Activation of free radical stress

Considering that one of the major pathogenetic mechanisms of neuropathy is oxidative stress (OS), the need for antioxidants prescription is obvious. Great therapeutic potential is observed in α -LA and creates pathogenic evidence for the use of this pharmacological agent^[63-65]. Mechanism of α -LA action is not fully developed, but specific attention should be paid to two hypotheses. Firstly, α -LA phenomenon causes dose-dependent proliferation of neuroblastoma

cultured cells. Changes in the membrane fluidity that are mediated through sulfhydryl groups α -LA are considered to cause this effect. This is confirmed by the following results of several studies, including experimental neuropathy induced by acrylamide, followed by a significant inhibition of proliferation of the above phenomenon; overlay and/or progression of experimental distal neuropathy, mainly caused by a decrease of content of substances in axons containing sulfhydryl groups (e.g., glutathione); α -LA *in vivo* and *in vitro* enhances spontaneous processes of expansion and improvement of the structural and functional nerve terminals membranes state; prescription of α -LA stimulates the regeneration of nerve terminals in case of the partial denervation, as well as experimental hexacarbon neuropathy. Secondly, and the most probable mechanism is the ability of α -LA to function as a radical binder ("cleaner")^[66-69].

Vitamins with antioxidant properties [a liposoluble vitamin B₁ (benfotiamin)], combined medications

There is enough experimental and clinical results of studies that suggest that the hyperinsulinemia, IR, and chronic hyperglycemia in T2DM have a negative impact on the metabolism of thiamine particularly due to the inhibition of the functional state of the thiamine transporter-1 and thiamine transporter-2, responsible for the reabsorption of vitamin in the proximal tubules of the kidneys; transketolase activity, which can lead to the congestion of intermediates in the initial stages of glycolysis [glyceraldehyde-3-phosphate (GA3P), fructose-6-phosphate (F6P) and dihydroxyacetone-phosphate]. Congestion of intermediates in case of chronic hyperglycemia increases the production of free radicals in the mitochondria, followed by inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Increased concentrations of GA3P, F6P and GAPDH can initiate induced hyperglycemia, metabolic fates that favor the overlay of vascular injury, including activation of protein kinase-C, accumulation of advanced glycation end products (AGEs) hexosamine biosynthetic fates activation, dicarbonyl compounds. Activation with dicarbonyl compounds is followed by further stimulation of the AGEs formation, which is also associated with functional impaired and structural state of cardiomyocytes^[70-72].

It is clear that the correction of thiamin deficiency must be performed using exogenous vitamin B₁, or benfotiamine (monophosphate S-benzoyl-thiamine, high-bioavailable liposoluble vitamin B₁ derivatives). Results of experimental and clinical studies suggest a positive effect of benfotiamine prescription on prevention of diabetic vascular disease progression. Benfotiamine broad therapeutic potential has a good efficiency on medications containing soluble thiamine derivatives for the purpose of regulating the activity of free radical processes; correction of endothelial dysfunction in case of CVD, stabilization of clinical and antioxidant effects. Benfotiamine favoring the transketolase (TK)

activity prevents the activation of pathophysiological mechanisms by reorientation towards of F6P and GAPDH metabolism^[73-75]. Benfotiamine can promote neuronal and vascular deficiency correction through participation of nitrogen oxide processes, which have a significant therapeutic potential for the treatment of CVD. The use of thiamine and α -LA combination has a great significance in the treatment of diabetic angio-neuropathy. In particular, it demonstrated that prescription of benfotiamine and α -LA to patients with T1DM was followed by normalization of hyperglycemia and for 4 wk it promoted the normalization of prostacyclin synthase suppressed by diabetes; increase of TK activity in monocytes in 2-3 times^[76-80].

Fatty acids metabolism disorders (γ -linolenic acid, acetyl L-carnitine)

Vasoactive prostanoids, metabolites and dihomogamma-linolenic acid (DGLA), including prostaglandins and other eicosanoids are necessary for the physiological behavior of nerve conductivity and blood flow. The results of double-blind, placebo-controlled studies showed that prescription of DGLA to patients with DPN is followed by positive dynamics in clinical course, as well as increase in the speed of nerve conductivity. L-carnitine's main function is to strengthen the metabolism of fatty acids, but there are experimental evidence of L-carnitine's ability to activate glucose metabolism. It is believed that T2DM is characterized by malfunction of L-carnitine exchange in the mitochondria. The results of several studies showed that prescription of L-carnitine helps to improve energy supplies and LV function. It is established that propionyl-L-carnitine improves the functional status, used as glucose energy oxidation in the rat's affected myocardium (despite the increased level of fatty acids). Nutrition of diabetic mice with obesity with L-carnitine addition increases the level of acyl-carnitine in the blood, muscle, liver and adipose tissue; increases levels of pyruvate dehydrogenase activity in the muscles; prescription of zinc-carnitine mixture reduces hyperglycemia and improves glucose tolerance. L-carnitine infusion with the help of hyperinsulinemic-euglycemic clamp improves glucose profile control, reduces the concentration of circulating lipids. L-carnitine prescription for 3 or 6 mo for newly diagnosed patients with T2DM with lipid metabolism disorders is followed by a statistically significant decrease in lipoprotein(a) [Lp(a)] levels. The results of double-blind, placebo-controlled studies among patients with verified hyperLP(a) established that L-carnitine (2 g/d) encouraged a significant decrease in the concentration of Lp(a) levels; L-carnitine incorporation into nutrition of patients with newly diagnosed T2DM is followed by similar changes; combined L-carnitine with simvastatin (20 mg/d) treatment is much more efficient in decreasing the concentration of lipids, including TG and Lp(a) than statin monotherapy. Thus, L-carnitine can be used as one of the components for lipid-modifying therapy among patients with T2DM^[81,82].

ω -3 and ω -6 PUFAs medications

A fundamentally new approach to assessing the biological role of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) is associated with long-term epidemiological studies results among Inuits, which established a small percentage of CVD. The Greenlandic Inuits were observed to have an increased bleeding duration, lower levels of TC, TG, VLDL-cholesterol and a significant increase in TC lipid membranes of EPA and DHA contents, arachidonic acid concentration reduction and linoleic acid. For the first time these results allowed to express a reasonable assumption about the protective effect of DHA and especially EPA from the damaging effects on the internal vessel wall cause capable of inducing experiment CAD-a phenomenon of TC activation and high blood viscosity, enhanced the cyclic endoperoxide synthase, including prostaglandin H₂, TXA₂ activation of endothelial cell proliferation, hypercholesterolemia and hypertriglyceridemia. Prescription of EPA and DHA is followed by a decrease in the "rigidity" of red blood cells, which is obviously associated with labilization of erythrocyte plasmolemma based on rapid and intensive incorporation of long-chain ω -3 PUFA phospholipids into membrane and decreased synthesis of vasoconstrictor active ingredients. The ability of exogenous EPA and DHA to incorporate phospholipid blood cell membranes and membrane phospholipids of endothelial cells blood vessels affects the fundamental plasmolemma properties and receptors function for the perception and processing of extracellular information. Accumulating long-chain polyenes acids, labilize plasmolemma, changing the microviscosity of its lipid matrix, which causes the transformation of the basic plasmolemma properties-permeability, generation of biopotentials, ions transit. Changes in the lipid environment of receptor structures affects their functional activity and enzyme systems control in the cell, which primarily relates to the corpuscular adenylate cyclase, whose function is related to the metabolism of phospholipids^[83-85].

Analysis of experimental and clinical studies proves that ω -3 PUFA inhibit the absorption of cholesterol in the intestine and its synthesis in the liver, lead to increased clearance of lipoproteins in the blood, prevent the development of IR in experimental diabetes, decrease level of BP, dose-dependently prevent the development of diabetes, improve the sensitivity of platelets to ADP and collagen, contribute to positive changes in the parameters of coagulation, endothelial cells migration, inhibits the proliferation of smooth muscle cells. However, the studies aimed to investigate the features of ω -3 PUFA in T2DM are numerically small and obtained results do not always testify to their effectiveness^[86-93]. In particular, the results of the ORIGIN trial demonstrated, that administration of 1 g ω -3 PUFA did not reduce the rate of death caused by cardiovascular reasons or their outcomes during a period of 6 years among patients with dysglycemia and additional cardiovascular risk factors. In this trial the dose of ω -3 PUFA was not chosen

Table 1 N-terminal fragment of the prohormone brain natriuretic peptide level and lipid metabolism parameters after 3-mo of omega-3 polyunsaturated fatty acid therapy

Parameter	Patients with T2DM and CAN (<i>n</i> = 36)	
	Control (<i>n</i> = 15) Group 1	ω -3 PUFA (<i>n</i> = 21) Group 2
NT-proBNP	-3.0 \pm 1.1	-6.8 \pm 1.1 ^a
LDL cholesterol	-8.3 \pm 1.4	-12.8 \pm 1.9
HDL cholesterol	4.1 \pm 1.0	7.1 \pm 0.5 ^a
TG	-8.3 \pm 1.2	-35.4 \pm 2.6 ^c
TC	-6.7 \pm 1.0	-8.2 \pm 1.1

The results are presented as % change from baseline, (Δ %, Mean \pm SEM); ^a*P* < 0.05, ^c*P* < 0.001. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; ω -3 PUFA: Omega-3 polyunsaturated fatty acid; NT-proBNP: N-terminal fragment of the prohormone brain natriuretic peptide; LDL cholesterol: Low density lipoprotein cholesterol; HDL cholesterol: High density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol.

on the basis of any estimate of its effect on TG levels, nevertheless, a significant reduction in the TG level was shown. However, this study did not apply to treatment of CAN and it was decided to continue the study for a few more years^[94]. In the same time, American Diabetes Association (ADA, 2005) recommend the prescription of α -LA and ω -3 PUFA in algorithms of DPN treatment^[95] and in ADA recommendations (2014) and results of some trials-prescription of ω -3 PUFA in DLP treatment among patients with T2DM and cardiovascular diseases^[2,90-92].

To explore the effectiveness of some above-mentioned compounds we examined 81 patients with T2DM and CAN, patients were aged between 50-59 years with disease duration 1-6 years and median HbA_{1c} 7.1% \pm 0.4%. CAN was diagnosed according to previously proposed criteria^[8,10,12]. The work was done according to the principles of the Declaration of Helsinki (2004) and all subjects signed an informed consent prior their inclusion in the study. Patients were allocated to five treatment groups: first group received traditional antihyperglycemic therapy (*n* = 15, control group); patients in group 2 (*n* = 21), received in addition to standard treatment 1 capsule/d of the ω -3 PUFA; patients in 3rd group (*n* = 12) - benfotiamine 300 mg/d; patients in 4th group (*n* = 18) -600 mg of α -LA, patients in 5th (*n* = 15) -1 capsule/d of the ω -3 PUFA, benfotiamine 300 mg/d and 600 mg of α -LA. Each one gram capsule of the ω -3 PUFA contains approximately 465 mg of EPA and 375 mg of DHA. The duration of the treatment was three months.

The concentration of glucose in the blood was determined by the glucose oxidase method while HbA_{1c} was assessed by using a highly sensitive method of ion-exchange liquid chromatography with D-10 analyzer and BIO-RAD reagents (United States). Determination of immunoreactive insulin (IRI) was performed using commercial kits from immunotech insulin immunoradiometric assay reagents (Czech Republic); leptin level-from Immunotech Leptin (Czech Republic) test

kits; TNF- α -from Vector-Best (Russia); high-sensitivity C-reactive protein (hsCRP)-from diagnosis-related group (United States); N-terminal fragment of the prohormone brain natriuretic peptide (NT-proBNP)-from Biomedica (Austria) kits and an enzyme-linked immunosorbent assay analysis technique. Lipid metabolism was assessed by the concentration of TC, LDL-, HDL-, VLDL-cholesterol measurements. The lipid fractions were determined by using HUMAN reagents (Germany) for the analyzer HUMANLAYZER 2000.

We found out that the HbA_{1c} of patients with T2DM and CAN was not statistically significant influenced by the treatment (*P* > 0.05). Treatment with the drug containing ω -3 PUFA among patients with T2DM and CAN (group 2) led to a significant increase of the HDL cholesterol level [7.1% \pm 0.5%, (*P* < 0.05)] and reduction of TG [-35.4% \pm 2.6%, (*P* < 0.05)]. The treatment also lead to a significant decrease of the NT-proBNP level [-6.8% \pm 1.1%, (*P* < 0.05)] compared to the control group. Changes of NT-proBNP and lipid metabolism parameters among patients with T2DM and CAN after 3-mo of ω -3 PUFA therapy are given in Table 1.

Benfotiamine prescription to patients with T2DM and CAN did not cause any significant changes in lipid profile and leptin levels (*P* > 0.05), while it probably helped reduce the IRI concentration [-12.7% \pm 1.4%, (*P* < 0.05)]. The use of benfotiamine in the comprehensive treatment of T2DM helped reducing hsCRP [-13.3% \pm 2.1%, (*P* < 0.05)] and TNF- α [-10.2% \pm 1.6%, (*P* < 0.05)] concentrations, but the prescription of α -LA was followed by a significant decrease in these parameters [-15.2% \pm 1.9%, (*P* < 0.01) and -14.7% \pm 1.8%, (*P* < 0.001), accordingly] and facilitated visible LDL cholesterol [-14.2% \pm 1.8%, (*P* < 0.05)], IRI [-15.9% \pm 1.6%, (*P* < 0.01)] and leptin [-16.3% \pm 1.2%, (*P* < 0.001)] reduction, also increased HDL cholesterol level [7.8% \pm 0.7%, (*P* < 0.01)]. Combined ω -3 PUFA, benfotiamine and α -LA prescription was followed by the more pronounced decrease of IRI, leptin and some inflammation factors (Table 2).

Obtained results of this study could prove that prescription of ω -3 PUFA is accompanied by more significant decrease of TG and increase of HDL cholesterol levels compared to patients in control group. The complex therapy with α -LA contributes to more evident antiatherogenic effect, in particular decrease of LDL and TC, increase of HDL cholesterol level (compared to patients of 1st, 2nd and 3rd groups). Combined prescription of ω -3 PUFA, benfotiamine and α -LA is followed by more statistically significant positive changes of lipid profile (Table 3).

In order to evaluate the artery stiffness parameters during active and passive periods of the day the 24-h blood pressure profile, aorta (AIXao) and brachial augmentation index (AIXbr), pulse wave velocity (PWV) and ambulatory arterial stiffness index (AASI) were

Table 2 Changes of the immunoreactive insulin, leptin, high reactive C-reactive protein and tumor necrosis factol alpha levels after 3-mo of treatment

Parameter	Patients with T2DM and CAN (n = 81)				
	1 st group (n = 15)	2 nd group (n = 21)	3 rd group (n = 12)	4 th group (n = 18)	5 th group (n = 15)
IRI	-6.8 ± 2.0	-10.3 ± 1.1	-12.7 ± 1.4 ^a	-15.9 ± 1.6 ^{b,e}	-20.9 ± 0.9 ^{c,f,i,j}
Leptin	-7.1 ± 1.8	-15.8 ± 1.7 ^b	-6.4 ± 1.4 ^f	-16.3 ± 1.2 ^{c,i}	-18.4 ± 1.4 ^{c,i}
hsCRP	-7.2 ± 1.6	-14.8 ± 2.4 ^a	-13.3 ± 2.1 ^a	-15.2 ± 1.9 ^b	-22.6 ± 1.6 ^{c,e,h,k}
TNF-α	-6.1 ± 1.0	-14.1 ± 2.1 ^b	-10.2 ± 1.6 ^a	-14.7 ± 1.8 ^c	-19.8 ± 1.6 ^{c,d,i,l}

The results are presented as % change from baseline, (Δ%, Mean ± SEM);
^aP < 0.05, ^bP < 0.01, ^cP < 0.001 - compared to 1st group; ^dP < 0.05, ^eP < 0.01,
^fP < 0.001 - compared to 2nd group; ^hP < 0.01, ⁱP < 0.001 - compared to
3rd group; ^jP < 0.05, ^kP < 0.01, ^lP < 0.001 - compared to 4th group. T2DM:
Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; IRI:
Immunoreactive insulin; hsCRP: High reactive C-reactive protein; TNF-α:
Tumor necrosis factol alpha.

Table 3 Changes of the lipid metabolism parameters after 3-mo of treatment

Parameter	Patients with T2DM and CAN (n = 81)				
	1 st group (n = 15)	2 nd group (n = 21)	3 rd group (n = 12)	4 th group (n = 18)	5 th group (n = 15)
LDL cholesterol	-8.3 ± 1.4	-12.8 ± 1.9	-7.6 ± 1.0 ^d	-14.2 ± 1.8 ^{a,h}	-33.1 ± 2.4 ^{c,f,i,j}
HDL cholesterol	4.1 ± 1.0	7.1 ± 0.5 ^a	5.7 ± 0.6	7.8 ± 0.7 ^{b,g}	13.9 ± 1.3 ^{c,f,i,j}
TG	-8.3 ± 1.2	-35.4 ± 2.6 ^c	-13.3 ± 3.4 ^f	-9.3 ± 1.1 ^f	-27.9 ± 3.9 ^{c,h,i}
TC	-6.7 ± 1.0	-8.2 ± 1.1	-7.1 ± 1.2	-10.7 ± 1.3 ^{a,g}	-27.2 ± 1.9 ^{c,f,i,j}

The results are presented as % change from baseline, (Δ%, Mean ± SEM);
^aP < 0.05, ^bP < 0.01, ^cP < 0.001 - compared to 1st group; ^dP < 0.05, ^eP <
0.001 - compared to 2nd group; ^gP < 0.05, ^hP < 0.01, ⁱP < 0.001 - compared
to 3rd group; ^jP < 0.001 - compared to 4th group. T2DM: Type 2 diabetes
mellitus; CAN: Cardiac autonomic neuropathy; LDL cholesterol: Low
density lipoprotein cholesterol; HDL cholesterol: High density lipoprotein
cholesterol; TG: Triglycerides; TC: Total cholesterol.

assessed by TensioMed™ Arteriograph 24 (Hungary). The program orders the values of the AIXbr and PWV into ranges as follows: optimal values: AIXbr > -30%, PWV < 7 m/s; normal values: -30% < AIXbr < -10%, 7 m/s < PWV < 10 m/s; elevated values: -10% < AIXbr < 9.8%, 9.8 m/s < PWV < 12 m/s; pathological values: AIXbr > 10%, PWV > 12 m/s^[96]. The study involved 51 patients with T2DM, among them 12 patients without CVD and CAN, 39 patients with moderate CAN. Patients with diagnosed CAN were allocated to two groups: control group (n = 18) received traditional antihyperglycemic therapy and treatment group (n = 21) received in addition to standard treatment 1 capsule/d of the ω-3 PUFA. Control-12 healthy volunteers. Artery stiffness parameters among patients with T2DM without CAN were within normal limits, but this group has a tendency toward increase of vascular wall stiffness parameters. The arterial stiffness parameters among patients with moderate CAN exceed the physiological values, in particular AIXao 26.2% (P < 0.01), AIXbr 66.2% (P < 0.001), PWV 24.7% (P <

0.001), AASI 30.6% (P < 0.01) compared to patients with T2DM without CAN and were considered as high (Table 4). After 1.5 mo of treatment we found out that there was a decrease of AIXbr (-10.0% ± 2.62%, P < 0.05) and PWV (9.8 ± 0.42 m/s, P < 0.01) values in treatment group. Prescription of ω-3 PUFA for three months was followed by more significant decrease of AIXao (27.8% ± 1.13%, P < 0.05), PWV (9.3 ± 0.42 m/s, P < 0.01) during the 24 h; decrease of AIXao (16.2% ± 3.12%, P < 0.01), PWV (-11.6% ± 2.09%, P < 0.05) during the day and decrease of AIXao (-11.2% ± 4.2%, P < 0.05), AIXbr (-98.0% ± 18.1%, P < 0.05), PWV (-18.9% ± 3.9%, P < 0.01) during the night. At the same time there wasn't significant influence on the AIXbr during the active period of day (Tables 5 and 6). Therefore, the administration of ω-3 PUFA to patients with T2DM for three months promotes arterial stiffness parameters improvement.

We previously reported that the use of ω-3 PUFA, which contains in one capsule approximately 90% ω-3 PUFA, mainly EPA and DHA, in the treatment of patients with T2DM and CAN improved the general condition of the patients. Thus, prescription of ω-3 PUFA contributed to significant decrease of mean diastolic blood pressure (DBP), time index of diastolic hypertension, diastolic hypertension area index and variability of DBP during the day and night hours and was followed by a tendency to a low pulse pressure^[97-101]. The influence of ω-3 PUFA on the dynamics of metabolism is probably caused by their effects on IR, glucose homeostasis and lipid metabolism (improvement of the lipid profile in patients with T2DM and DLP). In addition, ω-3 PUFA moderately reduce BP, improve endothelial function, reduce proinflammatory status and improve antioxidant protection. The combination of the positive influences of ω-3 PUFA on NT-proBNP, lipid profile and their moderate hypotensive effects suggests the feasibility of their use in the complex treatment of patients with T2DM and CAN. Further investigations aimed to establish the influence of ω-3 PUFA on dynamics of independent cardiovascular tests, daily monitoring of electrocardiography, daily monitoring of BP, arterial wall stiffness parameters among patients with T2DM and CAN are necessary^[102-104].

Orthostatic hypotension treatment

Postural hypotension syndrome is manifested by dizziness and possibility of consciousness loss. Hypovolemia and sympathoadrenal disorders are the most characteristic features among patients with T2DM and orthostatic hypotension. Postural hypotension among most diabetic patients progresses asymptotically and, therefore, does not require correction. However, in severe cases-it is key traumatic factor. Treatment of symptomatic postural hypotension among patients with CAN is very complicated because of the need to achieve a balance between changes in BP in the vertical and horizontal position. The increase of peripheral venous inflow is achieved through

Table 4 Arterial stiffness parameters in patients with type 2 diabetes mellitus and cardiac autonomic neuropathy

Parameter	Control (<i>n</i> = 12) 1 st group	Patients with T2DM without CVD and CAN (<i>n</i> = 12) 2 nd group	Patients with T2DM and CAN (<i>n</i> = 21) 3 rd group
Alxao (%)	20.6 ± 1.71	26.7 ± 1.84 ^a	33.7 ± 1.24 ^{c,e}
Alxbr (%)	-33.7 ± 2.86	-23.4 ± 1.91 ^b	-7.9 ± 2.67 ^{c,f}
PWV (m/s)	7.2 ± 0.31	8.9 ± 0.25 ^c	11.1 ± 0.39 ^{c,f}
AASI	0.3 ± 0.02	0.36 ± 0.02 ^a	0.47 ± 0.03 ^{c,e}

Δ%, Mean ± SEM; ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001 - compared to 1st group; ^d*P* < 0.01, ^e*P* < 0.001 - compared to 2nd group. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; CVD: Cardiovascular diseases; Alxao: Aorta augmentation index; Alxbr: Brachial augmentation index; PWV: Pulse wave velocity; AASI: Ambulatory arterial stiffness index.

Table 5 Changes of day arterial stiffness parameters after 3 mo omega-3 polyunsaturated fatty acid therapy

Patients with T2DM and CAN (<i>n</i> = 39)				
	Groups	Baseline	After treatment	% change from baseline
Alxao (%)	Control group	30.4 ± 1.97	28.4 ± 1.68	-4.3% ± 4.76%
	Treatment group	32.0 ± 1.32	26.4 ± 1.12 ^b	-16.2% ± 3.12%
Alxbr (%)	Control group	-10.6 ± 3.37	-12.0 ± 3.11	-19.3% ± 12.14%
	Treatment group	-9.8 ± 2.76	-14.3 ± 2.84	-42.8% ± 9.0%
PWV (m/s)	Control group	10.2 ± 0.4	9.6 ± 0.4	-6.0% ± 2.21%
	Treatment group	11.0 ± 0.35	9.7 ± 0.39 ^a	-11.6% ± 2.09%

The results are given as absolute values and as % change from baseline, (Δ%, Mean ± SEM); ^a*P* < 0.05, ^b*P* < 0.01, - compared to baseline. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; Alxao: Aorta augmentation index; Alxbr: Brachial augmentation index; PWV: Pulse wave velocity.

Table 6 Changes of night arterial stiffness parameters after 3 mo omega-3 polyunsaturated fatty acid therapy

Patients with T2DM and CAN (<i>n</i> = 39)				
	Groups	Baseline	After treatment	% change from baseline
Alxao (%)	Control group	33.2 ± 1.98	30.1 ± 1.27	-6.6% ± 4.15%
	Treatment group	36.6 ± 1.65	31.7 ± 1.23 ^a	-11.2% ± 4.2%
Alxbr (%)	Control group	-4.2 ± 2.8	-5.9 ± 2.48	-10.0% ± 17.23%
	Treatment group	-1.6 ± 2.79	-10.4 ± 3.23 ^a	-98.0% ± 18.1%
PWV (m/s)	Control group	10.9 ± 0.4	10.3 ± 0.36	-4.93% ± 1.41%
	Treatment group	11.3 ± 0.48	9.0 ± 0.44 ^b	-18.9% ± 3.9%

The results are given as absolute values and as % change from baseline, (Δ%, Mean ± SEM); ^a*P* < 0.05, ^b*P* < 0.01, - compared to baseline. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; Alxao: Aorta augmentation index; Alxbr: Brachial augmentation index; PWV: Pulse wave velocity.

the use of elastic tightening body linen. It is inappropriate to prescribe psychotropic and diuretic drugs, and eliminate the possibility of electrolyte disorders and/or reduce the fluid volume. Prescription of glucocorticoids is efficient among some patients with postural hypotension, but may be followed by the development of edema, risk of arterial hypertension. Metoclopramide is efficient among patients with excessive dopaminergic activity, or increased sensitivity to dopaminergic stimulation. The ineffectiveness of the above remedial measures requires the prescription of α₁-adrenergic agonists (midodrine) or dihydroergotamine combined with caffeine. Exceptional refractory to the treatment, often postprandial orthostatic hypotension forms determine the necessity of octreotide prescription^[105,106].

PROSPECTIVE DIRECTIONS OF CAN TREATMENT

The revival of interest in vascular hypothesis of CAN, OS index, neurotrophic hypothesis and importance of autoimmune disorders opens up new areas of treatment. The promising methods include research and use of tools that increase blood flow through the vasa vasorum, including butaprost (prostacyclin analogue), TXA₂ blockers and drugs that contribute into strengthening and/or normalization of Na⁺, K⁺-ATPase (cilostazol-a potential phosphodiesterase inhibitor), α-LA, DGLA, ω-3 PUFAs, and the simultaneous prescription of α-LA, ω-3 PUFA and DGLA^[107-112]. In addition, the combination of α-LA, ω-3 PUFAs, DGLA and ARI is the

most rational pathogenetically justified use.

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Short and long term neuro-behavioral alterations in type 1 diabetes mellitus pediatric population

Edna Litmanovitch, Ronny Geva, Marianna Rachmiel

Edna Litmanovitch, Ronny Geva, the Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 5290002, Israel

Ronny Geva, Department of Psychology, the Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 5290002, Israel

Marianna Rachmiel, Pediatric and Adolescents, Diabetes Mellitus Service, Division of Pediatrics, Assaf Harofeh Medical Center, Zerifin 70300, Israel

Marianna Rachmiel, Sackler School of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel

Author contributions: Litmanovitch E substantial contributed to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and final approval of the version to be published; Geva R substantial contributed to design of review and interpretation of data, revising it critically for important intellectual content, and final approval of the version to be published; Rachmiel M substantial contributed to conception and design, analysis and interpretation of data, revising it critically for important intellectual content, and final approval of the version to be published.

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Correspondence to: Marianna Rachmiel, MD, Head of Pediatric and Adolescents, Diabetes Mellitus Service, Division of Pediatrics (Building 131), Assaf Harofeh Medical Center, Zerifin section on 44 Road, Zerifin 70300, Israel. rmarianna@gmail.com
Telephone: +972-8-9542007

Fax: +972-8-9779136

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Abstract

Type 1 diabetes mellitus (T1DM) is one of the most prevalent chronic conditions affecting individuals under the age of 18 years, with increasing incidence worldwide, especially among very young age groups, younger than 5. There is still no cure for the disease, and therapeutic goals and guidelines are a challenge. Currently, despite T1DM intensive management and technological interventions in therapy, the majority of pediatric patients do not achieve glycemic control goals. This leads to a potential prognosis of long term diabetic complications, nephrological, cardiac, ophthalmological and neurological. Unfortunately, the neurological manifestations, including neurocognitive and behavioral complications, may present soon after disease onset, during childhood and adolescence. These manifestations may be prominent, but at times subtle, thus they are often not reported by patients or physicians as related to the diabetes. Furthermore, the metabolic mechanism for such manifestations has been inconsistent and difficult to interpret in practical clinical care, as reported in several reviews on the topic of brain and T1DM. However, new technological methods for brain assessment, as well as the introduction of continuous glucose monitoring, provide new insights and information regarding brain related manifestations and glycemic variability and control parameters, which may impact the clinical care of children and youth with T1DM. This paper provides a comprehensive review of the most recently reported behavioral, cognitive domains, sleep related, electrophysiological, and structural alterations in children and adolescences from a novel point of view. The review focuses on reported impairments based on duration of T1DM, its timeline, and modifiable disease related risk parameters. These findings are not without controversy, and limitations of data are presented in addition to recommendations for future research direction.

Key words: Type 1 diabetes mellitus; Cognitive; Behavioral; Brain; Alterations; Children; Adolescences

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Core tip: Neurocognitive and behavioral functions vary with age and depend on interactions between multiple endogenous (brain structure, integrity) and exogenous (metabolic) factors. Understanding of brain development with age is an emerging field of research, and delineation of type 1 diabetes mellitus (T1DM) impact on those processes is even more challenging and unclear. We review the most recent information in a novel format, relevant for clinicians practicing pediatric medicine and diabetologists taking part in clinical oriented research, in order to clarify: what is known, what is its association to modifiable diabetes related aspects, what should clinicians pay attention to, and what is needed in future research. The available studies presented hereby already indicate the need for a change in the care of pediatric population with T1DM. Periodic psychological and neurological ongoing evaluation of children and youth with T1DM, including cognition specific questionnaires and direct testing, should be performed as part of clinical care, especially while taking into account patients' daily and nocturnal glucose variability.

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INTRODUCTION

The notion that diabetes mellitus (DM) impacts brain function and structure is not new. The theory arose for the first time in 1922^[1], and since then the idea has intrigued many investigators, especially in regard to its effect on quality of life in young children and adolescents.

Although it is well-known that type 1 DM (T1DM) is associated with neuro-cognitive impairments, there are still some open debates regarding which abilities are impaired, their appearance according to disease acquisition, and their underlying mechanisms. Understanding the full impact of T1DM on the brain, and glycemic control in particular, is critical^[2], especially in children and adolescents, a key period for development of brain matter as well as cognitive functions^[3,4]. One should remember that this is the most challenging period of T1DM management due to the demanding and exhausting therapeutic self-care guidelines of this chronic disorder, especially in this pediatric and youth age group^[5].

The current review aims to explore the reported neuro-behavioral alterations in children and adolescences from a novel point of view. The review focuses on cognitive

and behavioral impairments according to duration of T1DM and its timeline, and according to modifiable disease related risk parameters. We do not present data regarding the physiological and tissue or cell related pathogenic processes, which are available elsewhere. Our aim is to summarize for clinicians the historical and novel data available, as compared to normal cognitive and behavioral age related development, in order to direct, modify and individualize the specific clinical goals in order to encourage both better outcomes in day-to-day life, as well as to increase long-term outcome, with a focus on age and developmental stage.

We wish to clarify: what is known about the disorder, what is its association to modifiable diabetes related metabolic aspects, what clinicians should pay attention to and prevent by clinical care and what is needed in future research.

BEHAVIORAL ALTERATIONS

Adolescence is a crucial time for the formation of healthy and responsible habits, yet it is an age characterized by an increasing tendency for risk-taking behavior. This is especially notable for adolescents with T1DM, who may require long-term healthcare support in creating healthy and responsible habits^[3].

Studies show that mastery over one's environment, an important component of resiliency, is related to life satisfaction, quality of life and improved daily functioning, including school functioning^[6,7]. Better glycemic control is related to resiliency and quality of life, which are both associated with better school functioning in youth with T1DM^[6].

Importantly, children and adolescents with T1DM show lower levels of life satisfaction than control populations^[8]. Life satisfaction is often discussed in the context of quality of life, which in children and adolescents with T1DM is associated with better glycemic control parameters, assessed as lower HbA1c (Glycated hemoglobin) levels^[9].

Unfortunately, poor health behaviors, such as drinking alcohol and smoking, are equally high among youth with T1DM as compared to their healthy peer counterparts, despite the increased risk associated with such behaviors in T1DM^[8].

Self-care

Older studies found that young adults with T1DM have a higher tendency to live at home and study at local colleges^[10,11], however, more recent research no longer shows such differences between those with T1DM and the typical population^[12]. This lack of a difference in more recent research may be explained by the progress in T1DM education, self-care empowerment and technological progress in T1DM management and glycemic control. These advances enable youth with T1DM to more flexible self-care and better glycemic control outcomes^[13].

School

Teachers often have limited knowledge about diabetes, leading to confusion in many areas associated with the disorder, particularly in regard to physical education. This lack of knowledge may have great implications on the child and their disorder. Teachers may wrongly exclude diabetic children from activities in which they are actually able to participate, and likewise children may get away with using their illness to avoid participation in activities that are in fact not harmful, and are possibly even favorable to their health. This may lead to skipping physical activity, which can in turn cause isolation among children with T1DM^[14]. Further, increased absences from school or missing class time in order to check glucose levels^[9,13,15] can contribute to lower academic achievement among students with T1DM^[7,13,15,16]. Importantly, overall it was found that children with fair to good glycemic control earn better grades in school as compared to children with poor glycemic control^[17]. The mechanism involved is not yet discerned. Both direct effects of glycemic control on the ability to learn may operate here, in addition to indirect paths, such as by affecting sleep-wake rhythms. School grades and school absences of youth with T1DM were found to be related to sleep duration and those, in turn, were found to be related to increased burden associated with diabetes management^[7]. Hence, a special focus on sleep quality is warranted for more optimal care of T1DM.

Physical activity

Results are inconsistent in regard to physical activity levels in those with and without T1DM. Few studies found that both groups showed similar levels of self-reported physical activity, while others found lower physical activity among youth with T1DM as compared to controls^[18], and one research reported higher activity among diabetic youth^[19]. It should be emphasized that physical activity has many health benefits for T1DM patients, in that it is known to improve their physical fitness, strength and overall well-being^[18,20-22], while decreasing long-term health deficiencies, such as vascular complications, for which T1DM patients are particularly vulnerable^[23].

Sleep

There are several reports regarding associations between sleep patterns and T1DM. Restricted sleep may contribute to reduced peripheral insulin sensitivity in T1DM^[24,25]. Children with T1DM experience ventilatory dysfunction during sleep, which is related to diabetes duration and to glycemic control during sleep^[26]. Adolescents with T1DM have more awakenings due to glucose fluctuations^[27] and they do not sleep as deeply as healthy population^[7,28]. Further, there may be increased sleep disturbances among adolescent males with T1DM as compared to females and healthy peers^[8]. In addition, children with T1DM are known to experience longer and more frequent

apnea (sleep disordered breathing) events, increased awakening from night sleep, more daytime sleepiness, decrease in total sleep time, decreased sleep efficiency and increased sleep latency, as compared to healthy children^[27]. Those with T1DM spend slightly less time in slow-wave sleep during the first half of the night and report less restorative sleep than their healthy peers as well^[25]. Based on the reported influence that T1DM may have on sleep, glycemic variation and poor glycemic control were associated with sleep-wake cycles in T1DM patients^[29], and associations between sleep loss and high-caloric intake as seen in Western lifestyles may reinforce this circle given that glycemic alterations during the night may affect the waking phase of the sleep-wake cycle^[29]. Youth with T1DM who spend less time in slow-wave sleep have higher average daily glucose values, higher HbA1c levels and more frequent hyperglycemia occurrences. More time in fast-wave sleep was associated with parental reporting of sleepiness, depressive moods, emotional and behavioral difficulties, lower grades in school and lower overall quality of life^[28].

Healthier life styles, which include proper quality and duration of sleep, may improve metabolic control, which in turn will further improve sleep quality. This suggests that proper diagnosis and treatment for sleep disorders among the T1DM population is paramount to improving outcome for the disorder.

Mood

During adolescents, particularly in patients who are challenged by school's academic and social demands symptoms of depression arise more frequently, these symptoms may lead to yet poorer glycemic control, and thus increase complications associated with diabetes^[30]. Youth with T1DM report more mental health symptoms as compared to healthy peers^[6]. This includes increased depression and more anxiety as compared to controls, although the differences seem to be smaller in more recent studies^[31]. Hanna *et al.*^[32] reported that depressive symptoms among adolescents with T1DM are associated with diabetes related weight control behavior. Depressive symptoms were found to be a predictor for poorer diabetic management and glycemic control among adolescences with T1DM, along with increased age, longer durations of diabetes, insulin *via* injections and diabetic specific family conflict^[33]. Further research found that depression and anxiety are associated with poorer glycemic control and more long term complications, which can apparently be explained by the negative impact these symptoms have on the capacity to follow diabetes treatment routines^[31,34,35].

Importantly, better parental relationships are associated with fewer depressive symptoms in emerging adulthood in this population^[36], and increased family support among youth with diabetes is related to better glycemic control^[37]. Mood and parental relationships then play an important role in good glycemic control, which can predict less diabetes related long term

damages. This underscores the importance of addressing family relations early during T1DM diagnosis and in management education.

Disturbed eating behavior

In considering family relations for glycemic control in T1DM, familial eating habits and familial management of gender specific maturational issues should be addressed. Importantly in this regard are the findings that adolescent females with T1DM tend to develop eating disorders twice as often as those without the disorder, and eating disorders are associated with poorer glycemic control^[38]. Therefore, disturbed eating behavior is another relevant aspect that has strong implications for diabetes related complications in T1DM^[39].

COGNITIVE ALTERATIONS

Taking together the physiological, psychological and academic issues reviewed above in T1DM, it is important to explore the cognitive alterations that may account for some of these symptoms. Many studies have reported cognitive impairments in people with T1DM, including children and adolescents. In adults, deficits in cognitive abilities are seen mostly in the domains of general intelligence, psychomotor speed and mental flexibility. In children, lower overall IQ scores were found in those with T1DM as compared to controls, and more noticeable difference were found in children with early onset diabetes (usually under 7 years old)^[40]. In addition, multiple studies have reported that children with T1DM show difficulties in response time, abstract reasoning, cognitive flexibility and verbal memory, as compared to controls; however, the differences reported are fairly small and inconsistent with borderline significance^[4,13,41-50].

The progression of these deficits when no other microvascular complications are present is generally present yet slow^[51]. Cognitive impairments may be seen within two years of diagnosis^[52], while after six years after diabetes onset, measures of intelligence, attention, processing speed, long term memory, executive function and self-monitoring were found to be lower than those of healthy peers, with stronger effects in attention, processing speed and executive function in children who were younger than 4 at disease onset^[53].

Twelve years after diagnosis, youth with T1DM showed impaired performance on working memory, compared to healthy controls. Early diabetes onset was associated with poorer attention, learning and mental efficiency. This has been thought to be related to both hypoglycemic and hyperglycemic events: Hypoglycemic events were associated with impaired learning and memory and slower processing speed, and hyperglycemia was associated with impaired working memory^[54]. Yet results are not consistent: In another study on preschoolers, T1DM patients showed no difference in neurocognitive performance as compared

to healthy controls, however, poor glycemic control was associated with lower cognitive abilities, slower fine motor speed and lower receptive language scores^[55]. Poor glycemic control in children 4-10 years old was associated with lower verbal comprehension scores, and a history of hypoglycemic seizures was associated with lower processing speed, lower full scale IQ score, impaired working memory and perceptual reasoning^[56]. A recent study on T1DM children from the same age group assessed the influence of hypoglycemic as well as hyperglycemic excursions on cognitive functioning, demonstrating a tendency for lower general IQ scores and executive functions compared to healthy peers^[57]. It may be the case that the presence of several risk factors rather than one or none is associated with poorer cognitive performance that may compromise learning and memory and executive functions in T1DM^[54].

Learning and memory

Diabetes effect on memory and learning was first raised in 1922 by Miles *et al.*^[1], who reported that people with diabetes complained of memory loss and attention difficulties. Later research found that early onset of T1DM was associated with verbal and visual learning and memory skills^[46], verbal and nonverbal intelligence, attention and psychomotor skills^[58]. In a recent study chronic hyperglycemia was associated with learning and memory in children^[57]. Further, one research found that in boys with T1DM, lower verbal intelligence was associated with poor glycemic control; however, most studies found no gender differences in neurocognitive abilities^[59].

Memory, especially working memory, is influenced by multiple risk factors^[60,61] including glycemic excursions, but by itself this was not proven to cause long term damage in T1DM, yet early diabetes onset may be a relevant additional risk factor in this respect^[45,62].

Executive functions

Early onset T1DM is associated with deficits in executive functioning^[46,58], which include the ability to initiate, plan, consolidate and sustain problem solving in working memory, to control emotions and behavior and to modify a cognitive set through proper inhibition control^[63]. The integrity of executive functions may be of particular importance for management of T1DM: People with T1DM have many daily tasks that must be organized, including timely insulin administration, blood glucose monitoring and regulating dietary intake^[64]. This suggests that executive functions are critically important in adolescents with T1DM during a time in which diabetes self-management must be learned and maintained. In fact, a relation was found between executive functioning and adherence to a self-management schedule in adolescents with T1DM^[64,65]. A mild trend was recently noticed in young children with T1DM, such that lower executive function was associated with hyperglycemia, a deficit that becomes more apparent and more debilitating

in older children or in those with longer durations of the disorder^[57]. Further, impaired decision making was found to be associated with T1DM in several studies, and was associated with comorbid depression, cognitive deficits and hypoglycemia unawareness^[59,66,67]. Impaired decision making may be related to white-matter microstructural deficits that were reported on neuroimaging studies in youth with T1DM^[66]. This cognitive disadvantage may cause poorer glycemic control, and thus elicit more brain alterations.

BRAIN STRUCTURE ALTERATIONS

Neuroplasticity is the ability of the brain to change its structure and function due to environmental changes. Deficiencies in brain trajectories involved in neuroplasticity were reported among T1DM, including hyperglycemia and hyperinsulinemia^[68]. Yet, the findings in this regard do not yet offer a coherent framework.

Consistent findings in neuroimaging research show structural changes, especially in cortical grey matter^[69]; however, very few studies have investigated participants younger than 20 years old^[70]. The few that exist show that brain volume alterations are detectable already in childhood^[70-72] and that these alterations have long term influences into adulthood^[51]. On the other hand, there is a consensus that some reported atrophic changes are short term and may be related to glucose excursion^[70]. For example, early results from a magnetic resonance imaging (MRI) study found that higher rates of ventricular atrophy and hippocampal white matter lesions were correlated with early diabetes onset^[62], and larger hippocampal volumes were associated with recurrent severe hypoglycemia^[73]. Future studies may explore a potential vicious circle between poor glycemic control in this population, learning and memory deficits and these brain structural changes. In this regard, it is important to consider both grey and white matter volume changes.

Grey matter volume

Smaller grey matter volume in the left superior temporal region and in the thalamus were reported among youth with T1DM, as well as among children ages 4-10, as compared to healthy controls^[56,74,75]. Those findings in both reports were associated with a history of severe hypoglycemic events. Smaller grey matter volume in the right cuneus and precuneus was also reported in T1DM patients, but associated with greater exposure to hyperglycemia^[74]. The decreased grey matter in those areas was also associated with high rest activity and dramatic decrease in brain activity during goal-oriented tasks^[70]. No other findings dealing with children and adolescents with T1DM support these associations to grey matter volume, however studies in adults showed similar findings^[70]. Smaller grey matter volume in bilateral temporal-occipital and cerebellar regions, and larger grey matter volume in left inferior

prefrontal, insula and temporal pole regions, associated with hyperglycemia, were seen in young children (mean age 7 years old) with early onset of T1DM^[71]. Similarly, higher HbA1c was associated overall decrease in grey matter volume^[76].

White matter volume

Perantie *et al.*^[74] reported that smaller white matter volume in the right posterior parietal region is associated with greater exposure to hyperglycemia in T1DM patients, and that distinct decrease in white matter volume, especially in the occipito-parietal cortex, is associated with severe hypoglycemia^[76]. Compared to healthy controls, young children between 4-10 years of age with T1DM who experienced hypoglycemic seizures showed significantly altered age-related white matter development (amygdala and hippocampus as well) and smaller white matter volumes^[56].

It is obvious that findings regarding T1DM, glycemic control and glucose excursions and their association to white and grey brain matter regions, are inconsistent and rely on few reports. In order to clear up these inconsistencies, further studies are required regarding the clinical and neurocognitive impact of such findings, and their cause. Whether there is any long term effect of severe hypoglycemia or of poor glycemic control on the developing brain, and whether the effect is subtle, as suggested by Arbelaez *et al.*^[70], should be clarified with further research. These different findings raise new questions regarding the possible explanations of increase or decrease in brain volume, the processes they may reflect as a function of age, gender, and severity, and the impact they may have on the lives and well-being of young patients with diabetes, in order to improve management at the various age groups^[77]. Some of these disparities concerning structural changes at different ages and glycemic control may be resolved in the future by adding brain properties into the model.

BRAIN PROPERTIES

White matter integrity

Barnea-Goraly *et al.*^[78] recently suggested that differences in fibers integrity and radial diffusivity are negatively associated with age of T1DM onset. Yet little is still known with regard to young patients.

Middle-aged adults with T1DM showed a decrease in white matter integrity of posterior parietal region^[51]. In children with T1DM, microstructural white matter integrity changes were seen in the thalamus, hippocampus and superior parietal regions^[72]. White matter microstructural changes were more apparent in children with higher HbA1c. White matter microstructural changes manifested especially in the frontal and temporal regions, including lower axial diffusivity values in diabetic children compared to controls, and in higher radial diffusivity among those who had higher HbA1c^[79]. These findings support the association between poor glycemic control

and demyelination and gliosis in frontal and temporal lobes^[80]. Lower axial diffusivities involving many white matter trajectories in all cortical lobes were observed in T1DM children as compared to controls. These white matter integrity alterations were associated with hyperglycemia and with cognitive impairments. White matter integrity alterations in the superior parietal and particularly in the precuneus and cuneus region, and decreased density in the hippocampus were associated with hyperglycemia^[72].

Connectivity

Alterations in neural networks were correlated with cognitive functions, such as with attention and memory^[81] and with mental illness, both of which are known to be associated with T1DM^[68]. In adults, abnormalities in functional magnetic fields and in the brain neural connectivity were detected in individuals with T1DM using magnetic encephalography (MEG)^[69]. Several studies employing various modalities and methodologies [electroencephalography (EEG), functional MRI (fMRI), MEG] agree on the overall influence that T1DM has on the brain's functional connectivity^[68,82-84]. These researches support the hypothesis that brain connectivity is altered due to diabetes, but with respect to affected brain regions, the results vary substantially^[51]. Additionally, these findings have not yet been replicated on children^[70]. The influence of hypoglycemia, glucose excursions or overall glycemic control on those findings requires further research.

BRAIN ACTIVITY ALTERATIONS

EEG differences have been seen between individuals with T1DM and healthy controls, including loss of high frequency activity in temporal, frontal and occipital regions and lower frequencies overall. These changes were attributed to the metabolic disturbances caused by a history of prolonged diabetes and past severe hypoglycemic events^[85-89]. However, most studies did not assess simultaneous glucose concentration, thus relation to hyperglycemic excursions could not be delineated, as well as duration of the disorder. Transient changes in electrical activity in various brain regions were reported during hyperglycemia during wakefulness and sleep among youth with T1DM. Glucose excursions above 280 mg/dL during wakefulness was associated with a decrease in the power of high frequency bands (α , β , and γ), and increase in the power of low frequency bands (δ and θ) in the EEG from central and occipital regions. Glucose concentration > 200 mg/dL during sleep was associated with increased power in high frequency bands in the EEG in frontal and central areas and more low frequencies generally. However, the clinical neurocognitive relevance of those findings is still not clear^[90].

T1DM RELATED RISK FACTORS

Glycemic control

Daily hypoglycemic and hyperglycemic excursions are frequent among T1DM patients despite efforts to keep glucose concentration within a narrow range, especially in the pediatric and adolescent population. Despite efforts to maintain strict intensive management, including dietary restriction, multiple daily glucose measurements and insulin injections in order to prevent glucose excursions in daily life, glucose concentration in children and young adolescents is more frequently in the hyperglycemic range^[91]. Possible effects of those excursions and their length on cognitive functions, brain activity and brain structure have mostly been examined in adults; yet, their effects on young children and adolescent brains may be critical. This age is a common period for marked developmental changes in the brain and in the maturation of cognitive functions, making the brain more susceptible to an array of pathogeneses, including metabolic instability^[3,4].

Hypoglycemic events

The anxiety associated with severe hypoglycemia is a major barrier in optimizing glycemic control, yet, a recent ten-year longitudinal study testing 1770 participants found that poor glycemic control was not associated with an increased risk for severe hypoglycemia^[92]. This is an important factor, since frequent mild hypoglycemic events are related to impaired cognitive functions, including abstract reasoning, motor responses, processing speeds, selective attention and behavioral inhibition^[40] all critical for problem solving, academic and social achievements and to well-being. Early studies reported that severe hypoglycemia is a primary cause of neurocognitive impairments^[42,93,94], but this was not supported by two meta-analyses that investigated poor glycemic control as a potential risk factor^[16,46]. Unfortunately, hyperglycemic and hypoglycemic events may be convolutedly intertwined, which makes it complicated to rule out severe hypoglycemia's role in cognitive impairments^[74].

Hyperglycemic excursions

Poor glycemic control, defined by high values of HbA1c, is often referred to as chronic hyperglycemia^[70]. Chronic hyperglycemia is associated with negative effects on memory^[95-97], and with lower estimated verbal intelligence^[49] in children with T1DM. The effects of chronic hyperglycemia on young children include lower cognitive abilities, slower fine motor speed and lower receptive language scores^[55]. Hence, hyperglycemia is a major risk factor for cognitive decrements.

Age of diabetes onset

A diagnosis of T1DM in the first 4-7 years of life appears to be a major risk factor for significant clinical neurocognitive deficiencies^[62]. Both severe hypoglycemia and

chronic hyperglycemia may impair cognitive abilities at this age range^[98].

Specifically, early onset diabetes, particularly before the age of 5, is associated with a significant reduction in IQ scores, slower motor speed, visuospatial processing deficits, selective attention, verbal memory deficits and executive function deficits. Not all domains of functioning were impaired in children with early onset diabetes, and different studies showed contradicting outcomes regarding the impact of early diabetes onset^[49,94]. From a neuro-structural perspective early onset (5-9 years old) was associated with bilateral grey matter decrease in the cerebellum and occipital regions and with grey matter increase in left insula, inferior frontal and temporal poles^[71]. The meaning of these findings has yet to be investigated in particular with regard to implications on cognitive function and on management.

DISCUSSION

Neuro-behavioral alterations and cognitive deficits associated with T1DM may be found in children and in adolescence soon after diabetes onset, as well as after long standing disease duration. However, its reported frequency and severity differ with age, T1DM duration and reported timeline according to medical therapeutic development. These clinical alterations seem to be correlated with brain changes (*i.e.*, structural and white matter integrity findings), and were found to be associated with diabetes metabolic consequences. By reviewing the recent literature we can emphasize some ideas and insights to help us understand the known findings that may be associated with T1DM, and to suggest future research goals.

Looking at the history of the disorder, less pervasive and behavioral findings are reported in children and adolescents with T1DM in more recent research. This is highly promising given the rise in affected children, and may be a result of better care, better education, and better health technologies that gradually became available, together with improved glycemic control management plans.

Overall, findings point to the notion that T1DM is associated with interconnected behavioral alterations and glycemic control pathologies, a timeline summarizing core finding is presented in Figure 1.

In the cognitive realm T1DM seems to be associated with impaired memory and learning, slow cognitive processing, reduced general intelligence and impaired attention and executive functions.

As for the psychological - emotional domain, T1DM seems to be tied to depression, anxiety and low life satisfaction, which may contribute to poorer glycemic control, but may also be exacerbated by it. Glucose concentration excursions may impair the length and efficiency of night sleep, affecting school achievements and physical activity, which are also in turn associated with yet poor glycemic control. Poor glycemic control

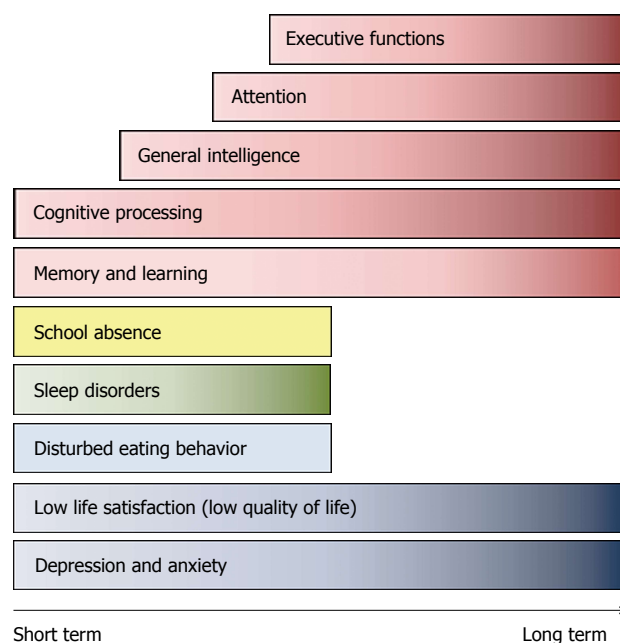


Figure 1 Short and long term behavioral and cognitive reported alterations following type 1 diabetes mellitus onset in children and adolescents according to type 1 diabetes mellitus duration. Figure is general and based on data retrieved from published reports as detailed in text.

may also affect mood, sleep, physical activity and grades in school, which once again circularly lead to poorer glycemic control, more depression and anxiety, cognitive impairments and eventually diabetes complications. The good news is that good glycemic control may balance self-control, mood and physical shape and moderate this escalating deterioration to some degree.

In recent studies, only mild cognitive deficits were observed as compared to healthy controls, which may become a marked deficit in the presences of additional risk factors. Combinations of risk factors are known to increase the probability of significant impairments in T1DM. Among these, we reviewed early onset diabetes from the ages 4-7 years of age, severe hypoglycemic seizures, hypoglycemia, hyperglycemia and poor glycemic control. Early diabetes onset, together with severe hypoglycemic seizures, may cause more significant impairments in some cognitive domains. Figure 2 summarizes the associations between cognitive domains and diabetes risk factor.

Overall, it seems that early achievement of glycemic control (*i.e.*, age dependent goal of HbA1c), may reduce the risk of cognitive impairments. Fear of hypoglycemia is considered to be a barrier to reaching good glycemic control, but recent studies support the understanding that though recurrent episodes of moderate hypoglycemia may compromise memory and executive function, in manners that are corroborated by alterations in the hippocampus, in the short term; implications for affects on long term cognitive abilities have not been proven. On the other hand, poor glycemic control and recurrent hyperglycemia seems to have long term effects on

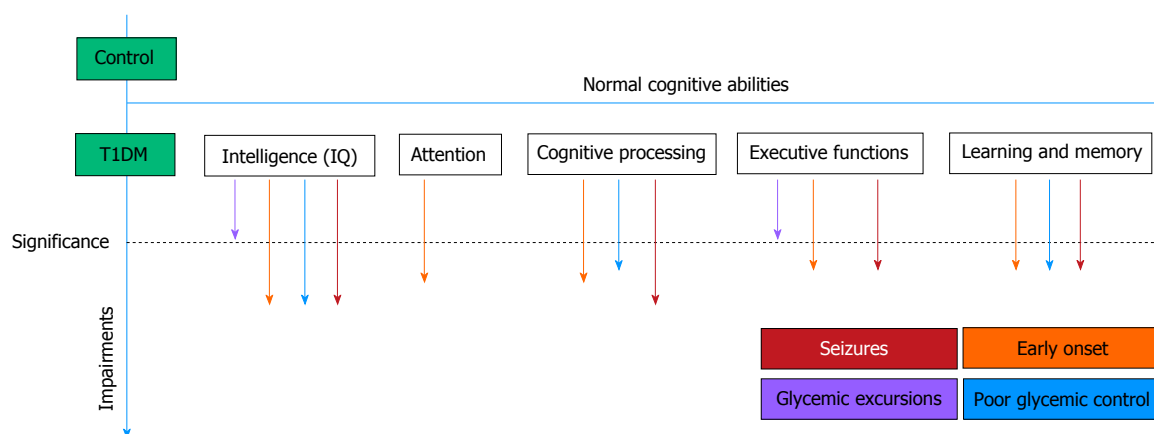


Figure 2 Impaired cognitive domains in children and adolescents with type 1 diabetes mellitus. Mild impairments were found compared to healthy controls, but several risk factors (marked by arrows) increase potential deficits. This figure is generalized, qualitative and not quantitative, based on reported studies. T1DM: Type 1 diabetes mellitus.

cognitive abilities.

Poor glycemic control is also associated with changes in brain structure, brain integrity and brain activity. Studies focusing only on the adult population demonstrated differences in connectivity underscoring the importance of extending this research line to younger patients in whom, plasticity is expected to be marked both as a function of poor glycemic control and by affecting cognitive competence.

PERSPECTIVES

Despite a wide range of previous research dealing with adults, this line of study in the pediatric population is still in its beginning phases for several reasons. First, glycemic control is accompanied with glycemic excursions, transient hypoglycemia and transient hyperglycemia, and their impact on brain functionality and on cognitive and behavioral domains has yet to be proven. Second, new studies with novel brain imaging data analysis methods have uncovered associations and impacts that diabetes has on brain properties and on brain function. Third, technological development introduced new modalities of brain study as well as ability to assess glucose excursions longitudinally and glycemic control parameters more accurately. Importantly, due to a lack of research dealing with developing age groups whose neurobehavior performances are essential to understanding the impact that T1DM, the brain the review revealed open questions regarding sensitivity and specificity of brain-behavior relations in typical and in atypical development in children and adolescents. Some reported findings with T1DM patients actually be part of normal variability of brain development, others may be a general response to stress that is not necessarily specific to T1DM.

Further research is required in a longitudinal prospective manner, beginning with T1DM diagnosis and continuing throughout the years of management. This research should include simultaneous objective

assessments of neurocognitive function, behavioral environmental objective questionnaire based assessment and brain function analysis. Brain function analysis may include novel modalities of fMRI, infusibility and connectivity assessment, and electroencephalographic assessment. Analysis should be performed in an attempt to assess simultaneous glucose excursions in a wide range of glucose concentrations, including the hypoglycemic and hyperglycemic ranges.

Studies should be performed in large populations, but within specific age groups to enable comparison with normal brain development and to avoid statements of possible abnormalities that may actually be part of normal brain function and development variability and not related to T1DM. On the other hand, this will elucidate the modifiable parameters of disease management.

In summary, review of neurobehavioral findings with pediatric populations with T1DM indicates that neurocognitive and behavioral function varies with age and depends on multiple endogenous and exogenous factors that are relevant to care methodologies. These functions are dependent on brain structure, integrity, connectivity, metabolic immediate and long term changes and vary constantly. Since understanding of brain maturation with age is an emerging field of research, delineation of T1DM impact on these processes is challenging but possible with current advances.

Importantly the available studies presented hereby already indicate the need for a change in the care of pediatric population with T1DM. Periodic psychological and neurological ongoing evaluation of children and youth with T1DM, including cognition specific questionnaires and direct testing, should be performed as part of clinical care, especially while taking into account patients' daily and nocturnal glucose variability. Clear goals of glycemic control criteria should be stated to families, including explanation of possible immediate cognitive impact in order to improve compliance. Gaining a deeper understanding of the effects of T1DM on cognitive functions, brain activity and brain connectivity may

deepen the understanding of the aftermaths of diabetes and eventually lead to better individual titration of management of young patients with T1DM in order to prevent long term and short term neurocognitive complications.

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Role of phytoestrogens in prevention and management of type 2 diabetes

Mohammad Talaei, An Pan

Mohammad Talaei, An Pan, Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117549, Singapore

An Pan, Department of Epidemiology and Biostatistics, MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

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Correspondence to: An Pan, PhD, Department of Epidemiology and Biostatistics, MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Rd, Wuhan 430030, Hubei Province, China. panan@hust.edu.cn

Fax: +86-27-83692560

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Abstract

Type 2 diabetes (T2D) has become a major public health threat across the globe. It has been widely acknowledged that diet plays an important role in the development and management of T2D. Phytoestrogens are polyphenols that are structurally similar to endogenous estrogen and have weak estrogenic properties. Emerging evidence from

pre-clinical models has suggested that phytoestrogens may have anti-diabetic function *via* both estrogen-dependent and estrogen-independent pathways. In the current review, we have summarized the evidence linking two major types of phytoestrogens, isoflavones and lignans, and T2D from epidemiological studies and clinical trials. The cross-sectional and prospective cohort studies have reported inconsistent results, which may be due to the large variations in different populations and measurement errors in dietary intakes. Long-term intervention studies using isoflavone supplements have reported potential beneficial effects on glycemic parameters in postmenopausal women, while results from short-term small-size clinical trials are conflicting. Taken together, the current evidence from different study designs is complex and inconsistent. Although the widespread use of phytoestrogens could not be recommended yet, habitual consumption of phytoestrogens, particularly their intact food sources like soy and whole flaxseed, could be considered as a component of overall healthy dietary pattern for prevention and management of T2D.

Key words: Type 2 diabetes; Phytoestrogen; Isoflavone; Lignan; Epidemiological study; Clinical trial

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Core tip: Phytoestrogens are a group of polyphenols that are structurally similar to endogenous estrogen. Animal experiments and pre-clinical models have provided strong evidence that phytoestrogens may have anti-diabetic function *via* both estrogen-dependent and estrogen-independent pathways. A number of epidemiological studies and clinical trials have thus been conducted in different populations linking two major types of phytoestrogens, isoflavones and lignans, to the prevention and management of diabetes. Although the current evidence is complex and inconsistent, habitual consumption of phytoestrogens, particularly their intact

food sources, could be considered as a component of overall healthy dietary pattern for prevention and management of diabetes.

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INTRODUCTION

Diabetes has become a global public health crisis, and the International Diabetes Federation estimated that 382 million adults were affected by diabetes in 2013, and 5.1 million deaths due to diabetes occurred annually. More than 90% of the diabetes cases are type 2 diabetes (T2D). The global prevalence of T2D has doubled in the last 30 years and is predicted to continue to rise at an alarming rate, and the number is projected to reach 592 million by 2035. The health and economic burden from diabetes is enormous^[1]. T2D is a constellation of disorders precipitated by complex and poorly understood interactions between environmental and genetic factors, leading to diminished insulin sensitivity and pancreatic β cell failure. However, diabetes is largely preventable by the adoption of a healthier lifestyle, including normal body weight, not smoking, regular exercise, and a balanced and healthy diet.

It has been widely acknowledged that diet plays an essential role in the development of T2D. Historically, the prevalence of T2D was very low in the traditional Asian society. One hypothesis speculates that the traditional Asian diet, characterized with high intakes of whole grains, large amount of vegetables and fruits, but small portions of meat products, contains many protective components against the development of T2D. Among the many food groups, soybean and soy products as the unique element of traditional Asian diet have aroused much interest because of considerable difference in its intake levels comparing with Western diet^[2]. Although there are several potential beneficial compounds (soy protein, dietary fiber, monounsaturated and polyunsaturated fat, vitamins and minerals) in soybean and soy products, one group of polyphenols concentrated in soy products, isoflavones, have been suggested to be beneficial for diabetes prevention and management^[2,3].

Isoflavones belong to a group of phytochemicals called phytoestrogens^[4,5]. Phytoestrogens are plant-derived compounds that are structurally similar to endogenous estrogen and also have weak estrogenic properties^[4,5]. There are two major types of phytoestrogens: isoflavones and lignans^[4,5]. The former is concentrated in beans and soy products, and the latter is concentrated in flaxseed, sesames, whole grain and other plant-based foods^[4,5].

The other types of phytoestrogens, like prenylated flavonoids and coumestans, are not commonly consumed in daily diet and are not discussed in this article.

In this review, we aimed to examine the current evidence linking phytoestrogens and T2D from epidemiological studies and clinical trials, to explore the potential underlying mechanisms of phytoestrogens' effect on glucose metabolism from animal and experimental studies, and to propose research priorities for future investigations in this field.

PHYTOESTROGENS

Isoflavones are primarily found in members of leguminosae family and occur in varying amounts in legumes consumed by humans, but soy exceptionally contains the highest isoflavone content^[6]. Isoflavone contents of soy food ranges from approximately 0.1 to 5 mg/g of soy protein^[7,8]. Asians generally consume very high amount of soy products, and studies have reported that the daily mean intake level of soy protein ranged from 2.0 g in Thailand to 9.6 g in North Korea^[9]. Other studies have reported similar results: 5-9 g in Japanese^[10] and Chinese^[11-13]. The mean isoflavone intake was reported to be from 6 to 75 mg/d in these countries^[9], while it was approximately 0.4 mg/d in Spain^[14] and Dutch^[15] populations, and approximately 0.3 mg/d in the United States population^[16].

There are three main soy isoflavones, genistin, daidzin, and glycitin, in which the first two are the major ones available as sugars conjugated form (glycosides) in soybeans and most soy foods in Asian cuisines^[4,5]. These biologically inactive forms are hydrolyzed in intestinal wall by the bacterial β -glucosidases and converted into the corresponding bioactive aglycones, daidzein and genistein, which then could be absorbed by intestine^[7]. After initial hydrolysis of the glucoside moiety in colon, daidzein can be further metabolized to equol by colonic bacteria. In addition to the conversion by intestinal microflora, genistin and daidzin can also be converted into bioactive forms by *in vitro* fermentation that is common in traditional Asian methods of preparing soy foods^[8]. The blood isoflavone concentration would be in the nanomolar range (< 40 nmol/L) in people who do not eat soy food, and can be increased to micromolar range by acute ingestion of dietary soy. Isoflavones and their metabolites are rapidly excreted in urine with a half-life of about 9 h for daidzein and 7 h for genistein^[7].

The other type of phytoestrogens, plant lignans, are more ubiquitous than isoflavones, and the common food sources include oilseeds (flaxseed, sesame, soy, rapeseed), whole-grain cereals (wheat, oats, rye), and various vegetables^[17,18]. Cereal fiber and wholegrain foods are among few food groups with established preventive effect for T2D^[19], and lignans may be partially responsible for protective effects of dietary fiber complex^[20]. Studies have suggested the use of urinary lignan excretion as a marker for fiber and whole

grain intake^[21,22]. Plant lignans (secoisolariciresinol and matairesinol) are converted to mammalian lignans, enterolactone and enterodiols, by mammalian gut microflora, and enterodiol can also be further oxidized to enterolactone^[4,5]. Like isoflavones, the main factors influencing circulating concentration of enterolactone are the food contents of lignans and microflora function^[20].

Because of the lack of complete databases of dietary phytoestrogens, large variations of phytoestrogen contents of foods, and comprehensive metabolism pathways influencing circulating concentrations of phytoestrogens, studies have started to use objectively measured blood or urinary phytoestrogen concentrations as a good indicator of dietary intake^[22-24]. In Asians with high variations of soy intakes, studies have reported a reasonably well correlation between urinary concentrations of isoflavone metabolites and dietary soy intakes (mostly assessed by food-frequency questionnaires), using morning spot urine^[25], or overnight urine samples^[26]. This was consistently observed in Japanese^[27] and other populations as well^[24]. Some studies in United States populations also confirmed that urinary concentrations of isoflavone metabolites are reasonable options for assessing isoflavone intake in epidemiologic studies^[28-30]. Studies also suggested that urine samples performed better than serum samples for correlating with dietary intakes^[31].

For lignans, studies in Western populations indicated that enterolactone concentrations in overnight urine samples moderately correlated with fruit and vegetables intake^[32], concentrations of enterodiol and enterolactone in spot urines were significantly correlated with dietary intakes of fiber^[32,33], vegetables and rye products^[34]. Few studies have been performed to estimate the urinary lignan metabolites in Asian populations. A small study in 19 Japanese adults found that concentration of urinary lignan metabolites was about one third of isoflavone metabolites, and was correlated with intakes of green and yellow vegetables, pulses and beans^[27]. A study in 75 Korean postmenopausal women found that the concentration of lignan metabolites in 24-h urine samples was about half of isoflavone metabolites^[35], similar results were found in a study among 68 Chinese T2D patients using first morning urine samples^[36]. In another large cross-sectional study of 2165 middle-aged and elderly Chinese women, despite that the concentrations of lignan metabolites were substantially lower compared to isoflavone metabolites in spot urines, the urinary enterodiol was higher than and enterolactone was similar to that among United States women of comparable age^[12]. This was observed in another study that collected urine samples from several Asian countries (Japan, Vietnam, India, and Cambodia) and United States^[37]: high concentrations of isoflavone metabolites were detected in urine samples from Japan and Vietnam, while the concentrations in urine samples from Cambodia and India were much lower and comparable to that found in United States samples;

the differences between urinary lignan metabolites were relatively small among samples from the five countries.

RECENT HUMAN STUDIES LINKING PHYTOESTROGENS TO DIABETES AND GLUCOSE HOMEOSTASIS

The epidemiological studies on the relation between phytoestrogens and risk of T2D or diabetes biomarkers are shown in Table 1. We have described the findings by study designs as below.

Cross-sectional study evidence

Several cross-sectional studies have assessed the association between soy protein and isoflavone intakes and diabetes related markers. In the Shanghai Women's Health study of 39385 women aged 40-70 years, it was observed that soy protein intake was inversely associated with glycosuria, an important indicator of diabetes, but only in normal weight postmenopausal women^[38]. However, in another study among 2811 Chinese adults, soy protein intake was significantly associated with increased odds of hyperglycemia in men, but null association in postmenopausal women^[11]. The median soy protein intake was around 8 g/d in both studies^[11,38]. The increased odds of hyperglycemia in men could be a chance finding, and residual confounding and reverse causation are possible in the cross-sectional studies. The sex-specific effects may also linked to the estrogen-like activity of isoflavones^[4,5], but the underlying mechanisms are complex and unclear^[11]. In a study of 208 American postmenopausal women who ate much lower levels of soy foods, genistein intake was significantly associated with 2-h post challenge insulin concentrations, but not fasting or 2-h glucose concentrations^[39]. This suggested that isoflavones may have direct effect on β -cell function and insulin secretion, which is supported by experimental studies^[40]. Among 299 pregnant women who participated in the United States NHANES 2001-2008 surveys, Shi *et al*^[41] found that urinary concentrations of total isoflavone metabolites were inversely associated with fasting glucose, insulin and homeostatic model assessment of insulin resistance (HOMA-IR).

Consumption of soy products is generally low in Western diet leading to modest effect of isoflavones on metabolic markers, while lignans may be the major form of phytoestrogens and exert a stronger effect. Dietary lignan intake was inversely associated with fasting insulin and C-peptide in 468 United States men, but the association was not found for isoflavones^[42]. In the Framingham Offspring Study with 939 postmenopausal women in United States, high intake of phytoestrogens was associated with a favorable metabolic cardiovascular risk profile (waist-to-hip ratio, triglyceride and overall metabolic score), with stronger association for lignans

Table 1 Epidemiological studies on the relation between phytoestrogens (lignans or isoflavones) and risk of diabetes or diabetes biomarkers

Ref.	Ethnicity	Population	Sample size, total (outcome)	Mean follow-up years	Main exposures	Outcome	Exposure level (mean or median)	Maximum effect (highest vs ref.)
Cross-sectional study Goodman-Gruen <i>et al.</i> ^[90]	Mix ¹	Postmenopausal women, aged 45-74 yr	208	-	Dietary isoflavones	Diabetes biomarkers	4.4 mg/d genistein (mean)	Inverse with 2-h insulin ($\beta = -0.2$); not significant for FG and insulin
Yang <i>et al.</i> ^[38]	Chinese	Women aged 40-70 yr	39385 (323)	-	Tofu and other soy products	Glycosuria	9 g/d soy protein	Inverse association in postmenopausal women
van der Schouw <i>et al.</i> ^[42]	Caucasian	Men aged 47-83 yr	468	-	Dietary lignans and isoflavones	Diabetes biomarkers	Approximately 1 mg/d total phytoestrogens	Inverse association of lignans with fasting insulin and C-peptide; no significant association with isoflavones
Pan <i>et al.</i> ^[11]	Chinese	Men and women aged 50-70 yr	2811	-	Dietary soy protein	Hyperglycemia (FG ≥ 5.6 mmol/L)	7.8 g/d soy protein	Increased odds in men, but not in women
Shi <i>et al.</i> ^[41]	Mix ¹	Pregnant women aged 28 yr	299	-	Urinary isoflavones	Diabetes biomarkers	502 mg/g creatinine	Inverse association with FG, insulin, and HOMA-IR
Longitudinal study Villegas <i>et al.</i> ^[44]	Chinese	Women aged 40-70 yr	64191 (896)	4.6	Soy protein, soybeans, soy products	T2D	7.7 g/d soy protein	Inverse association with soybeans; inverse but not significant relation with soy protein or other products
Nanri <i>et al.</i> ^[46]	Japanese	Men and women aged 45-75 yr	59791 (1114)	5	Soy products, daidzein, genistein	T2D	Approximately 73 g/d soy products, approximately 23 mg/d genistein, and 14.5 mg/d daidzein	No significant association
Morimoto <i>et al.</i> ^[47]	Mix ²	Men and women aged 45-75 yr	75344 (8564)	14	Soy products	T2D	Approximately 14.5 g/d in Japanese, approximately 8 g/d in Hawaiians, and 0 g/d in Caucasians	A modest increased risk in men and women
Mueller <i>et al.</i> ^[45]	Chinese	Men and women aged 45-74 yr	43176 (2252)	5.7	Isoflavones, unsweetened and sweetened soy products	T2D	Approximately 5.2 g/d for soy protein, 15.8 mg/d for soy isoflavones	Inverse association for soy isoflavones and unsweetened soy products, while increased risk for sweetened soybean drinks
Zamora-Ros <i>et al.</i> ^[48]	European whites	Men and women with mean age 52.4 yr	11559 cases and 15258 subcohort, case-cohort design	Approximately 12	Dietary isoflavones and lignans	T2D	0.9 mg/d isoflavones, 1.4 mg/d lignans	No significant association for isoflavones and lignans
Sun <i>et al.</i> ^[49]	Caucasian	Women aged 65.6 yr from NHS and 45.4 from NHS II	1107 cases and 1107 controls, nested case-control design	Approximately 6	Urinary lignin metabolites (enterodiol and enterolactone)	T2D	2.2 μ mol/g creatinine for NHS women, and 1.9 μ mol/g creatinine for NHS II women	Inverse association and odds ratio 0.64 (95%CI: 0.45-0.91) comparing extreme quartiles

¹Mostly non-Hispanic whites; ²Caucasian, Japanese American, and Native Hawaiian. FG: Fasting glucose level; NHS: Nurses' Health Study; T2D: Type 2 diabetes; HOMA-IR: Homeostatic model assessment of insulin resistance.

compared to isoflavones^[43]. No study has been conducted so far to investigate the cross-sectional relation between lignans and diabetes risk markers in Asian populations.

Prospective study evidence

A few larger prospective cohort studies have been conducted to investigate the relation between soy food consumption and risk of incident T2D in different populations. In a study with an average 4.6 years of follow-up among Chinese women from the Shanghai Women's Health Study, Villegas *et al.*^[44] reported that soybean and soymilk intakes were significantly associated with a lower risk of incident T2D, while soy protein and other soy products were related to a trend of reduced risk, although not statistically significant. In another large prospective study in Chinese population, the Singapore Chinese Health Study, Mueller *et al.*^[45] pointed out that consumption of unsweetened soy products was inversely associated with T2D risk in a graded fashion (P for trend = 0.02), while consuming sweetened soybean drink was positively associated with T2D risk. The findings underline the importance of food context and preparation method. Furthermore, after full adjustment including sweetened soy items, the authors observed a marginally significant inverse association between intake of isoflavones and T2D (relative risk comparing extreme quintile: 0.76; 95%CI: 0.58-1.00; P for trend = 0.08). In a large-scale study in middle-aged and elderly Japanese from the Japan Public Health Center-Based Prospective Study, Nanri *et al.*^[46] found no significant association between soy products and isoflavones with incident T2D in either men or women. The suggestive protective association in overweight women disappeared when energy-adjusted intake was considered^[46]. In the Multiethnic Cohort study in Hawaii with three ethnicities (Caucasian, Japanese American, and Native Hawaiian), Morimoto *et al.*^[47] reported a moderately elevated risk of T2D with soy food consumption and risk of T2D during 14 years of follow-up in men and women, particularly in overweight adults. However, the consumption level of soy products was substantially lower compared to that in the Asian populations. In the European populations, the recent EPIC-InterAct case-cohort study in 12403 incident T2D cases and a subcohort of 16154 participants found no significant association between isoflavones and risk of T2D, while a suggestive trend with lignans (the hazard ratio comparing extreme quintiles 0.88; 95%CI: 0.72-1.07; P for trend = 0.12)^[48].

Therefore, the current evidence from large longitudinal studies regarding the relation between phytoestrogen and related food sources and incident T2D is still inconsistent. One methodology challenge could be the measurement error of dietary assessment by questionnaire data. This may be due to the incomplete inclusion of phytoestrogen-enriched food items in the questionnaire and lack of comprehensive food composition databases of phytoestrogens. Furthermore,

phytoestrogen metabolism and circulating concentrations in human body can be influenced by many other factors in addition to dietary intake. Thus, studies have started to measure blood or urinary phytoestrogens and evaluate the relation with disease outcomes. Recently, a nested cases-control was conducted among 1107 T2D cases and 1107 control subjects from the Nurses' Health Study (NHS) and NHS II^[49]. Urinary concentrations of the lignan metabolites were assayed by liquid chromatography-mass spectrometry. After multivariate adjustment for lifestyle and dietary risk factors of T2D, the odds ratio for T2D was 0.70 (95%CI: 0.53-0.92) for each SD increment of urinary concentrations of total lignan metabolites. The association was seen in both enterolactone [odds ratio comparing the extreme quartiles 0.62 (95%CI: 0.44-0.88), P for trend = 0.003] and enterodiol [odds ratio comparing the extreme quartiles 0.67 (95%CI: 0.48-0.96), P for trend = 0.08]. Thus far, this is the only prospective study using objectively measured phytoestrogen biomarkers to link with diabetes risk. More studies are needed to examine the relation of urinary phytoestrogen excretion and risk of developing T2D in different studies and populations with varying intake levels.

Clinical trial evidence in participants without T2D

A meta-analysis of 24 intervention studies ($n = 1518$ in total) on soy intake and glycemic control was done including trials published before March 2010^[50]. While no significant effect on fasting glucose and insulin was generally observed for soy intake, the authors found 3.85 mg/dL (95%CI: 2.41-5.28) reduction in fasting glucose concentrations in a subgroup analysis of 9 studies that used whole soy foods or soy diets as the intervention regime. No statistically significant association was identified in 8 studies with isoflavone extract (ranged 40 to 132 mg/d isoflavones) or 6 trials with isolated soy protein containing isoflavones as the main intervention. This suggests that other components of soy like soy protein and fiber, polysaccharides, phytosterol, and unsaturated fatty acid or their interactions may play roles in glycemic control in addition to isoflavones. However, the majority of the studies in this meta-analysis had small sample size (ranged from 14 to 203) and short intervention period (ranged 4 to 52 wk). One of the largest studies so far was a 1-year double-blind, randomized, placebo-controlled trial in 203 Chinese postmenopausal women aged 48 to 62 years^[51]. They were randomly assigned to receive daily doses of 0 mg (placebo, $n = 67$), 40 mg ($n = 68$), and 80 mg ($n = 68$) isoflavone supplements along with 500 mg calcium in all groups. The mean differences in the changes of fasting glucose between the intervention and placebo groups were -5.2 mg/dL (95%CI: -9.4 to -1.0) and -3.3 mg/dL (95%CI: -7.5 to 0.9), respectively, for the mid-dose and high-dose groups, and the effect was much more significant in women with higher baseline glucose levels^[51].

Another meta-analysis of 12 clinical trials conducted before October 2010 focused on the effects of isoflavone supplementation on blood glucose and insulin in non-Asian postmenopausal women^[52]. Zhang *et al.*^[52] found that isoflavone supplementation significantly reduced fasting glucose by 0.19 mmol/L (95%CI: 0.03-0.34), and this effect was limited to the studies with more than 6-mo period of intervention. The meta-analysis also reported a significant reduction in fasting insulin by 0.94 μ U/mL (95%CI: 0.16-1.72). One of the largest and longest studies so far was done in Italian postmenopausal women with osteopenia^[53]. Participants were randomly assigned to receive genistein (54 mg/d; $n = 198$) or placebo ($n = 191$) for 2 years. Both groups received 500 mg/d calcium carbonate and 400 IU/d vitamin D. Compared with placebo, genistein significantly reduced fasting glucose and insulin as well as HOMA-IR after both 12 and 24 mo of treatment^[53].

Since 2010, a few more trials have been published on the effects of isoflavone supplementation on glucose homeostasis. Two long-term (24 mo) clinical trials by the same research group found that daily intake of 40 mg of soy isoflavones together with lifestyle modification (Mediterranean diet and exercise) reduced HOMA-IR compared to lifestyle modification alone among 116 Spanish postmenopausal women with insulin resistance^[54], this was confirmed using same study design (except for 80 mg/d of soy isoflavones) among 80 Spanish postmenopausal women^[55]. Improvement of fasting glucose and insulin was also reported^[55]. Another 1-year clinical trial among 120 postmenopausal women with metabolic syndrome revealed that 54 mg/d genistein supplements ($n = 60$) significantly reduced HOMA-IR, fasting glucose and insulin compared to placebo ($n = 60$)^[56]. However, the beneficial effects of isoflavones on glucose metabolism were not found in some short-term trials^[57-60]. Since S-equol is considered the most biologically active metabolite of isoflavones, a study was specifically designed to evaluate the effects of S-equol on metabolic profiles among 54 Japanese overweight/obese men and women using a cross-over study design^[61]. Significant improvement in HbA1c was observed using 10 mg/d S-equol for 12-wk compared to placebo^[61].

As for flaxseed and lignans, a meta-analysis found that flaxseed and/or flaxseed lignan intervention significantly improved lipid profiles^[62]. Two small cross-over clinical trials in overweight/obese glucose intolerant participants found that flaxseed reduced insulin resistance after 12-wk interventions^[63,64]. A large intervention study in 293 Chinese adults with metabolic syndrome found that 30 g/d flaxseed significantly reduced HbA1c and glucose levels among those with central obesity at baseline^[65]. A clinical trial in 55 hypercholesterolemic Chinese subjects found that 600 mg/d flaxseed lignan extract significantly lowered fasting glucose, particularly in those with a higher baseline glucose levels^[66]. Another cross-over clinical trial in 22 healthy postmenopausal women reported that

500 mg/d flaxseed lignan extract significantly reduced C-reactive protein levels after 6 wk^[67]. However, other studies have found null results^[68-71].

Taken together, long-term intervention studies using isoflavone supplements have reported potential beneficial effects on glycemic parameters in postmenopausal women^[51,53-56], while results from short-term small-size clinical trials are conflicting. Therefore, more high-quality long-term clinical trials are needed in men and premenopausal women, and to investigate the effect of lignans on glucose metabolism in humans.

Clinical trial evidence in patients with T2D

A number of clinical trials have been conducted in T2D patients to investigate the effects of phytoestrogens and related food sources on diabetes management. Jayagopal *et al.*^[72] found that 12-wk intervention of 30 g/d soy protein enriched with 132 mg isoflavones significantly reduced HbA1c (-0.6% vs 1.1% in placebo group), fasting insulin (-8.1% vs 9.9% in placebo group), and HOMA-IR (-6.5% vs 14.7% in placebo group) in postmenopausal women with T2D. Another long-term 4-year clinical trial among T2D patients with nephropathy reported a net change of -29 mg/dL in plasma glucose in the intervention group ($n = 20$; 0.8 g protein/kg body weight with 35% as soy protein, 35% as animal protein and 30% as vegetable protein) compared to the control group ($n = 21$; 70% as animal protein and 30% as vegetable protein)^[73]. However, some small short-term trials among T2D patients failed to observe significant improvement for isoflavone-containing soy protein on glucose, insulin resistance or HbA1c^[74-79]. On the other hand, clinical trials among T2D patients have reported improvement in lipid profiles^[73,74,77,78,80], kidney function^[73,78,81], endothelial function and blood pressures^[76].

The effects of other isoflavone-enriched foods in T2D patients have also been tested: a 1-year intervention with 27 g/d flavonoid-enriched chocolate (containing 850 mg flavan-3-ols and 100 mg isoflavones) significantly reduced insulin resistance and improved insulin sensitivity and lipid profile compared to placebo in 93 postmenopausal women with T2D^[82]. However, few studies have specifically investigated the effects of purified isoflavones supplements among T2D patients, and the available two interventions found no significant effects on glycemic control and lipid profiles^[83,84], but the intervention periods were short (4 and 12 wk) and sample sizes were small ($n = 16$ and 32).

A few studies of flaxseed or lignans among diabetic patients also found promising results. Daily supplementation with 10 g flaxseed powder for 4 wk decreased fasting glucose by 19.7% and HbA1c by 15.6% in T2D patients^[85], and also improved lipid profiles. Similarly, 5 g/d flaxseed gum for 12 wk significantly reduced serum glucose from 154 ± 8 mg/dL to 136 ± 7 mg/dL^[86]. Moreover, 360 mg/d lignan for 12 wk slightly decreased HbA1c^[36] and C-reactive

protein^[87], although fasting glucose and insulin and lipid profiles remained unchanged^[36]. Another study using 600 mg/d lignan for 3 mo found decreased HbA1c and glucose levels, but the results were not statistically significant after multivariate adjustment^[88].

In summary, isoflavone-enriched soy products and lignin-enriched flaxseeds provide promising benefits in glycemic control, lipid profiles and other cardiovascular markers in T2D patients, but the long-term effect of purified isoflavone or lignan supplements remains unknown.

POTENTIAL MECHANISMS LINKING PHYTOESTROGENS AND PREVENTION OF T2D

The potential mechanisms linking phytoestrogens and glucose metabolism and prevention of diabetes have been extensively reviewed elsewhere^[3,40,89,90], here we briefly discuss some animal studies and potential mechanisms on this topic.

A study in male C57BL/KsJ-*db/db* mice found that both genistein (0.02%, w/w) and daidzein (0.02%, w/w) supplements significantly decreased blood glucose and HbA1c levels, and this effect might be due to the suppression of hepatic glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), fatty acid synthase, β -oxidation and carnitine palmitoyltransferase activities^[91]. The same effects have been observed in the female non-obese diabetic mice as well, a T1D animal model^[92]. Some other studies suggested that isoflavones may exert antidiabetic effect *via* peroxisome-proliferator activated receptors (PPAR) pathway. In the obese Zucker rats, a T2D model, high-isoflavone soy protein diet improved glucose tolerance relative to low-isoflavone soy protein and casein diets^[93]. It was further found that genistein or daidzein significantly increased *PPAR* α - and *PPAR* γ -directed gene expression by 2-4 fold in RAW 264.7 cells^[93]. The increased *PPAR* α gene expression was also seen in another study^[94]. In streptozotocin-induced diabetic rats, 3-wk genistein supplementation decreased HbA1c levels and G6Pase activity, while increased glucokinase level and antioxidant enzyme activities^[95]. In an obese nongenetic T2D mouse model, dietary intake of genistein (250 mg/kg diet) improved hyperglycemia, glucose tolerance, and blood insulin level but did not affect insulin sensitivity, suggesting that genistein may increase the number of insulin-positive β -cells in islets, promote their survival, and preserve them by preventing apoptosis^[96]. Numerous studies have suggested that genistein may have direct effects on β -cell proliferation, glucose-stimulated insulin secretion and protection against apoptosis^[40]. Meanwhile, some other studies have shown insulin-sensitizing effect of genistein in male and female C57BL/6 mice^[97], as well as ovariectomized rats^[98].

Secoisolariciresinol diglucoside (SDG), the major dietary lignan in flaxseed, considerably reduced the incidence of diabetes in streptozotocin-induced diabetic rats^[99], diabetes-prone BioBreeding rats, a T1D model^[100], and ZDF rats, a T2D model^[101]. In these experiments, SDG significantly decreased oxidative stress by reducing malondialdehyde and pancreatic-chemiluminescence level. Sesamin, the most abundant lignan in sesame seed, showed hypoglycemic effect in a dose-dependent manner in KK-Ay mice, a T2D model^[102]. Sesamin was also found to attenuate vascular dysfunction and oxidative stress in streptozotocin-diabetic rats^[103].

The effects of phytoestrogens on glucose metabolism are thought to be *via* estrogen-dependent pathway and non-estrogen dependent pathways. Estrogens have been shown to modulate lipid and glucose metabolism directly through lipogenesis, lipolysis, and adipogenesis, or indirectly through their effect on central nervous system influencing appetite and energy expenditure^[104]. The relationship between endogenous sex hormones and development of T2D has been well established^[105,106]. Because of structural similarity, phytoestrogens could act as estrogen agonists or antagonists, depending on the target tissues^[107], doses^[108-110], and endogenous circulating sex hormone profile^[111]. Although the binding affinity to estrogen receptors (ERs) is much lower for phytoestrogens compared to 17 β -estradiol^[112], the concentration of phytoestrogens in blood is much higher than endogenous estrogens^[113], making it still possible to compete with 17 β -estradiol to bind the ERs. Therefore, it is hypothesized that phytoestrogens may influence glucose metabolism by directly modulating concentrations of circulating sex hormones, and this estrogenic effects of phytoestrogens have been supported by some human studies^[114-119]. Oxidative stress is considered as one of the causes for T2D and phytoestrogens are known to have strong antioxidant activity^[120]. For example, SDG, the major dietary lignan^[121] and its mammalian metabolites enterodiol and enterolactone^[122], were shown to have antioxidant activity even higher than that of vitamin E. Animal experiments found that lignans decreased lipid peroxidation in rats fed with docosahexaenoic acid^[123], and flaxseed increased activities of catalase, superoxide dismutase, and peroxidase^[124]. Similarly, isoflavones also showed antioxidant activity *in vitro*^[125] and *in vivo*^[95,126]. Several clinical trials in humans also found that high-isoflavone soy products increased antioxidant capacity^[127-130].

Phytoestrogens may influence glucose metabolism and insulin resistance through other non-estrogen dependent mechanisms. For example, both lignans and isoflavones were found to suppress the *PEPCK* gene expression^[92,131]. *PEPCK* enzyme catalyzes the first committed step in hepatic gluconeogenesis, and *PEPCK* gene transcription is induced by glucagon and glucocorticoids and inhibited by insulin. Thus, suppression of *PEPCK* gene will improve hyperglycemia through reduced gluconeogenesis^[132]. Furthermore, phytoestrogens, mostly isoflavones, activate

PPAR and increase the *PPAR* α - and *PPAR* γ -directed gene expression^[93,94,133,134], which is implicated in the glucose homeostasis and lipid metabolism. In the yeast model, genistein was shown to be a reversible, slow-binding, non-competitive inhibitor of alpha-glucosidase^[135], which breaks down starch and disaccharides to glucose. Therefore, the alpha-glucosidase inhibitors may reduce the postprandial glucose levels by slowing down the carbohydrate digestion and absorption. In the rabbit model, isoflavones were found to inhibit glucose uptake into rabbit intestinal brush border membrane vesicles *in vitro*^[136]. Genistein also directly acted on pancreatic β -cells, leading to activation of the cAMP/PKA signaling cascade to increase rapid glucose-stimulated insulin secretion^[137]. The increased insulin secretion was also reported elsewhere^[138-140]. Other studies have found that isoflavones may inhibit tyrosine-specific protein kinases^[141], induce adiponectin, leptin and *GLUT4* gene expressions in 3T3-L1 adipocytes^[142], promote postprandial carbohydrate oxidation and energy expenditure^[143], and protect against high glucose-induced pancreatic cell damage through ER β and Bcl-2 dependent pathways^[144].

IMPLICATIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

As summarized in this review, there has been a long-lasting interest to examine the relation of phytoestrogens and related food sources with diabetes risk. Although the current evidence is promising, there are some knowledge gaps that should be addressed in future investigations.

The food composition databases of phytoestrogens have become the major concern in many epidemiological studies on the relation between phytoestrogens and diabetes risk. The phytoestrogen contents vary dramatically in different food items, and are also influenced by the geographic location, harvest time, and food preparation methods, *etc.* Therefore, it is urgent to establish accurate, up-to-date, and comprehensive databases in different countries. Particularly for lignans, there is a lack of databases available for research. To the best of our knowledge, there has been no prospective longitudinal study in Asian population investigating habitual intake of lignans and risk of developing T2D.

More prospective studies are needed to use objective biomarkers of phytoestrogens exposure, *e.g.*, urinary excretion concentrations. One methodology challenge of the dietary assessment by questionnaire data is the large measurement error from incomplete inclusion of phytoestrogen-enriched food items in the questionnaire and lack of comprehensive food composition databases. Furthermore, phytoestrogen metabolism and circulating concentrations in human body can be influenced by many other factors (*e.g.*, bioavailability and microflora function) in addition to dietary intake. In addition, phytoestrogen biomarker measurements can be easily done in epidemiological studies with archived biospecimen

samples. Some large cohort studies have started to measure urinary concentrations of phytoestrogens and evaluate the relation with disease outcomes, but more investigations in different populations are still warranted. In these studies, repeated measures of phytoestrogen biomarkers are recommended to reduce measurement errors and address the issue of changes over time.

The results from clinical trials of the effects of phytoestrogens on glucose homeostasis are conflicting. Many trials have the limitations of small sample size and short intervention duration. Several recent trials in large sample size ($n > 100$) and longer duration (≥ 1 year) have produced more consistent and promising evidence to support the use of phytoestrogens. However, those trials were all in postmenopausal women and used isoflavones as the intervention supplements; thus, more high-quality long-term clinical trials are needed in men and premenopausal women, and to investigate the effect of lignans on glucose metabolism. Furthermore, clinical trials in T2D patients have supported the use of isoflavone-enriched soy products and lignin-enriched flaxseed for glycemic and lipid control, but whether the beneficial effects are due to phytoestrogens or other active components in soy or flaxseed remains unclear. Therefore, long-term and high-quality trials using purified phytoestrogen supplements are necessary to explore the possibility of their routine use for diabetes management.

Some studies hypothesized that the observed variations in effect of isoflavones on osteoporosis, cardiovascular disease, or some cancers could be attributed to the equol production ability in human^[145]. More investigations in epidemiological studies and clinical trials are needed to test whether this hypothesis is also true for T2D. Furthermore, some studies found that the effects of phytoestrogens on lipid profile^[146], endometrial cancer^[8], or breast cancer^[147] could be modified by various polymorphisms in genes relevant to estrogen or sex hormone binding globulin, like *CYP1A1*, *CYP1B1*, and *COMT*. However, there are few studies that directly assess gene-phytoestrogen interaction for the T2D outcome or glucose metabolism. This line of investigation is important to help understand the potential mechanisms and design personalized interventions.

Although there are many *in vivo* and *in vitro* studies to explore the potential pathways for the effects of phytoestrogens, the exact anti-diabetic mechanisms are still unclear. Furthermore, the effective doses used in many experimental studies far exceed the physiological concentrations in human circulation. Thus, it is recommended to consider dosages applicable for human in future animal studies.

CONCLUSION

In conclusion, the current evidence of phytoestrogen and T2D from different study designs are complex and inconsistent. Findings from some high-quality prospective cohort studies and clinical trials are promising, but more

studies are needed to fill the aforementioned knowledge gaps. Although the widespread use of phytoestrogens could not be recommended due to the controversies, habitual consumption of phytoestrogens, particularly their intact food sources like soy and whole flaxseed, could be considered as a component of overall healthy dietary pattern for prevention and management of T2D.

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Changing trends in management of gestational diabetes mellitus

Gunasekaran Kala Poomalar

Gunasekaran Kala Poomalar, Department of Obstetrics and Gynaecology, Sri Manakula Vinayagar Medical College Hospital, Madagadipet, Puducherry 605107, India

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Correspondence to: Gunasekaran Kala Poomalar, DGO, DNB (OG), Associate Professor, Department of Obstetrics and Gynaecology, Sri Manakula Vinayagar Medical College Hospital, Kesavan Road, Madagadipet, Puducherry 605107, India. poomalarpragash@gmail.com

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Abstract

Gestational diabetes mellitus (GDM) is on the rise globally. In view of the increasing prevalence of GDM and fetal and neonatal complications associated with it, there is a surge of research in this field and management of GDM is undergoing a sea change. Trends are changing in prevention, screening, diagnosis, treatment and future follow up. There is emerging evidence regarding use of moderate exercise, probiotics and vitamin D in the prevention of GDM. Regarding treatment, newer insulin analogs like aspart, lispro and

detemir are associated with better glycemic control than older insulins. Continuous glucose monitoring systems and continuous subcutaneous insulin systems may play a role in those who require higher doses of insulin for sugar control. Evidence exists that favors metformin as a safer alternative to insulin in view of good glycemic control and better perinatal outcomes. As the risk of developing GDM in subsequent pregnancies and also the risk of overt diabetes in later life is high, regular assessment of these women is required in future. Lifestyle interventions or metformin should be offered to women with a history of GDM who develop pre-diabetes. Further studies are required in the field of prevention of GDM for optimizing obstetric outcome.

Key words: Gestational diabetes mellitus; Prevention; Vitamin D; Probiotic; Insulin analogs; Oral hypoglycemic agents

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Core tip: To summarize, the use of probiotics and vitamin D supplementation may help in preventing gestational diabetes mellitus (GDM) in high risk women. Glycemic targets need to be lower than current recommendations. Oral hypoglycemic agents are an effective and safe alternative to insulin in managing GDM. Newer insulins, aspart, lispro and detemir, provide better glycemic control than routinely used insulin. Continuous glucose monitoring systems and insulin pumps may be of use in women who require a very high dose of insulin. Lifestyle interventions in GDM women help to reduce the subsequent development of diabetes. Further studies are required in the field of prevention of GDM for optimizing obstetric outcome.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable degrees with onset or first recognition during pregnancy. The prevalence of gestational diabetes mellitus is increasing globally. In the United States, up to 14% of pregnancies are complicated by GDM, accounting for 200000 cases annually^[1]. The prevalence of GDM in Canada is 8%-18%^[2] and in China it varies between 6.8% and 10.4%^[3]. In India, there is an exceptionally high estimated prevalence of GDM (27.5%) when compared to 9.9% in Sri Lanka and 9.8% in Bangladesh^[4]. Recently, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended new screening criteria for GDM based on the hyperglycemia and adverse pregnancy outcomes (HAPO) study. Based on these criteria, the total incidence of GDM reaches almost 15%-20%^[5].

The offspring of a diabetic mother is at increased risk for fetal, neonatal and long term morbidities. They are at higher risks of developing macrosomia and stillbirths. Macrosomia is the most common morbidity, occurring in 15%-45% of infants, leading to shoulder dystocia and trauma during delivery^[6-8]. Hypoglycemia, hyperbilirubinemia, respiratory distress syndrome, hypocalcemia, polycythemia and hypertrophic cardiomyopathy are complications expected in the immediate postnatal period. They are also at increased risk of developing diabetes, obesity and metabolic syndrome in childhood and adult life^[9]. The magnitude of fetal and neonatal risks is proportional to the severity of maternal hyperglycemia.

With this background of increasing prevalence of GDM worldwide and its established association with adverse fetal, neonatal and their long term complications, we need to look into available options for preventing and managing GDM.

PREVENTION OF GDM

High risk groups for development of GDM are those with a high body mass index (BMI) (BMI cutoff of 30 kg/m² in non-Hispanic whites, 26 kg/m² in African Americans, 25 kg/m² in Chinese and 24 kg/m² in South Asians)^[10], history of diabetes in a first degree relative, previous macrosomic infant, history of GDM in previous pregnancies and women belonging to the ethnic origin of Asia, the Caribbean and Arabia, particularly of the Middle East. Much research is underway in the field of prevention of GDM. Various modalities like exercise and diet have been found to be useful for prevention of GDM in high risk groups. More recently, probiotics and vitamin D are being studied for the same. The effectiveness of the preventive modalities in pre-

pregnant and pregnant women are detailed below.

Diet

Dietary fiber and glycemic index of food: Less dietary fiber intake has been associated positively with GDM. Intake of dietary fiber, in particular cereal and fruit fiber, were strongly and inversely associated with GDM risk. Each 10 g/d increment in total fiber intake was associated with a 26% reduction in risk; each 5 g/d increment in cereal fiber and fruit fiber was associated with 23% and 26% reduction in GDM risk, respectively. Increased dietary fiber intake leads to decreased appetite and thus lowers total energy intake. It also delays gastric emptying and slows glucose absorption, resulting in less absorption of glucose leading to glucose homeostasis and less increase in insulin levels^[11].

Both dietary glycemic index and glycemic load have an influence on postprandial glycemia. The combination of high glycemic load and low cereal fiber diet was associated with a 2.15 fold increased risk compared with the low glycemic index and high cereal fiber diet^[11]. These findings suggest that the pre-pregnancy diet pattern might be associated with women's GDM risk. In particular, diet with low fiber and high glycemic load was associated with an increased risk.

Western diet pattern: A study was conducted comparing the Western pattern and the prudent dietary pattern. The prudent dietary pattern includes high intake of poultry, fish, fruit and green leafy vegetables. The Western pattern includes high intake of red meat, processed meat, French fries, pizza, refined grain products and sweets. There were strong positive associations between the Western dietary pattern and GDM, whereas the prudent dietary pattern was significantly and inversely associated with GDM^[12].

Pre-pregnancy intakes of red and processed meats were both significantly and positively associated with GDM risk^[12]. It might be related to the presence of saturated fat and cholesterol content in red and processed meats affecting insulin sensitivity^[13].

Exercise

Physical activity was not encouraged during pregnancy due to the fear of adverse effect on the fetus and mother, but various studies show no adverse maternal and fetal effects on women engaged in mild and moderate physical activities^[14,15].

Daily stair climbing, when compared with no stair climbing, was associated with a 49%-78% reduction in GDM risk ($P < 0.011$). The amount of hours spent in recreational physical activity during the year before the index pregnancy was associated with statistically significant reductions in the incidence of GDM, but the greatest reduction in risk was observed in women who engaged in physical activity before and during pregnancy (OR = 0.40; 95%CI: 0.23-0.68)^[16]. A

study conducted among Hispanic women by Chasan-Taber *et al*^[17] also showed that exercise reduces the incidence of GDM. Vigorous physical activity before pregnancy and continuation of physical activity in early pregnancy may reduce a woman's risk for developing GDM^[18]. In the absence of either medical or obstetric complications, moderate exercise for ≥ 30 min/d on most of the day of the week is recommended for a pregnant woman^[19].

Pre-pregnancy BMI and GDM

Pre-pregnancy BMI has significant influence on GDM. Compared to women with a normal BMI, the odds ratio of an underweight woman developing GDM was 0.75 (95%CI: 0.69-0.82). The odds ratio of overweight, moderately obese and morbidly obese women to develop GDM were 1.97 (95%CI: 1.77-2.19), 3.01 (95%CI: 2.34-3.87) and 5.55 (95%CI: 4.27-7.21) respectively^[20]. The risk of GDM is positively associated with pre-pregnancy BMI. This information is important when counseling women planning for a pregnancy.

Another parameter which is as important as pre-pregnancy weight is an acceptable weight gain during pregnancy. Guidelines for weight gain during pregnancy were released by the Institute of Medicine in 2009. These guidelines are based on pre-pregnancy BMI. Those who have a normal BMI between 18.5-24.9 kg/m² should aim for a weight gain of 25-35 lb (11-15 kg) and those who are underweight with BMI < 18.5 kg/m² should aim for 28-40 lb (12-18 kg) weight gain during pregnancy. Those who are overweight with a pre-pregnancy BMI between 26 and 29.9 kg/m² should aim for a weight gain of 15-25 lb (6.8-11.4 kg). Those who are obese with a pre-pregnancy BMI of ≥ 30 kg/m² should aim for 11-20 lb (5-9 kg) weight gain^[21]. In women who have excessive weight gain in the first trimester, there is increased risk of development of GDM^[22,23]. Counseling regarding acceptable weight gain in pregnancy is also required during the pre-pregnancy period.

Probiotics

Numerous studies show that probiotics can reduce the incidence of GDM. A systematic review by Lindsay *et al*^[24] demonstrated that probiotic use in pregnancy could significantly reduce maternal fasting glucose levels. Also, there is a significant decrease in the incidence of GDM and pre-eclampsia rates. By supplementing the gut bacteria, they change the metabolism in individuals and help to prevent GDM. Among the list of various organisms, *Lactobacillus rhamnosus* and *Bifidobacterium lactis* are found to have an anti-diabetic effect^[25]. A randomized controlled trial was conducted in 256 pregnant women randomized to receive probiotics or placebo. Probiotics given in that study were *Lactobacillus rhamnosus* GG (10^{10} colony forming units) and *Bifidobacterium lactis* Bb12 (10^{10} colony forming units). Significantly reduced plasma glucose ($P = 0.025$) and improved insulin sensitivity ($P = 0.028$) were

observed in the diet/probiotic group^[26]. In yet another study, daily intake of *Lactobacillus rhamnosus* GG (10^{10} colony forming units) and *Bifidobacterium lactis* Bb12 (10^{10} colony forming units) reduced GDM frequency in the diet/probiotic group (13%) compared to the diet/placebo (36%) and control/placebo (34%) groups ($P = 0.03$)^[27]. There were no adverse events in mothers or children who took probiotics during pregnancy. No significant difference in prenatal or postnatal growth rates among the study groups was detected. This shows that probiotics are a safe and cost-effective tool in preventing GDM.

The Cochrane review also states that the use of probiotics is associated with a reduction in the rate of GDM (relative risk 0.38). There was also a reduction in the birth weight of infants of women who were supplemented with probiotics^[28]. All this evidence suggests that daily intake of probiotic capsules with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 with 10^{10} colony forming units may be effective in pregnant women who are at high risk for GDM.

SPRING, a multicenter randomized control trial of a high-risk group of overweight and obese pregnant women, is being conducted which will provide a clear idea about the usage of probiotics in preventing GDM in that group^[29].

Various studies support the beneficial relationship between fermented dairy products and a reduced risk of diabetes^[30-32]. An observational study conducted in > 6500 individuals found that yogurt consumers had reduced levels of glucose and insulin resistance compared with non-consumers^[33]. A study shows a daily consumption of 200 mL of a shake containing *Lactobacillus acidophilus* (4×10^8 CFU/100 mL) and *Bifidobacterium bifidum* (4×10^8 CFU/100 mL) resulted in a blood glucose reduction in type 2 diabetes individuals^[34]. It has also been shown that consumption of yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* for a duration of 6 wk by type 2 diabetes individuals resulted in reduced fasting glucose, reduced glycosylated hemoglobin (HbA1c) levels and higher activity of superoxide dismutase and glutathione peroxidase when compared to a control group^[35].

Probiotic food supplements are available from many sources but effectiveness is dependent on various factors like temperature, anaerobic storage conditions, the initial dose of the strain and its quality. It is not known to what extent these food sources alter the gut microbes and thereby have biological effects outside research settings.

Vitamin D

All over the world, there is a high prevalence of vitamin D deficiency irrespective of age. A study done in United States adults showed that the overall prevalence rate of vitamin D deficiency was 41.6%^[36]. Vitamin D deficiency is known to cause musculoskeletal problems. Apart from that, it is associated with various cardiovascular

problems like myocardial dysfunction, heart failure and sudden cardiac deaths. Its deficiency also leads to respiratory problems, autoimmune diseases, certain cancers, hypertension and diabetes mellitus^[37]. Various studies have shown that there is an increased prevalence of pre-eclampsia^[38], gestational diabetes^[39,40], preterm labor^[41] and intrauterine growth restriction^[42] in pregnant women with vitamin D deficiency. Supplementation of vitamin D during pregnancy may reduce these complications. Safe dosage of vitamin D in pregnancy has been studied. A dose of 4000 IU/d or 50000 IU every 2 wk was able to raise serum 25-hydroxyvitamin D levels to > 30 ng/mL, leading to a decrease in insulin resistance^[43]. This dosage is associated with reduction in pregnancy complications without producing toxicity but, as there is insufficient evidence supporting a vitamin D role in GDM, the Cochrane database has concluded that more randomized trials are required to evaluate its role in pregnancy^[44]. Results in a European multicenter study of vitamin D and lifestyle interventions are awaited, which may throw light on our dilemma about the role of vitamin D in the prevention of GDM^[45].

DIAGNOSIS OF GDM

There is a dilemma whether to perform selective screening or universal screening for GDM in pregnant women. By screening only the high risk population, up to 30% of GDM women may be missed. In areas where the incidence of GDM is $< 3\%$, selective screening in a high risk population is acceptable, but where the prevalence of GDM is $> 3\%$, universal screening may be considered^[46].

Diagnostic criteria

Various criteria are being utilized worldwide for diagnosing GDM.

American College of Obstetricians and Gynecologists (ACOG): It recommends a 1 h glucose challenge test for screening GDM. If plasma glucose value is ≥ 140 mg/dL, a 3 h 100 g oral glucose tolerance test (OGTT) should be performed for diagnosis. Carpenter Coustan criteria are used for diagnosing GDM. Abnormal values are as follows: Fasting plasma glucose (FPG) ≥ 5.3 mmol/L (95 mg/dL); 1 h ≥ 10 mmol/L (180 mg/dL); 2 h ≥ 8.6 mmol/L (155 mg/dL); 3 h ≥ 7.8 mmol/L (140 mg/dL). GDM is diagnosed with ≥ 2 abnormal values.

World Health Organization (WHO) 1999: (2 h 75 g OGTT). Fasting plasma glucose FPG ≥ 6.9 mmol/L (125 mg/dL); 2 h ≥ 7.8 mmol/L (140 mg/dL).

Australasian Diabetes in Pregnancy Society (ADIPS) 1991 Criteria: (2 h 75 g OGTT). FPG ≥ 5.5 mmol/L (100 mg/dL); 2-h ≥ 8.0 mmol/L (144 mg/dL).

Diabetes in Pregnancy Study Group India dia-

gnostic criterion: Seventy-five gram oral glucose load is performed irrespective of the last meal and blood sugar level is taken 2 h later. A value of ≥ 7.8 mmol/L (140 mg/dL) is diagnosed as GDM. This may be of use in limited resource settings and where patient compliance is doubtful.

International Association of Diabetes and Pregnancy Study Groups two-phase strategy for the detection of hyperglycemia in pregnancy

HAPO, a multicenter study, was done in a cohort of 25505 pregnant women. Participants were tested with 2 h, 75 g OGTT and their pregnancies were followed for adverse maternal and neonatal outcomes^[47]. Based on the study results, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus panel formulated the new diagnostic criteria for GDM^[48]. The cut-off points were derived based on infant birth weight, cord blood C-peptide and neonatal body fat composition.

At the first antenatal visit: Screening is done with either FPG, HbA1c or random plasma glucose. Based on the prevalence of GDM in the locality, either all women or only high-risk women are screened. (1) GDM is diagnosed if FPG is between 5.1 and 7.0 mmol/L (92 to 126 mg/dL). A diagnosis of overt diabetes is made with one of the following values; (2) FPG ≥ 7.0 mmol/L (126 mg/dL); (3) HbA1c $\geq 6.5\%$; and (4) RPG ≥ 11.1 mmol/L (200 mg/dL), then confirm with FPG or HbA1c.

At 24-28 wk gestation: In all women previously not found to have GDM/overt DM, 2 h 75 g OGTT is done. If FPG ≥ 7.0 mmol/L (126 mg/dL), overt diabetes is diagnosed. GDM was diagnosed with \geq one abnormal values: (1) FPG ≥ 5.1 mmol/L (92 mg/dL); (2) 1-h ≥ 10 mmol/L (180 mg/dL); and (3) 2-h ≥ 8.5 mmol/L (153 mg/dL).

Australasian Diabetes in Pregnancy Society (ADIPS) criteria 2013: It also recommends the same cut-off points suggested by IADPSG. A diagnosis of GDM is made if ≥ 1 of the following glucose levels is elevated: (1) FPG ≥ 5.1 mmol/L; (2) 1-h ≥ 10.0 mmol/L; and (3) 2-h ≥ 8.5 mmol/L.

Unlike IADPSG, ADIPS does not recommend the use of the term "overt diabetes" to describe marked hyperglycemia first detected during pregnancy. Glucose intolerance of any severity with onset or first recognition during pregnancy is labelled as GDM by this guideline.

WHO 2013 recommendations^[49]

In the interest of moving towards a universal standard recommendation for the diagnosis of GDM, the WHO guideline development group has decided to accept the IADPSG criteria rather than introduce another set of arbitrary cut-off values.

The difference from IADPSG guidelines is that these new WHO guidelines provide a range of plasma

glucose levels to distinguish diabetes in pregnancy and GDM. Likewise, instead of using the terminology "overt diabetes", WHO uses "diabetes mellitus in pregnancy".

For diagnosing diabetes mellitus in pregnancy, WHO recommends its 2006 criteria.

Diabetes is diagnosed if one or more of the following criteria are met: (1) FPG ≥ 7.0 mmol/L (126 mg/dL); (2) 2 h plasma glucose ≥ 11.1 mmol/L (200 mg/dL) following a 75 g oral glucose load; and (3) Random plasma glucose ≥ 11.1 mmol/L (200 mg/dL).

Gestational diabetes mellitus should be diagnosed at any time during pregnancy based on any one of the following values: (1) Fasting plasma glucose = 5.1-6.9 mmol/L (92-125 mg/dL); (2) 1 h post 75 g oral glucose load ≥ 10.0 mmol/L (180 mg/dL) (there are no established criteria for the diagnosis of diabetes based on the 1 h post-load value); (3) 2 h post 75 g oral glucose load between 8.5-11.0 mmol/L (153-199 mg/dL).

As IADPSG guidelines use only one abnormal value to diagnose GDM, its prevalence is expected to significantly increase from 5%-6% to 15%-20%.

American Diabetes Association

The American Diabetes Association (ADA) also recommends these IADPSG cut-off points in its 2011 position statement. Although ADA recognizes that with these cut-off points there would be a significant increase in the incidence of GDM, it recommends these changes with the intention of optimizing gestational outcomes for women and their babies^[5].

Falavigna *et al*^[50] compared IADPSG criteria with WHO 1999 criteria. The IADPSG criteria gave better results than the WHO 1999 criteria. The adoption of the IADPSG criteria instead of the WHO criteria would reduce the incidence of LGA births by 0.32% ($P < 0.001$) and of pre-eclampsia by 0.12% ($P = 0.007$).

Other evidence also suggests that adopting IADPSG criteria may be more cost effective^[51,52].

As IADPSG diagnostic criteria are based on prognostic accuracy (the risk of pregnant women developing an adverse outcome in a certain period of time), these guidelines appear to be more logical and can be adopted worldwide.

MicroRNA profiling in preventing GDM

Now there is emerging evidence that the use of microRNAs (miRNAs) is useful in predicting GDM. It has been generally assumed that most genetic information is transacted by proteins, but recent evidence suggests that the majority of the genomes of mammals and other complex organisms are in fact transcribed into non coding RNAs (ncRNAs), many of which are alternatively spliced and/or processed into smaller products. These ncRNAs include miRNAs, snoRNAs and many others. They contain internal signals that control various levels of gene expression in physiology and development^[53]. The miRNAs have recently been demonstrated to

abundantly and stably exist in serum and to be potentially disease-specific. Specifically, miRNAs are required for pancreatic development and the regulation of glucose stimulated insulin secretion^[54,55]. Serum miRNAs are differentially expressed between GDM women and controls and could be candidate biomarkers for predicting GDM. Particularly, the use of miR-29a, miR-222 and miR-132 as serum-based biomarkers in early second trimester (16-19 wk) has been shown to be effective, which warrants further evaluation and optimization^[56].

TREATMENT FOR GDM

There is a greater dilemma whether it is worth treating mild cases of GDM if the outcome does not change much. The Cochrane review states that there are various benefits of diagnosing and treating mild GDM cases. There is a reduction in the proportion of infants weighing more than 4000 g (RR = 0.46, 95%CI: 0.34-0.63) and the proportion of infants weighing greater than the 90th birth centile (RR = 0.55, 95%CI: 0.30-0.99) in mothers receiving specific treatment for GDM compared to those not receiving specific treatment. Perinatal morbidity (shoulder dystocia, bone fracture, nerve palsy and death) was significantly reduced in women receiving intensive treatment for mild GDM compared to routine antenatal care (RR = 0.32, 95%CI: 0.14-0.73)^[57].

Glycemic targets during pregnancy

Glycemic targets as recommended by the fifth international workshop conference on GDM^[58] are capillary glucose concentrations of: (1) Pre-prandial: ≤ 5.3 mmol/L (96 mg/dL); (2) 1 h post meal: ≤ 7.8 mmol/L (140 mg/dL); and (3) 2 h post meal: ≤ 6.7 mmol/L (120 mg/dL).

In the metformin in gestational diabetes (MIG) trial, analysis of glycemia and its outcome showed that a fasting capillary glucose < 4.9 mmol/L (88 mg/dL) had significantly better outcomes than women with a fasting capillary glucose between 4.9 and ≥ 5.3 mmol/L (88 and ≥ 95 mg/dL). Two hours postprandial, capillary glucose values of ≤ 6.4 mmol/L (≤ 115 mg/dL) were associated with improved outcomes. Further improvement was seen with mean postprandial capillary glucose < 5.9 mmol/L (< 106 mg/dL)^[59].

A review by Hernandez *et al*^[60] states that average glucose values in pregnant non-diabetic women are 4.5 mmol/L (81 mg/dL) for fasting, 6.8 mmol/L (122 mg/dL) for 1 h postprandial and 6.1 mmol/L (110 mg/dL) for 2 h postprandial.

All these studies implicate that targets for fasting and postprandial capillary glucose may need to be lower than what current guidelines recommend. Further studies are required in this field.

Adjustment of insulin therapy based on postprandial glucose values improves glycemic control better than pre-prandial glucose values and decreases the risk of neonatal hypoglycemia, macrosomia and cesarean

delivery^[61].

Continuous glucose monitoring systems (CGMS) may be of use in pregnant women with overt diabetes who require a very high dose of insulin to achieve good glycemic control. CGMS during pregnancy is associated with improved glycemic control and a reduced risk of macrosomia^[62]. A study in Finland showed that CGMS detects a markedly higher proportion of GDM mothers needing antihyperglycemic medication compared with self-monitoring of plasma glucose^[63]. Further large scale studies are needed to evaluate whether CGMS guided initiation of antihyperglycemic therapy results in less macrosomia and perinatal complications related to GDM.

Diet

Calorie requirement for GDM women is 30-35 kcal/kg for normal weight, 25-30 kcal/kg for overweight and 35-40 kcal/kg for underweight subjects. Severe calorie restriction to < 1500 cal/d is not advisable. A study by Rizzo *et al*^[64] has shown that severe calorie restriction to < 1500 cal/d is associated with increased incidence of ketonemia, resulting in lowered mental developmental index scores and average Stanford-Binet scores in the babies. The American Diabetes Association recommends a 30%-33% calorie restriction in obese women with GDM, with a minimum of 1800 cal/d^[65].

Diets composed of 50%-60% carbohydrates will often result in hyperglycemia and excessive weight gain. So, calorie intake from carbohydrate has to be limited to 33%-40%, with the remaining calories divided between protein (20%) and fat (40%)^[61]. Three meals and two to three snacks are recommended to distribute glucose intake and to reduce postprandial glucose fluctuations. Glycemic index of food may also be an important factor for sugar control. Low glycemic index (< 55) foods produce a better sugar control than foods with a high glycemic index (> 70). Studies have shown that the glycemic index of food has an influence on birth weight of the baby^[66,67]. Studies on the effect of a high fiber diet on outcome of pregnancy in GDM have shown mixed results^[68,69].

Insulin

When glycemic targets are not achieved by 1-2 wk of the diet, pharmacological treatment is recommended^[70]. Short acting insulin is used to cover glucose excursions following the meal and intermediate acting insulin for hepatic glucose production in the fasting state. Regular human insulin (RHI) is a shorter acting insulin and neutral protamine hagedorn (NPH) is the intermediate acting insulin in common practice to date. Following each meal, glucose levels peak at approximately 1 h after the start of the meal and then return to pre-prandial levels within 2-3 h^[71]. Short acting insulin starts its action 1/2 to 1 h after injection and its effect reaches a peak at 2-4 h. Therefore, at times, the pre-prandial administration of RHI is not able to control the peak

postprandial blood glucose. At the same time, delayed peak action and a longer duration of action may result in inappropriate hyperinsulinemia before the next meal, resulting in pre-prandial hypoglycemia^[72].

Rapid acting insulin analogs

In order to overcome this problem, newer rapid acting insulin analogs can be used instead of short acting insulin. These rapid acting insulin analogs start their action within 15 min, reaches a peak by 31-70 min and acts for 2-4 h. Several studies have proven the safety of insulin aspart and lispro in pregnancy. United States Food and Drug Administration (FDA) has approved both insulins for use during pregnancy. Several clinical studies have shown fewer episodes of hypoglycemia, strict sugar control and higher reduction in HbA1c levels in pregnancy.

The latest rapid acting insulin analog, insulin glulisine, is available with the same action profile as that of aspart and lispro. As there are no clinical data available, to date, United States FDA has not approved it for use in pregnancy.

Long acting insulin

Commonly used NPH insulin starts its action in 1-2 h, with peak action at 4-8 h and effective up to 12-18 h. The night dose of NPH has its peak action in the early morning hours and produces hypoglycemia^[72].

Compared to NPH, newer long acting insulin analog detemir starts its action in 1-2 h and has a flatter profile with a more even distribution of metabolic effect over 24 h^[73]. Insulin detemir also shows lower rates of hypoglycemia. Various studies have proved the efficacy and safety of insulin detemir in pregnancy. Moreover, the United States FDA has approved insulin detemir as class B in pregnancy. Although the insulin glargine has same pharmacodynamic properties as detemir, the use of insulin glargine is not approved in pregnancy. Well controlled trials are needed to determine its safety in pregnancy.

Premixed insulin preparations

Premixed insulin preparations are commonly used everywhere. A combination of short acting and intermediate acting injections are available in different ratios of 30/70, 25/75, 50/50. Premixed insulin analogs provide better postprandial coverage and less hypoglycemic episodes between meals. Biphasic insulin aspart (BIAsp 30) comprises rapid acting aspart combined with protamine-crystallized insulin aspart in a 30:70 ratio. It requires twice daily dosing and provides better sugar control^[74]. It has been found to be safe during pregnancy.

Insulin pump

Insulin pumps allow for flexible insulin administration with a profile that resembles the physiological insulin profile of the beta cells of the pancreas very closely.

Major advantages of administering insulin by continuous subcutaneous insulin infusion include decreased variability in insulin absorption (due to small insulin doses administered at one time), decreased risk of hypoglycemia (due to the lower total doses of insulin) and improved control of the dawn phenomenon^[75].

CGMS and insulin pumps used together during pregnancy help improve glycemic control. Together, these devices could potentially constitute an artificial pancreas^[76]. Real-time blood glucose readings are continuously relayed to the insulin pump. Based on glucose values, the insulin pump delivers an accurate dosage of insulin needed by the patient's body. A closed-loop system with physiologically responsive insulin adjustments capable of maintaining near normal glucose levels could be of great benefit for pregnant women with type 1 diabetes or GDM with high plasma glucose values.

The Helen Murphy group evaluated closed-loop insulin delivery with a model predictive control (MPC) algorithm^[77]. The basal insulin infusion rate was calculated using women's weight, basal insulin requirements (measured by continuous glucose monitoring) and total daily insulin dose during the preceding 3 d. A nurse adjusts the basal insulin infusion rate from continuous glucose measurements and feeds into the MPC algorithm every 15 min. The total daily insulin dose was reduced by 30% for conversion to insulin pump. The advantage found with a closed-loop insulin delivery system over conventional multiple injections is its ability to respond rapidly to glucose excursions with more flexible insulin infusion rates despite comparable overall insulin doses.

Pump therapy can be especially beneficial for women who require high doses of insulin and experience repeated episodes of hypoglycemia with intermittent insulin injections^[78]. Large scale trials are needed before widespread use.

Oral hypoglycemic agents for GDM

The traditional management of women with GDM is to treat with insulin if diet therapy fails. However, insulin therapy has its own drawbacks. Now there is emerging evidence for the role of oral medications in these women. ACOG and National Institute for Health and Care Excellence (NICE) guidelines suggest that insulin and oral medications are equivalent in efficacy and either can be an appropriate first-line therapy^[61,79].

Metformin

Su *et al*^[80] conducted a systematic review of six randomized clinical trials involving 1420 subjects. They found that by using metformin in women with gestational diabetes there was no increase in adverse maternal and neonatal outcomes compared to insulin. Moreover, metformin usage in pregnancy is associated with less weight gain and neonatal hypoglycemia.

A randomized control trial by Niromanesh *et al*^[81] showed that the birth weight of the neonate was lower

in the metformin group compared to the insulin group, although not statistically significant. Maternal weight gain was significantly reduced in the metformin group.

A retrospective study done by Marques *et al*^[82] showed that there was no statistical differences between insulin and metformin groups with regard to the rates of abortion, preeclampsia, macrosomia, prematurity, small for gestational age or large for gestational age newborns, perinatal deaths, cesarean deliveries, neonatal intensive care unit admissions and birth malformations or neonatal injuries.

There is no significant difference in postprandial glucose control between women on insulin and those taking oral hypoglycemic agents. This finding is reflected in similar rates of fetal macrosomia and mean birth weight in women receiving insulin or oral hypoglycemic agents as first-line treatment^[83].

However, there are studies which show that metformin can induce neural tube defects when it is taken during first trimester of pregnancy. Expression of *Pax3* gene is essential for neural tube closure. Adenosine monophosphate-activated protein kinase (AMPK) is stimulated in embryos during diabetic pregnancy by maternal hyperglycemia^[84]. This stimulated AMPK inhibits expression of *Pax3* gene and induces neural tube defects. Studies have shown that apart from maternal hyperglycemia, metformin has also been shown to stimulate AMPK activity in skeletal muscle and liver^[85,86]. This stimulation of AMPK activity by metformin causes neural tube defects. However, a study by Lee *et al*^[87] showed that metformin increases activated AMPK in the maternal liver but it did not have an effect on AMPK in embryos or maternal skeletal muscle. Because of the absence of AMPK activity, metformin did not inhibit *Pax3* expression in embryos and thus did not cause neural tube defects. The absence of metformin responsiveness on embryos may be explained by insufficient expression of metformin transporters during neurulation. Thus, in their study, they showed that metformin does not stimulate embryo AMPK activity and consequent embryopathy.

The MIG trial by Rowan *et al*^[88] showed that women receiving metformin had less weight gain between the time of enrollment and 36 wk of gestation than those receiving insulin. Similarly, there was a greater weight loss between the time of enrollment and the postpartum visit in women receiving metformin compared to insulin. There were significantly less episodes of neonatal hypoglycemia events in infants of women taking metformin. The MIG trial concluded that metformin had similar perinatal complications compared to insulin. Due to the convenience, women preferred metformin to insulin treatment.

The follow up of exposed children up to 2 years in the MiG-TOFU trial revealed that metformin-exposed infants had more subcutaneous fat and less visceral fat. This may result in an increased insulin sensitivity pattern of growth in future^[89].

Glyburide

In the treatment of GDM, metformin and glyburide were equally efficacious to insulin in blood sugar control^[90-92]. The only significant difference in outcome between the 2 drugs was that maternal weight gain during pregnancy was lower with metformin. A few studies showed that the incidence of macrosomia^[91,93] and neonatal hypoglycemia^[91] is higher in babies of GDM mothers treated with glyburide. In contrast to this, a meta-analysis showed that there is no consistent evidence for increase in any adverse maternal or neonatal outcomes with the use of glyburide or metformin compared with the use of insulin^[94].

Oral agents are better in pregnancy because they are patient friendly and convenient, resulting in increased compliance with treatment regimens. Oral agents do not require instruction at the time of initiation of therapy. Glycemic control and perinatal outcomes produced by oral hypoglycemic agents (OHAs) were comparable to insulin. Insulin is costlier, inconvenient to use and needs ideal storage conditions, which makes it difficult in developing countries. So, OHAs should be considered safe alternatives to insulin which should be reserved as a second-line agent for patients in whom oral treatment does not achieve glycemic control^[83].

FETAL SURVEILLANCE

There is an increased risk of fetal demise in patients with poor glycemic control. To improve fetal outcome, all women with GDM should be instructed to monitor fetal movements during the last 8-10 wk of pregnancy. They should report immediately if there is any reduction in the perception of fetal movements. Antepartum fetal surveillance should be started from 32 wk in insulin treated GDM women. Non-stress testing should be done after 32 wk gestation in women on insulin. Biophysical profile testing and Doppler velocimetry to assess umbilical blood flow may be considered in those with associated intra uterine growth restriction, macrosomia or in those with co-morbid conditions, such as pre-eclampsia. There is no consensus regarding antepartum testing in women with GDM well controlled with diet.

INDUCTION OF LABOR

The timing and the mode of delivery is still debatable. For the timing of delivery, the ADA in 2004 recommended delivery at 38 wk unless obstetric considerations dictated alternative management^[65]. Similarly, NICE in 2008 recommended that pregnant women with diabetes should be offered elective birth through induction of labor after 38 completed weeks^[79]. The ACOG did not recommend routine delivery before 40 wk in GDM women well controlled with diet or medication^[61]. Induction of labor at term helps to reduce the incidence of shoulder dystocia in women with gestational diabetes^[95]. The option of induction of labor in GDM is still controversial. Further studies are needed in this field.

For the mode of delivery, a cesarean delivery would only be suggested for an estimated fetal weight of ≥ 4500 g in mothers with GDM^[79,96].

GLYCEMIC CONTROL DURING LABOR

During labor and birth, capillary blood glucose should be monitored on an hourly basis in women with diabetes and maintained at 4-7 mmol/L. Intravenous dextrose and insulin infusion is recommended during labor and birth in women with diabetes whose blood glucose is not maintained between 4-7 mmol/L.

FUTURE PREVENTION

Women with a history of GDM have a greatly increased subsequent diabetes risk and should be followed up with subsequent screening for the development of pre-diabetes or diabetes. Women who had GDM in pregnancy should have a 75 g 2 h OGTT (WHO criteria), preferably at 6-12 wk post-partum. Women with a normal result should be reassessed once in 3 years. For those who are diagnosed with impaired fasting glucose or impaired glucose tolerance, annual testing should be done.

For women who had GDM in the first pregnancy, there is 13.2 fold increased risk (95%CI: 12.0-14.6) of developing GDM during the second pregnancy. The recurrence risk of GDM in the third pregnancy was stronger when women had GDM in both prior pregnancies (25.9 fold increased risk; 95%CI: 17.4-38.4)^[97]. The cumulative proportion of women developing diabetes at 1 year postpartum was 1.7%. At the end of 10 years, 17% of women developed diabetes and at the end of 15 years, 25% developed diabetes^[98].

Lifestyle interventions or metformin should be offered to women with a history of GDM who develop pre-diabetes. Subsequent diabetes risk after a history of GDM was significantly lower in women who followed healthy eating patterns^[99]. In the diabetes prevention program, administering metformin and intensive lifestyle modification in women with a history of GDM led to 50% reduction in diabetes risk^[100]. Metformin therefore might reasonably be recommended for very high-risk individuals (those with a history of GDM or very obese women)^[5].

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Prediabetes diagnosis and treatment: A review

Nidhi Bansal

Nidhi Bansal, Department of Pediatrics, Section of Pediatric Diabetes and Endocrinology, Baylor College of Medicine, Texas Children's Hospital, Houston, TX 77030, United States

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Correspondence to: Nidhi Bansal, MD, MPH, Assistant Professor, Department of Pediatrics, Section of Pediatric Diabetes and Endocrinology, Baylor College of Medicine, Texas Children's Hospital, 6701 Fannin St., Suite 1020, Houston, TX 77030, United States. nbansal@bcm.edu

Telephone: +1-832-8244104

Fax: +1-832-8253903

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there is increasing evidence to prove the efficacy of pharmacotherapy in prevention of diabetes in adults with prediabetes, pharmaceutical treatment options other than metformin are associated with adverse effects that limit their use for prediabetes. There are no reports of systematic evaluation of health outcomes related to prediabetes in children. The effects of pharmacotherapy of prediabetes on growth and pubertal development in children remains unknown. Secondary intervention with pharmacotherapy with metformin is advocated for high-risk individuals but criteria for such consideration benefit of early intervention, long term cost effectiveness of such interventions and the end point of therapy remain unclear. Pharmacotherapy must be used with caution in children with prediabetes. Prediabetes is a condition defined as having blood glucose levels above normal but below the defined threshold of diabetes. It is considered to be an at risk state, with high chances of developing diabetes. While, prediabetes is commonly an asymptomatic condition, there is always presence of prediabetes before the onset of diabetes. The elevation of blood sugar is a continuum and hence prediabetes can not be considered an entirely benign condition. This aim of this review is to describe the challenges associated with diagnosis of prediabetes, the possible adverse medical outcomes associated with prediabetes and the treatment options and rationale for their use in context of prediabetes.

Key words: Impaired fasting glucose; Impaired glucose tolerance; Diabetes; Metformin; Lifestyle intervention; Prediabetes

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Core tip: Prediabetes is a state of intermediate hyperglycemia. While there are several controversies about the diagnosis of prediabetes, it remains an at-risk state for development of diabetes. Several adverse health outcomes have been associated with prediabetes. This review provides a detailed description of the current literature regarding diagnosis, health consequences and treatment of prediabetes and also

Abstract

Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold. While, the diagnostic criteria of prediabetes are not uniform across various international professional organizations, it remains a state of high risk for developing diabetes with yearly conversion rate of 5%-10%. Observational evidence suggests an association between prediabetes and complications of diabetes such as early nephropathy, small fiber neuropathy, early retinopathy and risk of macrovascular disease. Several studies have shown efficacy of lifestyle interventions with regards to diabetes prevention with a relative risk reduction of 40%-70% in adults with prediabetes. While

provides an insight for clinical management.

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DIAGNOSIS OF PREDIABETES

Various organizations have defined prediabetes with criteria that are not uniform. The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia using two specific parameters, impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) of 6.1-6.9 mmol/L (110 to 125 mg/dL) and impaired glucose tolerance (IGT) defined as 2 h plasma glucose of 7.8-11.0 mmol/L (140-200 mg/dL) after ingestion of 75 g of oral glucose load or a combination of the two based on a 2 h oral glucose tolerance test (OGTT)^[1]. The American Diabetes Association (ADA), on the other hand has the same cut-off value for IGT (140-200 mg/dL) but has a lower cut-off value for IFG (100-125 mg/dL) and has additional hemoglobin A1c (HbA1c) based criteria of a level of 5.7% to 6.4% for the definition of prediabetes^[2]. Several studies have shown poor correlation between HbA1c and IFG and IGT^[3-5]. The usefulness of diagnosis of diabetes or prediabetes on basis of IFG and IGT have been challenged due to inability of these blood glucose cut points to capture pathology related to diabetes and probability of developing diabetes in future^[6]. These cut-offs further loose their credibility due to poor reproducibility of these tests in adults and children^[7,8]. Although, HbA1c is believed to represent an average blood sugar level and should ideally represent hyperglycemia more accurately, this may not be entirely true. HbA1c is substantially determined by genetic factors independent of blood glucose levels and may be an imprecise tool to measure average blood sugar^[9,10]. While there are valid concerns about diagnostic criteria of prediabetes, prediabetes remains to have a lower reproducibility (approximately 50%) than diabetes (approximately 70%). Based on the available evidence, it appears that prediabetes defined by various alternative criterions consists of an overlapping group of individuals with one or more abnormalities in their glucose excursions. It is possible that presence of IFG and IGT identifies subjects with different pathological abnormalities in their glucose metabolism and presence of both of these signifies more advanced impairment in overall glucose homeostasis.

PREVALENCE OF PREDIABETES

There have been reports of increased mean FPG and prevalence of diabetes in developed as well as developing countries^[11]. The Centers of Disease Control and Prevention National Diabetes Statistics Report

suggested that 37% of United States adults older than 20 years and 51% of those older than 65 had prediabetes in 2009-2012 defined by fasting glucose or HbA1c levels^[12]. When applied to the entire United States population in 2012, these estimates suggest that, there are nearly 86 million adults with prediabetes in United States alone^[12]. The world wide prevalence of IGT in 2010 was estimated to be 343 million (7.8%) ranging from 5.8% in South East Asia to 11.4% in North American and Caribbean Countries of the nation's population^[13]. International Diabetes Federation projects an increase in prevalence of prediabetes to 471 million globally by 2035^[13].

HEALTH RISKS ASSOCIATED WITH PREDIABETES

Progression to diabetes

The conversion rate of individuals from prediabetes to diabetes changes with population characteristics and the criteria used to define prediabetes^[14,15]. In a meta-analysis evaluating the progression of prediabetes to diabetes published in 2007, the annual incidence rate of diabetes was found to be 4%-6% for isolated IGT, for isolated IFG 6%-9% and for both IGT and IFG was 15%-19%^[16]. This meta-analysis only consisted of studies published prior to 2004. In subsequently reported major studies, the annual incidence rates of conversion from prediabetes to diabetes were similar. In the Diabetes Prevention Program (DPP) Outcomes Study, the incidence of diabetes was noted to be 11% in the control group^[17]. In the United States Multi-Ethnic Study of Atherosclerosis the annual incidence of diabetes in IFG group slightly above 4%^[18]. In the The Toranomon Hospital Health Management Center Study the incidence of diabetes was reported as 7% in the group with an HbA1c 5.7%-6.4% and 9% in the IFG group^[19]. In the China Da Qing Diabetes Prevention Study (CDQDPS), the cumulative incidence of diabetes over a 20 years period, was noted to be higher than 90% among subjects with IGT defined by repeated OGTT in the control group^[20]. The use of ADA vs WHO criteria to define prediabetes has also been shown to affect the incidence rate of diabetes with lower incidence in individuals defined by ADA criteria compared to WHO criteria^[21].

According to an expert panel, continuous rather than dichotomous risk scores are more useful for predicting the risk of developing diabetes^[22]. A diabetes risk score based more easily accessible variable such as age, sex, ethnicity, fasting glucose, systolic blood pressure, HDL cholesterol, BMI and history of diabetes in parents or siblings has been shown to have better predictive value than either IFG or IGT^[23].

Nephropathy and kidney disease

Several studies have shown an association of increased risk of chronic kidney disease and early nephropathy with

prediabetes^[24-28]. The causal nature of this relationship remains unclear as this association may be due increased incidence of diabetes in this group or the presence of other factors associated with both hyperglycemia and nephropathy rather than the effect of prediabetes itself^[29,30].

Neuropathies

Prediabetes is found to be associated with dysfunction of cardiac autonomic activity, reflected by reduced heart rate variability^[31-35], decreased parasympathetic modulation of the heart^[35] and increased prevalence of male erectile dysfunction in individuals with prediabetes^[36]. Non-invasive evaluation of neural impairment in subjects with IGT has shown significantly greater abnormalities detected by four of five cardiovascular reflex tests, increase prevalence of both hyperesthesia and hypoesthesia, and increased heat detection thresholds^[37]. There is also increasing evidence to demonstrate a higher frequency of idiopathic polyneuropathy, painful sensory neuropathy^[38-43] and small fiber neuropathy^[38,40,41] among prediabetic individuals with IGT. These findings suggest an involvement of the small unmyelinated nerve fibers that carry pain, temperature, and regulate autonomic function during prediabetes, prior to development of diabetes.

Retinopathy

Nearly 8 percent of participants with prediabetes in the DPP study were found to have evidence of diabetic retinopathy^[44]. While prediabetes has been associated with an increased risk of diabetic retinopathy in some studies, these findings vary depending on the method used for detection^[24,45-49].

Macrovascular disease

Prediabetes has been associated with increased risk of developing macrovascular disease but whether this elevated risk is due to prediabetes itself or due to development of diabetes remains unclear^[50,51]. While cross sectional studies have shown an increased prevalence of coronary heart disease in individuals with prediabetes^[52,53] but this relationship may be confounded by the common risk factors present between cardiovascular diseases and prediabetes.

TREATMENT OPTIONS FOR PREDIABETES

Lifestyle interventions

The encompassing theme of lifestyle intervention programs is to change the modifiable risk factors of prediabetes and diabetes by targeting obesity with increase in physical activity and dietary changes. The two largest diabetes prevention studies, the United States DPP and the Finnish Diabetes Prevention Study (DPS) have both shown beneficial effects of lifestyle interventions^[54,55]. In the DPP study, after a 3 year

follow-up, intensive lifestyle interventions (ILS) lead to a 58% risk reduction. The ILS involved changes in diet and physical activity aimed at producing weight. The biggest determinant of risk reduction was note to be weight loss. This study showed that for every 1 kg decrease in weight, the risk of developing diabetes in future was reduced by 16%^[56]. In the DPS, the benefits were found to be dependent on achievement of the number of pre-defined goals of the intervention by the participant. These goals consisted of weight reduction greater than 5 percent, total fat intake less than 30 percent of energy intake, saturated-fat intake less than 10 percent of energy intake, fiber intake greater than or equal to 15 g per 1000 kcal, and exercise greater than 4 h/wk^[55]. While both of these studies were largely among Caucasians, studies in Asian population have also shown similar benefits^[57,58].

Pharmacotherapy

Several groups of antidiabetic drugs such as Biguanides, Thiazolidinediones, α -Glucosidase Inhibitors, GLP-1 analogies and non-antidiabetic drugs and therapies such as anti-obesity drugs, and bariatric surgery have been studied in context of prediabetes.

Metformin has been used for several decades for treatment of diabetes and has been noted to have additional favorable outcomes such as body mass index (BMI) reduction and improved cholesterol profile. The collective evidence of trials among subjects with IGT, suggests a 45% risk reduction for development of type 2 diabetes^[59]. Metformin was noted to be less effective than lifestyle in the United States DPP trial but in the indian DPP (IDPP) trial it was noted to be as effective as lifestyle intervention^[54,57]. Metformin has been found to be more beneficial to individuals with higher BMI and higher FPG^[54]. Metformin has also been studied in obese children by several investigators. The collective evidence shows a slight benefit in BMI reduction over lifestyle interventions, while the benefit was statistically significant it was noted to be only short term with the greatest benefit at 6 mo and no difference at 12 mo duration^[60].

The glitazones are synthetic ligands for peroxisome proliferator-activated receptors- γ . They increase glucose uptake and utilization in the peripheral organs and decrease gluconeogenesis in the liver, thereby reducing insulin resistance^[61]. In the double blind placebo controlled Diabetes Reduction Assessment with Ramipril and rosiglitazone Medication study, rosiglitazone was found to be effective in decreasing incidence risk of diabetes by 60% over a 3 year period but was associated with significant side effects such as an additional average weight of 2.2 kg in intervention group compared to controls and a higher incidence of heart failure (0.5% vs 0.1%) and total cardiovascular events (2.9% vs 2.1%)^[62,63]. Pioglitazone was found to decrease the risk of diabetes by > 70% in obese subjects with IGT in the ACT NOW study. Some of the added benefits were, decrease

in diastolic blood pressure, reduction in rate of carotid intima-media thickness and a greater increase in HDL cholesterol but it was associated with increased weight gain (approximately 3 kg more than placebo) and edema (13% vs 6% in controls)^[64]. In the double blinded placebo controlled 3 years prospective IDPP-2 study, there was no difference in incidence of diabetes between subjects receiving lifestyle intervention and placebo and subjects receiving lifestyle intervention and pioglitazone^[65]. In the more recent Canadian Normoglycemia Outcomes Evaluation trial, low dose combination of rosiglitazone and metformin was tested against placebo to investigate whether low dose combination therapy would decrease the incidence of type 2 diabetes with a lower risk of adverse events. Incident diabetes occurred in significantly fewer individuals in the active treatment group (14%) than in the placebo group (39%). The relative risk reduction was 66% and the absolute risk reduction was 26%, and 80% subjects in the treatment group reverted to normoglycemia compared to 53% in the control group, but the subjects in active treatment group had increased reports of diarrhea (16% vs 6% in controls)^[66]. Overall, there are safety concerns for thiazolidinedione such as weight gain, liver toxicity, increased cardiovascular risk and possible link with bladder cancer which have limited the use of these medications for treatment of prediabetes.

α -glucosidase inhibitors such as acarbose and voglibose, prolong the overall carbohydrate digestion time, and reduce the rate of glucose absorption, thus decrease the postprandial rise in blood glucose^[67]. In the STOP-NIDDM trial, acarbose was found to decrease the relative risk for diabetes by 25% among subjects with IGT during a 3.3 years of follow-up^[68,69]. The medication was associated with several gastrointestinal side effects such as flatulence and diarrhea and 31% of the participants in the acarbose arm dropped out before completion of the study^[68]. A Japanese trial found a 40% risk reduction in incidence of diabetes in high-risk individuals with IGT with voglibose over a 48 wk period. Voglibose was noted to have a similar side effect profile as acarbose but only 7% subjects discontinued the use of drug due to adverse effects^[70].

GLP-1 analogs exploit the physiological effects of GLP-1, they have been shown to augment post prandial insulin secretion, suppress glucagon and hepatic glucose production, slow gastric emptying, and reduce appetite^[71]. Exenatide and liraglutide have been demonstrated to have long term efficacy for sustained weight loss in obese subjects and reduce prevalence of prediabetes over a follow-up period of 1-2 years. The most common side effects with these drugs are nausea and vomiting and they remain injectable preparations^[72-74].

Anti-obesity drugs Orlistat has also been studied in context of prediabetes. Orlistat is a gastrointestinal lipase inhibitor used for treatment of obesity that acts by inhibiting the absorption of dietary fats by approximately 30%. Research has shown that over a 1.5 year follow-up

period, use of Orlistat in conjunction with low energy diet is associated with greater weight loss as compared to placebo (6.7 kg vs 3.8 kg) and a decrease in conversion rate from IGT to overt diabetes (7.6% vs 3.0%) in obese adults^[75]. Similar findings have also been reported by the XENDOS trial regarding the efficacy of Orlistat with a 37% relative risk reduction in development of diabetes after 4 years of treatment^[76].

Bariatric surgery

Bariatric surgery includes a variety of procedures aimed at either creating a mal-absorptive state, a restrictive state or a combination of the two to limit caloric intake. The procedures commonly used include Roux-en-Y gastric bypass, Laparoscopic adjustable gastric banding, Sleeve gastrectomy, and Duodenal switch with biliopancreatic diversion. In the Swedish Obese Subject, bariatric surgery was found to result in sustained weight loss (23.4% at 2 years and 16.1% at 10 years) and a 75% relative risk reduction of diabetes compared to controls^[77]. Bariatric surgery was also associated with a lower 2 year and 10 year rate of development of type 2 diabetes, cardiovascular disease and reduced number of cardiovascular deaths in obese adults^[77,78]. A previous study had demonstrated that after gastric bypass surgery, 78% subjects with previous diabetes and 98% subjects with IGT reverted to normoglycemia^[79].

PROS AND CONS FOR TREATMENT OF PREDIABETES

The rationale behind treatment of prediabetes includes, prevention of development of diabetes, prevention of consequences of diabetes and prevention of the consequences of prediabetes itself. Several research studies have shown success of interventions designed for treatment of prediabetes with sustained reduction in incidence of diabetes^[20,54,80-82]. The CDQDPS study, with lifestyle intervention and 20 year follow-up showed nearly 50% relative risk reduction in incidence of severe retinopathy, but there was no difference between the intervention and control groups in the risk of developing other microvascular complications, such as neuropathy and nephropathy^[83]. The evidence regarding effects of interventions on macrovascular complications is inconsistent. The Malmo Preventive Project with a 12 year follow-up, showed reduced mortality in subjects with IGT after a long-term lifestyle intervention program, with emphasis on dietary counseling and physical activity, but this was not a randomized trial^[84]. The Collective evidence of all randomized control trials among prediabetic subjects with lifestyle and drug based interventions in a recent meta-analysis showed that these interventions resulted in reduction in stroke risk but did not result in any risk reduction for all-cause mortality, cardiovascular death or myocardial infarction over a mean follow-up period of 3.8

years^[80]. While, the current evidence suggests efficacy of several treatment modalities regarding prevention of progression to diabetes, the long term benefits on microvascular or macrovascular complications remains debatable. There is no evidence to suggest that early intervention is better than late intervention and long term studies looking at cost vs benefit and long terms of outcomes related to the point at which glycemic intervention should begin are lacking. Majority of published literature and guidelines support that lifestyle interventions focusing on dietary modification and increased physical activity should be the foundation of therapy for diabetes prevention in patients with prediabetes. Although, lifestyle interventions are safe and have proven efficacy in prevention of diabetes, these programs are not reimbursed by most health care insurance plans. There is increasing evidence to prove the efficacy of pharmacotherapy and support its use in adults with prediabetes. Due to the favorable long term safety profile and observed positive outcomes with metformin, organizations such as ADA have recommended the use of metformin in certain high risk individuals^[85] but the end point of pharmacotherapy is yet to be defined. The concept of prediabetes or its treatment has not been systematically studied in children with prediabetes. Long term effect of common medications used for prediabetes on growth and pubertal development in children have not been studied. Moreover in children, due to puberty related insulin resistance, incidence of diabetes may be over all inflated. There is lack of evidence with regards to long term efficacy as well as safety for use of pharmacotherapy in children with prediabetes.

CONCLUSION

In conclusion, there remains a need of systematic evaluation of the health outcomes of prediabetes and benefits if any with its early treatment. It is very important to choose the right outcomes for such a study. Moreover, the criteria used to define prediabetes needs to be refined in accordance to the long term medical outcomes. While, these studies seem essential, the length of duration needed to study the adverse outcomes of prediabetes and low frequency of some of these outcomes may be a limiting factor for such studies. At present there is no concrete evidence to formulate clinical guidelines for treatment of prediabetes. Lifestyle interventions remain an essential part of management of prediabetes. The use of pharmacotherapy should be on an individual case based approach. When pharmacotherapy is used to treat prediabetes, such treatment plan should be initiated with predefined goals and end points by the physician. A cautious approach is warranted for use of pharmacotherapy in children and youth.

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Economic development and diabetes prevalence in MENA countries: Egypt and Saudi Arabia comparison

Sherif Shalaby, Bauer E Sumpio

Sherif Shalaby, Bauer E Sumpio, Section of Vascular Surgery, Yale School of Medicine, New Haven, CT 06510, United States
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Correspondence to: Bauer E Sumpio, MD, PhD, Professor of Vascular Surgery, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510, United States. bauer.sumpio@yale.edu
 Telephone: +1-203-7856217

Fax: +1-203-7857556

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economic development, labor force diversity and the prevalence of diabetes.

Key words: Diabetes; Obesity; Health-related behavior; Burden of disease; Middle East and North Africa region

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Core tip: The prevalence of diabetes across the Middle East and North Africa (MENA) region has been significantly rising with an increasing burden of healthcare costs. The economic changes occurring in the past decade throughout the MENA region have directed more of the labor force towards the service sector and low physically active lifestyle.

Shalaby S, Sumpio BE. Economic development and diabetes prevalence in MENA countries: Egypt and Saudi Arabia comparison. *World J Diabetes* 2015; 6(2): 304-311 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i2/304.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i2.304>

Abstract

Diabetes is increasing in epidemic proportions globally, exhibiting the most striking increase in third world countries with emerging economies. This phenomena is particularly evident in the Middle East and North Africa (MENA) region, which has the highest prevalence of diabetes in adults. The most concerning indirect cost of diabetes is the missed work by the adult population coupled with the economic burden of loss of productivity. The major drivers of this epidemic are the demographic changes with increased life expectancy and lifestyle changes due to rapid urbanization and industrialization. Our focus is to compare MENA region countries, particularly Egypt and Saudi Arabia, in terms of their

INTRODUCTION

The Middle East and North Africa (MENA) region countries spans from the Pacific Ocean to the Persian Gulf and extending from the North Africa shores to the sub-Saharan desert. The Gulf Cooperation Council (GCC) region is part of MENA region but exclusive to countries surrounding the Persian Gulf Sea. The GCC consists of Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and the United Arab Emirates. The climate, traditions, religious, and dietary habits are intertwined and shared across the region for eons^[1].

The MENA region had the highest comparative prevalence of diabetes in the world in 2012, with four countries in the region among the top ten worldwide

in terms of prevalence^[2]. The International Diabetes Federation (IDF) estimates that by 2030, patients with diabetes will double to current estimates of up to 59.9 million in the MENA region^[3]. Even though epidemiological studies document high diabetes prevalence in each country, there are specific regions within each country that have higher prevalence of diabetes than originally stated. For instance, one study reported a prevalence of diabetes in Basra, Iraq of more than 19%^[4], while, the national prevalence of Iraq is 7.4%^[2]. Thus, the diabetes epidemic in the region could be much worse than anticipated and the differences between rural or urban centers are yet to be investigated.

A number of reports have documented a relationship between increased per capita income and economic development on the drastic increase in diabetes prevalence^[5]. There is evidence that urbanization and economic progress in emerging economies, such as China, have also led to a drastic reduction in overall and occupational physical activity^[6,7]. In West Africa, major urbanization have enabled physical inactivity and diabetes is a major health concern^[8,9]. However, it is important to question the contributions of the sedentary lifestyle or economic development on the epidemic of diabetes *per se*. Developed countries have many similarities including demographic, economic, and population genetics. However, these countries also display varying proportions of diabetes prevalence, as the United States is leading with 12.3%^[10] while Japan has a diabetes prevalence of 7.6%^[2] with very similar economic status and urbanization demographics. These countries have high-income economies with very high Human Development Index. Yet, with their high economic development, they are not regarded as having a rapidly growing diabetes epidemic. The Middle East has the highest prevalence of diabetes in the world with Egypt leading the MENA region in the number of patients with diabetes and with Saudi Arabia leading in the highest prevalence of diabetes in its the adult population.

In this paper we review the economic development, physical activity, and prevalence of diabetes across the MENA and specifically its largest two states, Egypt and Saudi Arabia. We will attempt to understand whether the economic development and westernization of the region is a curse or a blessing concerning diabetes and its epidemic progression?

RESEARCH

We searched PubMed and Google for articles, reports, and major organization statistics related to diabetes mellitus in the MENA region published from 1990 to 2014 without language restriction. We used the following keywords: diabetes, MENA region, physical inactivity, and noncommunicable diseases and found 46 relevant publications. Linear correlation coefficient and statistical analysis was assessed by Minitab (State College, Pennsylvania).

FINDINGS

Diabetes prevalence varies greatly across the MENA region

Egypt and Saudi Arabia are on the separate ends of the spectrum with regards to diabetes prevalence in the MENA region with Egypt having the lowest rate of 7.2% and Saudi Arabia the highest 21.8% (Figure 1)^[11]. There is no significant difference in diabetes prevalence between genders within the Egyptian and Saudi population. The urban population within Egypt have higher prevalence of diabetes compared to the rural population (4.9%), regardless of higher (20%) or lower socioeconomic statuses (13.5%)^[12]. The increased prevalence of diabetes in urban areas could be partly explained by higher socioeconomic status was associated with decreased physical activity and increased prevalence of obesity^[13]. In Saudi Arabia, the same trend can be observed where diabetes was more prevalent among Saudis living in urban areas (25.5%) compared to rural Saudis (19.5%). Despite the readily available access to healthcare facilities, a large number of diabetics (27.9%) were unaware of having diabetes^[14]. Thus, not only diabetes prevalence varies greatly between countries but also among urban and rural regions. This may suggest that lifestyle changes associated with urbanization plays a pivotal role in the progression of the diabetes epidemic.

Arabs are genetically predisposed to diabetes

The incidence of type 2 diabetes is determined by the complex interplay between multiple genetic, epigenetic and environmental factors^[15]. The prevalence of diabetes was highest in the Eastern Mediterranean Region, which includes most MENA region countries, (11% for both sexes) and lowest in the World Health Organization (WHO) European Region (7% for both sexes)^[11]. Whether genetics are a contributing factor remains unknown but recent research may shed some light on this issue.

Approximately 40 single-nucleotide polymorphisms (SNPs) that display genome-wide associations with type 2 diabetes have been identified^[16]. How these SNPs predispose to type 2 diabetes is still limited and is only available for a few variants that mainly affect insulin secretion and sensitivity^[17]. However, there are patterns of SNPs across the MENA region which may bear a role in the diabetes prevalence diversity across the region and on the differences between Arabs and Caucasians.

A unique risk of SNPs for type 2 diabetes that are exclusive to Arab ethnicities has been identified, through a comparative meta-analysis of previously identified SNPs among Arabs and Caucasians^[18]. The study demonstrated diversity in the MENA region with countries on the Mediterranean Sea such as Egypt, Palestine, and Tunisia having similar SNPs as Caucasians. Whether the low prevalence of diabetes in those countries is

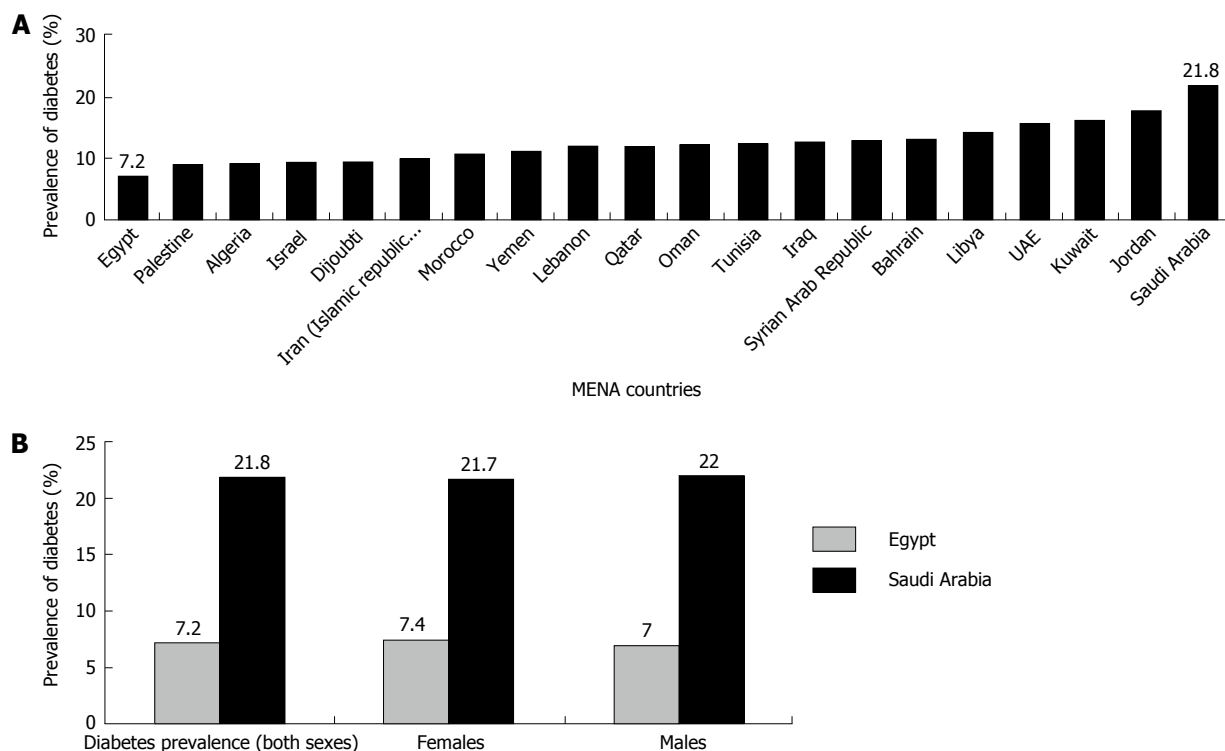


Figure 1 Prevalence of diabetes in Middle East and North Africa region. A: Middle East and North Africa (MENA) countries as defined by the World Bank arranged in increasing prevalence of diabetes; B: Prevalence of diabetes in both genders of Egyptian and Saudi Arabia population. From World Health Organization, 2008. UAE: United Arab Emirates.

influenced by genetic factors is yet to be determined. Conversely, countries from the GCC have different SNPs from Caucasians. North African countries have lower prevalence of diabetes than GCC nations and a similar pattern with the Organization for Economic Co-operation and Development (OECD) countries, which are predominately Caucasian. Egypt, with its low prevalence of diabetes, has a different pattern of SNPs to Saudis.

Other clinical studies comparing Arabs to Caucasians have confirmed that Arabs are more susceptible to diabetes^[19,20]. In particular, when comparing Iraqi immigrants (a state located on the Persian Gulf but not a member of GCC) with native Swedes, type 2 diabetes mellitus was twice as prevalent in Iraqis and the people were at a higher risk of developing diabetes^[21]. To underscore the implications of genetics on diabetes, consanguinity is prevalent in the Arab culture and that may enhance the diabetic genetic predisposition^[22]. A recent study investigated possible mechanisms of consanguinity on the etiology of diabetes in a Saudi population by genotyping SNPs associated with a higher risk of diabetes and measuring other risk factors. It concluded that consanguinity might increase the risk of diabetes by an earlier onset of the disease and by strengthening possible genetic effects on fasting blood glucose^[23]. It may explain why a family history of diabetes increases the risk of diabetes by 1.6, 1.8, and 2.4 times in studies among Palestinians, Iranians, and Kuwaitis, respectively^[24-26]. However, there are

variations in the degree consanguinity across the MENA region. In Saudi Arabia, the prevalence of consanguinity is as high as 60%^[22]. On the other hand, Egypt ranges from 8.3% to 17.2% for the urban and rural regions respectively^[27].

MENA region westernization and lifestyle changes due to its economic growth

Three out of the world's top five oil exporting countries are located in the GCC region^[28], with the world's second largest exporter of natural gas, Qatar. The diabetic population is increasing overall across the MENA region^[2] but the GCC states in particular are increasing at a staggering rate compared to others^[29]. Some have proposed that the degree of westernization and economic development of the GCC has reached comparable levels to OECD with respect to per capita income and have fueled this diabetes epidemic. However, the economic development in the OECD countries is based on industrialization, manufacturing, and other manpower dependent activities. On the other hand, the major driver of GCC economic development is export of raw materials, primarily crude oil and natural gas.

These resources varying allocation within the region created great disparities among MENA region countries. Not only is there a large difference between Egypt and Saudi Arabia with respect to diabetes prevalence but there is also a wide economic gap. Saudi Arabia has more than double the Gross National Income (GNI) of Egypt (Figure 2). Overall, the gap is wide across the

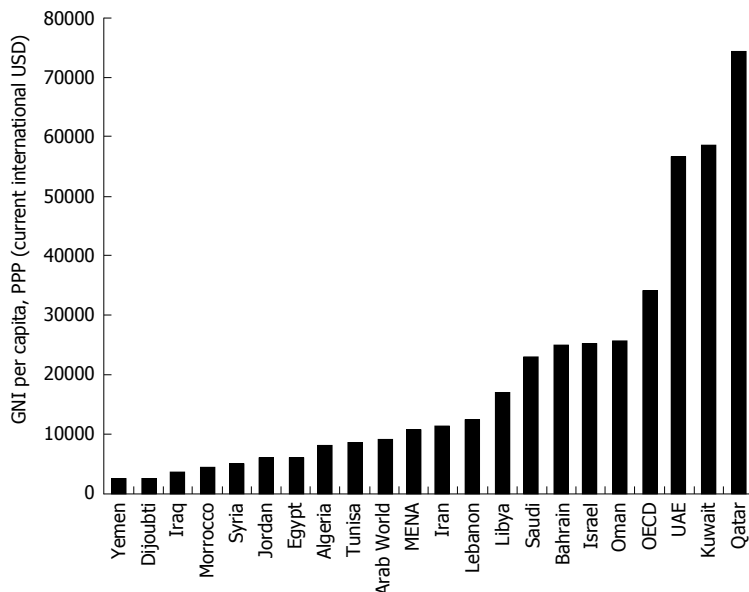


Figure 2 Gross national income per capita based on purchasing power parity of Middle East and North Africa countries. Egypt falls belows Arab world average (\$5710 vs \$8882) where Saudi Arabia is well above average (\$22760). OECD: Organization for Economic Co-operation and Development. From World Bank, 2008. UAE: United Arab Emirates; PPP: Purchasing power parity; GNI: Gross national income.

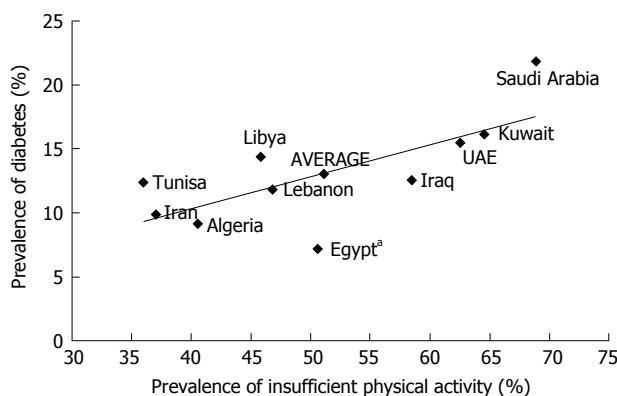


Figure 3 Prevalence of insufficient physical activity in Middle East and North Africa countries compared to prevalence of diabetes. The region's average of prevalence of insufficient physical activity is estimated to be 51.1%. Tunisia and Saudi Arabia are on the two ends of prevalence of physical inactivity in the region (35.9% vs 68.8%). Egypt's prevalence of insufficient physical activity is approximately at the region's average (50.6%). The correlation coefficient of the relationship between prevalence of insufficient physical activity and prevalence of diabetes indicate a moderate positive linear relationship ($R^2 = 0.51$). Regression Analysis of prevalence of Insufficient physical activity vs prevalence of diabetes showed statistical significance ($P = 0.01$). From World Health Organization, 2008. ^aMinistry of Health and population, Egypt Preventive Sector Central Epidemiology and Disease Surveillance (ESU). Non-Communicable Disease Surveillance Unit Community based survey study on non-communicable diseases and their risk factors, Egypt, 2005-2006. UAE: United Arab Emirates.

region with Qatar's GNI over USD 70000 while Yemen is as little as USD 2000, creating financial strains for some nations and lavish lifestyles for others. Furthermore, this overall economic progress has altered the lifestyle of the region. Where the Arab population once frequently participated in outdoor activities, everyone is now almost always inside in an air-conditioned environment, and physical activity has declined^[30].

Increased service sector jobs contributes to higher prevalence of physical inactivity and diabetes

Figure 3 shows that the prevalence of diabetes in a MENA country correlates with a higher prevalence of physical inactivity. Countries with the higher prevalence of physical inactivity and diabetes are mostly GCC countries which are also high income countries. We speculate that the exclusive reliance on export of raw resources by these GCC countries may have detrimental effects on the health of the adult population by decreasing their physical activity.

The statistics on the labor force compiled by the Arab Labor Organization, confirm the diversity of labor in countries that have a lower diabetes prevalence compared with the Gulf region with an economy that is entirely reliant on the service sector with low physically demanding occupations (Figure 4). In contrast, Egypt depends on manufacturing, fishing, and agricultural labor. Egypt's agricultural sector is reliant predominantly on manual labor and old farming techniques that require heavy manpower; mechanical farming is not prevalent nor invested in as labor cost is inexpensive. The labor pattern across the MENA region impacts the gross national income of the countries illustrating the economic disparity between Egypt and Saudi Arabia. The service sector tends to predominate in countries with higher GNI, whereas in Egypt, there is a demand for hard laborious jobs for the underprivileged class.

To emphasize the impact of physical inactivity on mortality, a recent study utilized life-table analysis to estimate gains in life expectancy if physical inactivity were to be eliminated^[31]. Saudi Arabia, with the highest prevalence of physical inactivity and diabetes, had the highest gain in the region, with an estimated additional 1.5 years of life expectancy (1.0 average for region).

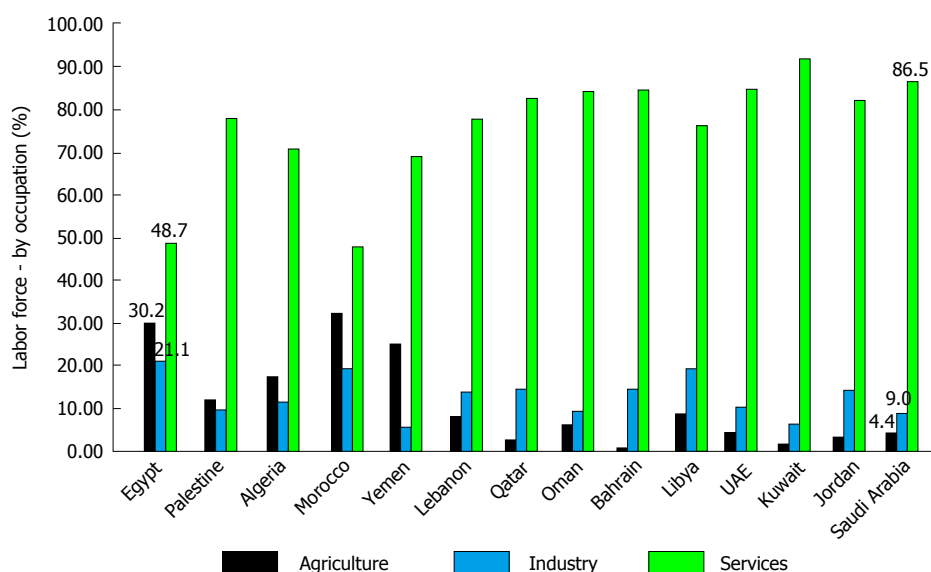


Figure 4 Middle East and North Africa countries labor force by occupation. Diversity of the workforce of Middle East and North Africa countries arranged horizontally from lowest diabetes prevalence (left) to highest diabetes prevalence (right). Data from Arab Labor Organization, but data collected from each country varies from 2006 -2008 (Egypt, 2008; Saudi Arabia, 2007). UAE: United Arab Emirates.

Tunis, with the region's lowest prevalence of physical inactivity, benefited the least with only 0.6 years gain of life expectancy. Iran, second in terms of lowest prevalence of physical inactivity in the region, only gained 0.7 years of life expectancy. Other studies have confirmed the magnitude of health benefits of physical activity with respect to the prevalence and complications of diabetes on the MENA region population. One large study, with an average 3.2 years follow up, has reported that diabetes can be prevented solely by lifestyle changes even in high risk subjects^[32]. Currently we cannot identify which labor force sector contributed significantly to the increasing diabetes prevalence in the MENA region due to lack of epidemiological studies. However, a study identified the highest prevalence of diabetes was in the service sector above all sectors in the sub-Saharan country of Namibia, also a developing country^[33].

Serious consideration need to be taken to diversify the labor force in countries heavily affected by the diabetes epidemic. This will not only maintain economic growth and provide jobs but may also induce a physically active lifestyle that will eventually enhance the longevity of the adult population and decrease morbidity, mortality, and health care expenditure.

Direct healthcare cost of diabetes

Diabetes presents a severe economic burden as patients with diabetes require at least 2-3 times the health care resources compared to people who do not have diabetes^[34]. The direct diabetes healthcare expenditure varies across the MENA region due to economic inequalities. In the United Arab Emirates (UAE) treatment costs are USD 1605 per person^[35] vs USD 175^[36] in Sudan. The economic disparity and the different prevalence of diabetes between Egypt and Saudi Arabia

affects their healthcare expenditure for diabetes. Based on a large population study on global health care in diabetes^[37], Saudi Arabia's healthcare expenditure for diabetes per person has reached USD 682, which is 21% of total healthcare expenditure. On the other hand, Egypt's healthcare expenditure for diabetes has reached only USD 116 per person which is 16% of total healthcare expenditure. It is difficult to assess the exact economic burden of diabetes in each individual country across the region because not a single country has optimally invested in accordance to the magnitude of its diabetic epidemic. Further investigations needs to be conducted to identify risk factors within the region that propagate the diabetes epidemic and optimal healthcare strategies and investments tailored specifically to each country.

MENA region underinvests in diabetes related healthcare expenditure

When comparing OECD and GCC countries, there is a large gap in healthcare expenditure even though some GCC states surpass OECD's GDP per capita. High incomes states such as, Kuwait, the UAE, and Qatar's per capita health expenditures were USD 1500, 1640, and 1776, respectively, compared to an average of USD 4593 in OECD countries^[38].

This underinvestment in health care is likely the responsible for diabetes being the fifth leading cause of death in 2010 in the MENA region compared with 11th place in the 1990s^[39]. Epidemiological studies have noted that in high income countries, such as Oman, only 2% of the diabetes population surveyed had their blood glucose levels controlled^[40]. Such studies indicate the dire need for MENA countries, especially the GCC states, to invest in primary healthcare, outreach programs, lifestyle coaching, and self-management

education to produce long term healthy gains. This will not only enhance the longevity and quality of life of patients with diabetes but also ultimately reduce the economic burden of healthcare expenditure.

Improvement in life expectancy across MENA region despite increase in noncommunicable diseases

Economic development across the region has dramatically improved the overall life expectancy of many MENA countries. According to WHO, Lebanon, with the region's highest life expectancy at 80 years in 2012, has sharply risen from 67 years in 1990. Lebanon falls below the MENA region's average prevalence of diabetes (11.9% vs 12.4%) and physical inactivity (46.8% vs 51.1%) contributing to the high life expectancy^[11]. Even countries with the lowest life expectancy in the region have dramatically improved. For example, Yemen had a life expectancy of 58 years in 1990 but has risen to 64 years in 2012^[41]. When comparing GCC countries with lower middle income countries in the MENA region, there is also a difference in the life expectancy based on differences in overall economic development. Egypt's life expectancy is lower than Saudi Arabia, 71 compared to 76 in 2012, despite the higher prevalence of diabetes and physical inactivity. However, Egypt has an overall higher noncommunicable disease death rate than Saudi Arabia^[42].

Increased work hours does not increase risk of developing diabetes

A study was conducted on 40861 employees from four large companies in Japan to determine the effect of long work hours on the prevalence of diabetes^[43]. It was hypothesized that longer work hours would predispose employees to diabetes. Surprisingly, there was a decreasing trend of diabetes prevalence with increasing hours of overtime of up to 100 h/mo. In the participating companies, however, occupational physicians recommended employers to shorten working hours for patients with advanced disease, including diabetes. Exclusion of workers taking medication for diabetes did not appreciably change the results. Of the measured risk factors, short sleep duration and leisure time physical inactivity were associated with long working hours, but BMI was not. The physical demand of the job description was not considered in the study. Whether shorter periods of sleep and longer working hours (implying a more vigorous lifestyle) would have a direct causality in prevention of diabetes mellitus is yet to be determined. Taking these studies in account it is important not only to diversify the labor force in MENA region but to consider prolonging work hours for possible additional health benefits.

Dietary habits and prevalence of diabetes

Egypt is one of the few countries in the MENA region that has strictly adhered to the Mediterranean diet^[44] which consists primarily of raw vegetables, fruits, moderate amount of fat, and low amounts of meat^[45].

It has been suggested that the Mediterranean diet has numerous health benefits including diabetes prevention and management^[46,47]. The Egyptian population has a low alcohol consumption of 0.5% which will further decline to 0.3% by 2015^[48]. Low alcohol consumption is not specific to Egypt but is characteristic of the MENA region. This eliminates the risk of alcohol attributable and nonattributable diseases such as diabetes, cancers, and liver cirrhosis. However, infection with hepatitis B and C viruses (HCV) continues to be a risk factor^[49]. As the prevalence of diabetes is increased in cirrhosis due to HCV infection (compared to cirrhosis to other etiologies), this may be conducive to the development of type 2 diabetes *via* insulin resistance^[50]. The prevalence of HCV in Egypt is ten-fold higher than that in other countries^[51]. The contribution of HCV infections in Egypt on the prevalence of diabetes is not precisely known and remains to be investigated.

In contrast, the Saudi population, with its desert environment, infrequently consume vegetables and fruits and has been reliant on meat and heavy carbohydrates, primarily dates, as its main diet. When taken in the context of the country's current westernization, this unbalanced diet has progressively worsened to include high carbonated drink such as soda and processed food. Whether the dietary contrast of Saudi Arabia to Egypt may have direct causality to the large difference of diabetes prevalence between the two countries remains unknown but is an interesting speculation.

CONCLUSION

Diabetes inflicts unacceptable high human, social and economic costs on MENA region countries at all income levels. Increased economic development and the subsequent adaptation of a Western lifestyle and the emergence of low physically demanding job sectors on the expense of manual labor may have causal effects on the increased diabetes prevalence in the population independent of other lifestyle habits. Diabetes is more than a health issue and serious consideration need to be taken to initiate diversity in the labor force in the MENA region that might require change in current economic policies. This strategy would produce direct health benefits with lower healthcare expenditure and improved quality of life on the overall population.

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Current knowledge on diabetic retinopathy from human donor tissues

Jessica H Eisma, Jennifer E Dulle, Patrice E Fort

Jessica H Eisma, Jennifer E Dulle, Patrice E Fort, Departments of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, MI 48105, United States
Author contributions: Eisma JH wrote the first draft of the paper; Dulle JE and Fort PE edited, corrected and formatted the manuscript.

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Correspondence to: Patrice E Fort, PhD, Departments of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, 1000 Wall Street, Ann Arbor, MI 48105, United States. patricef@umich.edu

Telephone: +1-734-2328225

Fax: +1-734-2328030

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complications including kidney failure, heart attacks, and retinal degeneration. In order to better understand the molecular basis of this disease and its complications, animal models have been the primary approach used to investigate the effects of diabetes on various tissues or cell types of the body, including the retina. However, inherent to these animal models are critical limitations that make the insight gained from these models challenging to apply to the human pathology. These difficulties in translating the knowledge obtained from animal studies have led a growing number of research groups to explore the diabetes complications, especially diabetic retinopathy, on tissues from human donors. This review summarizes the data collected from diabetic patients at various stages of diabetic retinopathy and classifies the data based upon their relevance to the main aspects of diabetic retinopathy: retinal vasculature dysfunction, inflammation, and neurodegeneration. This review discusses the importance of those studies to discriminate and establish the relevance of the findings obtained from animal models but also the limitations of such approaches.

Key words: Retina; Diabetic retinopathy; Human donor; Physiopathology; Vascular disease; Inflammation; Neurodegeneration

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Abstract

According to the American Diabetes Association, diabetes was the seventh leading cause of death, and diabetic retinopathy the leading cause of blindness in working age adults in the United States in 2010. Diabetes is characterized by hyperglycemia associated with either hypoinsulinemia or insulin resistance, and over time, this chronic metabolic condition may lead to various

Core tip: This review summarizes the current state of the knowledge on the physiopathology of diabetic retinopathy directly obtained from the analysis of tissues from human patients strongly complementing what has been gathered from animal models. The review discusses the vascular, inflammatory and neurodegenerative aspects of the disease, their inter-relation and the advantages and limits of such studies compared to the ones using animal models. Altogether, these analyses clearly demonstrate the complexity of the disease mechanisms but also the somewhat

still limited knowledge and the need for additional complementary studies.

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INTRODUCTION

Diabetic retinopathy (DR) is one of the most prevalent complications of diabetes, and while it is now well recognized that this disease involves perturbations of all the components of the retinal tissue, its diagnosis still mostly relies on the detection of damages associated with the vascular network. Based on those clinical features, diabetic retinopathy has been subdivided into two main stages: non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR). In the primary stages of NPDR, microaneurysms, or the swelling of small blood vessels, are the first detectable clinical signs of DR, and are associated with hypoperfusion of discrete regions of the retina. While the clinical gradation is still made based on vascular pathology, recent studies have shown that ganglion cell sensitivity as well as dark adaptation is altered in patients with NPDR, thus confirming early alteration of neuronal function^[1] and raising questions regarding the relative causality of those phenotypes. The progression of swelling can also be associated with leakage of fluid into the eye and increased inflammation, which can ultimately lead to swelling of the macula, the most sight-threatening stage of NPDR called diabetic macular edema (DME). While not detectable on fundus photographs, several studies showed that DME was also characterized by dramatic loss of neuronal function and increased inflammation, confirming the progression of the non-vascular alterations at this stage of the disease^[2,3]. In some cases, it is believed that progression of NPDR yields a severe ischemic state in specific areas of the retina, causing production of various factors leading to growth of new blood vessels in a process called neovascularization, marking the transition to PDR^[4,5]. These new vessels grow in a less controlled manner and disturb vision by developing in normally avascular regions, such as the vitreous cavity, often leading to hemorrhage, or the macula, leading to dramatic and persistent vision loss. Lastly, scarring or gliosis associated with retinal neovascularization can cause traction between the vitreous and the retina, which can ultimately lead to detachment of the neural retina from the retinal pigment epithelium, a phenomenon called tractional detachment. When taking place in the macula, this detachment is a major cause of severe vision loss in DR patients.

While the diagnosis and associated grading of DR has been more clearly defined over the years, the molecular mechanisms responsible for the different stages of this disease have been more difficult to characterize. Various studies have been conducted using animal models of diabetes to explore the molecular mechanisms of retinal vascular changes and neurodegeneration central to this disease. In contrast, and despite the limitations of the studies based on animal models, only a small number of studies have been conducted focusing specifically on the human pathology. This review will summarize what is presently known about DR from studies using human donor tissues. We will describe how the use of post-mortem tissues has allowed investigation of the vascular, inflammatory, and neuronal aspects of the disease and how it compares to data collected from animal models. Lastly, this review will briefly discuss how this approach enables a better understanding of the pathological mechanisms associated with the individual stages of DR and will explore both the advantages and limitations of using human donor tissues.

VASCULAR ALTERATIONS OF DR

In order to comprehend how changes in the vascular integrity can lead to DR, it is important to understand the basic properties and function of the neuroretina and its vasculature. Retinal capillaries are composed of endothelial cells located on the basement membrane, which is surrounded by pericytes. Connecting neighboring endothelial cells are tight, gap, and adherens junctions, which are responsible for maintaining the integrity of the blood-retinal barrier (BRB). The BRB tightly regulates the permeability of those vessels and is responsible for allowing nutrients and certain elements to reach neuroretinal cells. Blood flow is in turn mostly controlled by the surrounding pericytes through the regulation of the diameter of the vessels. Chronic conditions, such as diabetes, can lead to the disruption of the retinal vasculature over time, leading to the blockage of vessels, leakage of capillaries, and other vascular complications, ultimately resulting in conditions like DR.

Retinal microvascular cell death

Progressive retinal microvessel obliteration marks one of the most significant effects of DR in its early stages, resulting in increased vascular permeability as demonstrated clinically on retinal angiograms. The association between DR and pericyte or endothelial cell death was first described by Mizutani *et al.*^[6] using trypsin digest preparations and retinal cross-sections from diabetic patients and age-matched non-diabetic controls^[6]. Combining TUNEL staining on retinal cross-sections and histological analysis of trypsin digests, the authors first demonstrated an increased number of dying cells in the vascular network of diabetic patients. Morphological analysis coupled with cell-specific immunostaining

demonstrated that both pericytes and endothelial cells were dying in tissues from diabetic patients. To control for structural and functional changes primarily due to aging, rather than diabetes, or even simply post-mortem artifacts, the authors reproduced those findings in two rodent models. The authors reproduced those findings in two rodent models, the alloxan-induced diabetic and galactose-fed rats. This study was one of the first demonstrating the death of endothelial cells and pericytes in retinal tissue from human diabetic patients, and that this phenomenon occurred during the early stages of DR, even before clinical signs could be detected. Subsequently, several studies confirmed those results in independent sets of postmortem human tissues from diabetic patients^[7,8]. Detection of a decreased pericyte/endothelial cell ratio was also found, suggesting a higher sensitivity of pericytes compared to endothelial cells that could be associated with their lower capacity to replicate^[7].

Neo-angiogenesis

Endothelial and pericyte cell death and the subsequent retinal microvessel degeneration results, among other things, in ischemic foci, which, by way of unmet metabolic needs, can ultimately lead to the initiation of angiogenesis. Previous studies investigating the molecular mechanisms of vascular growth and permeability in DR have identified some of the growth factors involved in angiogenesis in response to diabetes^[9]. Non-human primate models involving retinal hypoxia were among the first in which increased mRNA levels of vascular endothelial growth factors (VEGFs) were detected in retina and intraocular fluids after vaso-obliteration^[10,11]. Soon after, VEGF was reported to be almost exclusively detected in the intraretinal and choroidal vasculature of diabetic patients^[12]. To acquire further insights specific to human vasculature, Mathews *et al.*^[13] analyzed the retinal vasculature of human post-mortem eyes to determine if a correlation existed between VEGF-positive vessels and vascular permeability. Supporting this hypothesis, the authors observed an increase in the number of VEGF-positive vessels in diabetic eyes compared to non-diabetic eyes. Furthermore, the greatest concentration of VEGF-positive vessels were found in the central retina of diabetic eyes, consistent with prior observations that angiogenesis occurs mainly in this region^[14]. Conversely, non-diabetic eyes displayed higher numbers of VEGF-positive vessels within the peripheral retina, results reported in previous studies and likely attributed to degeneration of the eye caused by age or other vascular diseases^[15]. Additionally, the authors reported that vascular permeability, assessed by human serum albumin levels, was far greater in diabetic eyes than in non-diabetic eyes. Upon further statistical analysis, the levels of VEGF and human serum albumin in diabetic eyes were directly correlated, whereas no pattern existed in non-diabetic eyes. This represented some of the first evidence that increased permeability of diabetic retinal

vessels correlated with increased VEGF levels, even prior to the detection of clinical signs of PDR^[13].

While VEGF is a central player in PDR and DME, some evidence points to other factors being involved, including the cytokines tumor necrosis factor (TNF)- α and transforming growth factor (TGF)- β or other growth factors such as platelet-derived growth factor^[16]. Analysis of vitreous fluid from diabetic patients with PDR showed increased levels of TNF- α ^[17] which could be part of the pathological mechanism of DR due to its known role in vascular and inflammatory regulation that will be expanded upon later in this review. As for TGF- β , it has previously been shown to have a critical role in the maintenance of the BRB and endothelial cell barrier function^[18]. However, one study reported that TGF- β was detected in the photoreceptor layer of both diabetic and non-diabetic human retinal tissue^[19], and thus, its exact role in DR remains to be clarified.

Angiogenesis relies not only on different growth factors such as VEGF, TGF- β and TNF- α , but also cell adhesion molecules. The role of cell adhesion molecules is to connect the cell to the external environment as well as neighboring cells by binding proteins that make up the extracellular matrix (ECM) *via* membrane-associated proteins on the cell surface. One class of cell adhesion molecules, integrins, is critical for cell-cell and cell-ECM interaction, thus significantly influencing cellular responses and physiology. Interaction of integrins with the ECM has been shown to influence angiogenesis through regulation of intracellular signaling that affects replication and differentiation of endothelial cells and pericytes. A study conducted by Friedlander *et al.*^[20] in animal models of angiogenesis suggested that since $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins were involved in angiogenesis, these specific integrins could be critical in PDR. Ning *et al.*^[21] utilized this data to expand upon and determine the co-localization of five different integrins with the retinal vascular endothelium of four patients with PDR. Contrary to what was found in the animal models, no staining was observed for $\alpha_v\beta_5$ integrins, possibly attributed to inter-species variability. As for the other integrins tested, $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins were detected in all tissues, at moderate level and without co-localization with endothelial cells. Only $\alpha_v\beta_3$ and β_3 integrin proteins were found to be moderately induced and specifically co-localized with endothelial cell markers in two of the four patients with PDR tested. While further validation is required, these data suggest a specific impact of PDR on a subset of integrins, which could reflect their role in pathological angiogenesis^[21].

Perturbations of the retinal vasculature associated with diabetes have long been known from animal models, but studies using tissues from human donors continue to be key to our understanding of the molecular mechanisms underlying the vascular changes associated with the different stages of DR. These studies highlight the complexity of those mechanisms and the interconnection of the vascular pathology with other aspects of the disease

such as the inflammation and neurodegeneration.

INFLAMMATION ASSOCIATED WITH DR

Astrocytes and Müller glial cells, the main macroglial cells of the retina, represent another contributing factor to the diabetes-associated pathology of the neuroretina. In their position between the vasculature and retinal neurons, glial cells play important roles in retinal physiology including regulating permeability of the BRB, supporting neuronal cells, and sensing the extracellular environment, the latter being critical to their regulatory function of retinal inflammation during chronic diseases such as diabetes.

Glial cells dysfunction

Müller cells have long been thought to be affected by diabetes, mostly in their capacity as a support network for the rest of the retina. The first study to investigate the impact of PDR on Müller cells was conducted by Nork *et al.*^[22] almost 30 years ago, using four post-mortem human eyes. The authors reported the first evidence in human tissues from PDR patients of reactive gliosis demonstrated by the formation of intra-retinal bridges between cystic spaces; and Müller cell dysfunction as suggested by disorganization and migration of their nuclei^[22]. More recently, two independent studies sought evidence of abnormalities within Müller cells during the early stages of NPDR. Both studies confirmed reactive gliosis, as demonstrated by increased glial fibrillar acidic protein (GFAP) immunoreactivity in tissues from patients with no to mild NPDR^[23,24]. The two studies, however, reported different outcomes regarding the expression of apoptotic regulatory markers, which will be discussed later in this review. GFAP upregulation in diabetic eyes has been demonstrated in various animal models of diabetes and is classically recognized as an indication of cell and tissue reactivity to environmental stress^[25,26]. Collectively, these early studies not only confirmed that DR affects Müller cells, but that diabetic conditions promote increased glial cell activation. One of the main functions of glial cells is to regulate the communication between blood vessels and neurons to respond to changes in the environment and maintain retinal homeostasis. Previous studies have suggested that in the early stages of DR, the number of Müller cells increases as astrocyte population decreases, likely as a reaction to the increase in vascular permeability^[25,27]. The reported loss of astrocytes in response to diabetes could be enhancing the increased vascular permeability both through mechanical perturbations induced by their absence and loss of the important role they play in the induction of tight junctions within inner retinal blood vessels and the maintenance of the BRB^[28,29]. However, the astrocyte loss could also play a pivotal role in the increase in the number of Müller cells, and thus, the enhanced inflammatory response that occurs in the

retina in DR.

Inflammatory response

In their original work, Rungger-Brändle *et al.*^[25] showed that the number of microglial cells, the innate immune cells of the retina, was increased in diabetic rodents. As importantly, they showed that those cells became activated, a state characterized by morphological changes as well as an increase in cytokine production and secretion^[25]. While resting microglial cells have multiple, long processes to sense the surrounding environment, activated microglial cells become compact and respond to stress signals by producing various signaling molecules including pro-inflammatory cytokines such as TNF- α , proteases, and reactive oxygen species. There is increasing evidence that inflammation in general, and microglial cell activation in particular, may play an important role in the pathogenesis of DR. One of the premier findings that hinted to this was a study in 1964, when diabetic patients on a salicylate diet, a known anti-inflammatory drug, for treatment of rheumatoid arthritis, reported lower incidence of DR^[30]. Subsequent studies displayed similar results, wherein inflammation increased in retinas in response to diabetes; however, most of those have been done in animal models rather than human donor tissues. One such study used streptozotocin-induced diabetic rats to support previously reported findings of an increase in diabetes-associated pro-inflammatory cytokines. Interestingly, and for the first time, this study specifically reported the increase of chemokine (C-C motif) ligand 2 (CCL2) mRNA in the retina of diabetic rats^[31]. To this day, data from human retinas remain to be obtained to confirm these observations; however it is supported by human data that were collected by multiple groups from vitreous samples. Interleukin-1 β and TNF- α , two pro-inflammatory cytokines have been shown to be increased in vitreous samples from patients with DR, even more so in patients with PDR^[17]. Several studies also reported CCL2, also known as monocyte chemoattractant protein 1 (MCP1), and other cytokines to be elevated in the vitreous of patients with DR^[2,32-37]. Muramatsu *et al.*^[37] also reported that increased levels of cytokines such as MCP-1 correlated with increased VEGF and complement factors levels in the vitreous of PDR patients, while Funatsu *et al.*^[2] demonstrated an association of cytokine levels with DME, both providing evidence of a link between increased vascular permeability and the inflammatory response in PDR. Of note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, a known negative regulatory mechanism of cytokine signaling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye^[38].

In addition to these inflammatory mediators, enhanced expression of intracellular adhesion molecule-1 (ICAM) and P-selectin has been linked to the progression of

DR^[39]. ICAM is believed to play a critical role in leukocyte adhesion, one of the initial steps of the inflammatory response allowing leukocytes to cross the BRB in response to increased stress signals. Increased expression of these molecules has been shown to promote the release of inflammatory cytokines, which interfere with endothelial cell tight junction integrity, and thus, increase vascular permeability^[40]. While not clearly established, this could be linked with the basement membrane thickening, known to be associated with increased permeability and changes in ECM content in DR patients^[41].

As suggested by the observation made by Powell *et al.*^[30] in 1964, for diabetes as for a wide range of chronic pathologies, a proper control of inflammation is critical to maintaining cellular and tissue homeostasis. While inflammation is a protective mechanism of most complex organisms in response to injury or infectious disease, an uncontrolled inflammatory response becomes part of the pathological mechanism in a variety of chronic diseases such as multiple sclerosis and diabetes. In a study conducted by Krady *et al.*^[42], the anti-inflammatory drug, minocycline, was suggested to have therapeutic potential in treating/preventing the progression of DR. The authors showed that minocycline treatment leads to a decrease in diabetes-induced cytokine production and reduces microglial cell activation in a rodent model of diabetes. These results were the first to suggest that regulating the inflammatory response could be an important strategy for DR treatment, and that minocycline may be a viable drug to prevent the advancement of DR^[42]. More recently, a proof of concept study reported that minocycline oral administration had been associated with improved visual function and regression of central macular edema and vascular leakage in diabetic patients^[43] providing further evidence that regulating the inflammatory response can be beneficial to preventing irreversible vascular and neuronal degradation over time.

Overall, the data collected from tissues from human donors along with the data from animal models of diabetes strongly support a role of inflammation in the progression of DR. Seminal work has now been performed that suggests that anti-inflammatory drugs could represent a key component of future therapies for the treatment of DR in order to protect retinal function from the adverse effects of diabetes-associated conditions.

NEURODEGENERATION IN DR

While DR has generally been considered a micro-vascular complication of diabetes, degeneration of the neuroretina has been known for over 50 years. The primary histological analysis of retinal tissue from patients with DR resulted in reports characterizing the loss of cell bodies in every nuclear layers of the retina suggesting a significant loss of retinal neurons in response to diabetes^[44,45].

Neuronal cell death

Ganglion cell atrophy and inner nuclear layer (INL) degeneration in retinal tissues from diabetic patients was first described in 1961 and was suggested to happen even prior to vascular changes^[46,47]. Soon after, the degeneration of the INL and ganglion cell layers was confirmed in a study using 295 postmortem human eyes and documented the fragmentation of ganglion cell nuclei, which is a characteristic of cells undergoing apoptosis^[45]. Following these seminal reports, numerous studies only focused on understanding the vascular aspect of the disease, and additional investigation regarding the neurodegenerative mechanisms only occurred recently. The nature of neuronal cell dysfunction and death associated with diabetes began to be better characterized in a study by Barber *et al.*^[48] when they reported reduced thickness of the retinal inner nuclear and plexiform layers in 7.5 mo diabetic rats. Retinal ganglion cell (RGC) survival was also decreased by 10% compared to non-diabetic rats. The authors discovered that apoptosis began soon after the induction of diabetes and that the population of cells undergoing apoptosis potentially included ganglion and photoreceptor neuronal cells^[48]. More recent findings using rodent models of diabetes have confirmed the diabetes-associated alteration of RGC function and survival (reviewed in^[49]). These findings are interesting with regards to reports analyzing flat-mounts and cross-sections of human retinas by immuno-based assays. Consistent with the first report of apoptotic cells in retinas from diabetic human donors distinct from vascular lesions^[48], expression of the pro-apoptotic protein Bax was shown to be increased in RGCs of diabetic patients^[50]. This study supported the previous findings wherein the progression of diabetes paralleled increased levels of the pro-apoptotic protein Bax in diabetic human retinas, specifically in the inner retina and concentrated in ganglion cells^[51]. A subsequent study revealed an increase in levels of Bax, cleaved caspase-3, and caspase-9 in RGCs of diabetic patients supporting the hypothesis that RGCs specifically are undergoing apoptosis during diabetes and providing evidence of neurodegeneration during the early stages of DR^[52]. This activation of the apoptotic pathway could be associated with a loss of trophic factors. While the exact impact of diabetes on local insulin signaling in the human retina remains to be fully characterized, rodent models strongly suggest that it is affected as demonstrated by significant reduction of kinase activity of the whole signaling cascade - insulin receptor (IR), insulin receptor substrate 2, phosphoinositide 3-kinase, Akt, and mechanistic target of rapamycin^[53,54]. While this could be directly due to loss of insulin signaling, it could also be a result of loss of activation by insulin-like growth factors (IGF). Retinal IR is known to be a spliced variant that is equally susceptible to insulin and IGFs, and IGF1 levels of expression was shown to be decreased in retina from rodent models and human diabetic patients^[51]. These

data support a diabetes-associated decrease of trophic factors and subsequent molecular signaling that could lead to retinal cell death.

Apoptosis regulation

In addition to their role in the regulation of inflammation, glial cells and more specifically Müller cells, are involved in supporting neuronal cells, including the regulation of their survival. Regulation of the intrinsic apoptotic pathway is dependent upon expression of members of the anti-apoptotic Bcl-2 protein family. Two independent groups reported Bcl-2 expression in the human retina to be confined to Müller cells; however, one study by Mizutani *et al.*^[23] reported no change in the expression of the anti-apoptotic protein while the second study by Abu-El-Asrar *et al.*^[24] reported a small but significant induction of Bcl-2 in samples from diabetic patients compared to age-matched non-diabetic patients. The authors of the second study also reported changes in expression of other anti-apoptotic proteins, which suggests activation of survival pathways, but also increased expression of the cytotoxic effector Fas ligand, which reflects the ambiguous and complex role of Muller glial cells in the molecular mechanisms leading to neuronal cell death^[24].

Neurodegeneration is a cumulative process resulting from the complex interplay of several independent stress mechanisms, one of which is oxidative stress. In animal models of diabetes, various cellular stresses have been shown to alter the oxidative state of retinal cells and lead to accumulation of reactive oxygen species (ROS), promoting damages to the cell machinery and ultimately leading to increased cell death. Several studies have reported increased superoxide production, a known marker of oxidative stress, in diabetic rats^[55,56]. One of these reported further increased production of superoxide in hypertensive rats in response to diabetes. Additionally, this study showed that those animals presented with increased levels of gliosis and neuronal apoptosis suggesting a link between superoxide production and neurodegeneration in an animal model of diabetes, especially under hypertensive conditions^[56]. Despite the growing number of reports suggesting a direct role for oxidative stress in promoting retinal cell death in animal models of diabetes, evidence of such a link between increased oxidative stress and neurodegeneration associated with DR has not been directly shown in human tissues. Indeed, the only data collected so far and supporting this hypothesis come from indirect measures of oxidative stress *via* analysis of the levels of ROS in the retinas of diabetic human donors^[57,58]. It is interesting to note that an anterior study using retina samples from twelve human donors with varying durations of diabetes, showed that, within the retina, rod photoreceptors are the most vulnerable to oxidative stress, likely attributable to the high concentration of polyunsaturated fatty acids within their

membranes^[59]. High lipid concentration, specifically low-density lipoprotein, was shown to be associated with the progression of DR, and recent studies theorize that the retina is highly susceptible to oxidative stress due to its composition of polyunsaturated fatty acids and high oxygen usage^[60,61].

Altogether these data strongly support that retinal neurons are highly affected by diabetes and that neurodegeneration is a key aspect of diabetic retinopathy. While it suggests that oxidative stress and loss of trophic factors could play an important role in the induction of apoptosis, it also clearly shows how little we still know about this aspect of the disease and the need for additional studies.

ADVANTAGES AND LIMITATIONS OF POST-MORTEM TISSUES

Advantages

The primary advantage to using human donor samples is that the data collected are directly representative of the disease pathology as opposed to mechanisms uncovered in artificially-induced or genetically-modified animal models of the disease. In addition, the retinas from the majority of the animal models of diabetes do not have the same structural and cellular properties as the human retina. For example, rabbit and guinea pig models are more similar to human in regards to the type and repartition of photoreceptors, but lack an intraretinal vascular network, as opposed to rodent models that possess this vascular network but have a very different rod/cone repartition and properties. Additionally, neither of those models has a macula and thus does not allow investigation of the specific impact of diabetes on this central and key region of the human retina. Another reason of the limited success in translating data obtained from animal models is the heterogeneous nature of the human pathology^[62]. In addition to the human-specific variable environmental conditions, none of the animal models routinely used recapitulate the anatomical and regional specificity of the human retina and how it is impacted by diabetes; *i.e.*, peripheral vascular hypoperfusion, non-homogenous visual field impairment, local hemorrhage and lipid exudates, and macular edema.

Limitations

However, it is also important to note the limitations when working with post-mortem human tissues. The first and main concern is the difficulty in promptly processing fresh tissues from human donors compared to animal models, where no consent is needed, and experiments can be planned ahead. Moreover, there is less control over inter-sample experimental variability due to the very diverse background and health history of human donors. In contrast, studies using animal models can be tightly controlled by using inbred strains that have identical

genetic background and are maintained in identical experimental conditions.

CONCLUSION

The goal of this review was to produce an overview of the current state of knowledge regarding the human specificity of the pathophysiology and molecular mechanisms of DR. This review summarizes what has been discovered regarding the impact of diabetes on the vascular, inflammatory, and neuronal components of human retinal tissue. Overall, this review demonstrated that while animal model-based studies can be utilized to address a variety of disease-related questions, studies using human donor tissues are necessary to validate the conclusions from animal models, as well as characterize different molecular mechanisms associated with the individual stages of DR pathology. It also demonstrates the importance for continuous evaluation of the various disease models to assess their efficacy and limitations for investigating specific pathological mechanisms. Finally, this review underscores the gaps in our knowledge concerning even the basic mechanisms regulating vascular alteration, retinal cell survival, and the interplay of various components of the retina in response to diabetes that underline the progression of DR.

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Hepatic glycogenosis: An underdiagnosed complication of diabetes mellitus?

María Teresa Julián, Núria Alonso, Isabel Ojanguren, Eduarda Pizarro, Enric Ballestar, Manel Puig-Domingo

María Teresa Julián, Núria Alonso, Manel Puig-Domingo, Department of Medicine, Universitat Autònoma de Barcelona, 08035 Barcelona, Spain

María Teresa Julián, Eduarda Pizarro, Enric Ballestar, Department of Endocrinology, Hospital de Mataró, 08034 Mataró, Barcelona, Spain

Núria Alonso, Manel Puig-Domingo, Department of Endocrinology, Hospital Germans Trias i Pujol, 08916 Badalona, Barcelona, Spain

Isabel Ojanguren, Department of Pathology, Hospital Germans Trias i Pujol, 08916 Badalona, Barcelona, Spain

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Correspondence to: María Teresa Julián, MD, Department of Endocrinology, Hospital de Mataró, Carrer Prolongació Cirera s/n, 08034 Mataró, Barcelona, Spain. mjulian@csdm.cat

Telephone: +34-93-7417700

Fax: +34-93-741733

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glycogen accumulation in hepatocytes and represents a hepatic complication of diabetes that particularly occurs in patients with longstanding poorly controlled type 1 diabetes (T1D). HG has been reported to be a very rare disease, although it is believed to be extremely underdiagnosed because it is not possible to distinguish it from non-alcoholic fatty liver disease (NAFLD) unless a liver biopsy is performed. In contrast to HG, NAFLD is characterized by liver fat accumulation and is the more likely diagnosis for patients with type 2 diabetes and metabolic syndrome. The pathogenesis of HG involves the concomitant presence of insulin and excess glucose, which increases glycogen storage in the liver. HG is characterized by a transient elevation in liver transaminases and hepatomegaly. Differentiating between these two conditions is of the utmost importance because HG is a benign disease that is potentially reversible by improving glycemic control, whereas NAFLD can progress to cirrhosis. Therefore, HG should be suspected when liver dysfunction occurs in patients with poorly controlled T1D. The aim of this article is to review the epidemiology, clinical characteristics, pathogenesis and histology of HG.

Key words: Hepatic complications; Diabetes mellitus; Type 1 diabetes; Hepatic glycogenosis; Non-alcoholic fatty liver disease

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Core tip: Hepatic glycogenosis (HG) is a complication of diabetes mellitus that is often underdiagnosed. It is defined as pathological glycogen storage in hepatocytes with hepatomegaly and elevated liver enzymes and mainly occurs in patients with longstanding poorly controlled type 1 diabetes. HG cannot easily be distinguished from non-alcoholic fatty liver disease (NAFLD) by history, physical examination or ultrasound; only liver biopsy can provide a definitive diagnosis. The hallmark of this condition is its reversibility with improved glycemic control; in contrast, NAFLD can progress to fibrosis.

Abstract

Hepatic glycogenosis (HG) is characterized by excessive

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INTRODUCTION

Diabetes mellitus (DM) is associated with various structural and functional liver abnormalities, including non-alcoholic fatty liver disease (NAFLD) and hepatic glycogenosis (HG). NAFLD represents the most common liver disease associated with DM, especially in patients with type 2 diabetes (T2D) and metabolic syndrome^[1]. HG involves pathological glycogen storage in hepatocytes and is associated with poorly controlled DM, particularly type 1 diabetes (T1D). This condition is believed to be extremely underdiagnosed because it is indistinguishable from NAFLD in the absence of a liver biopsy. However, the distinction between these two diabetes-related complications is important: whereas NAFLD may progress to fibrosis and cirrhosis, HG is potentially reversible with sustained glycemic control^[1,2]. This review aims to provide an overview of the clinical characteristics and pathological features of HG to improve recognition of this diabetes-related complication.

DEFINITION AND EPIDEMIOLOGY

HG is defined as pathological excessive glycogen accumulation in hepatocytes and is characterized by hepatomegaly and a transient elevation in liver transaminases^[2]. Glycogen accumulation in the liver was first described in children by Mauriac^[3] in 1930 as a component of Mauriac's syndrome. He observed glycogen accumulation in a child with T1D and poor diabetic control that was associated with hepatomegaly and abnormal liver enzymes as well as other features such as growth retardation and/or dwarfism, delayed puberty, cushingoid features and hypercholesterolemia. Currently, HG is a well-recognized disease that occurs at any age and can be present without the full spectrum of features described for Mauriac's syndrome. Although numerous case reports and several series have been published^[2,4-24], the exact prevalence of HG is unknown, but it is considered to be the primary cause of hepatomegaly in children and adolescents with T1D^[24]. This condition has been given many labels, such as hepatic or liver glycogenosis^[4,11,12,15,16], glycogenic hepatopathy^[2,5,17,19,22,23], liver glycogen storage^[6,13], and DM-associated glycogen storage hepatomegaly^[10].

HG was first described in association with acute ketoacidosis or recurrent hypoglycemia^[7-10,15] in cases presenting with excessive insulin and/or elevated glucose. However, hepatic glycogen accumulation also develops in diabetic patients with long-term

poor control and several hospitalizations for diabetic ketoacidosis^[4,5,11,16-21]. Although HG is more common in patients with T1D, it has also been described in insulin-dependent type 2 diabetic patients during ketosis or poor diabetic control requiring increasing amounts of insulin^[25]. In addition, HG has also been reported in other clinical settings, such as in three children after short-term, high-dose steroid therapy without insulin treatment^[26] and in a patient with dumping syndrome associated with gastrostomy^[27].

PATHOGENESIS

Although the underlying mechanisms by which HG develops have not yet been fully elucidated, wide fluctuations in glucose and insulin concentrations seem to be essential for its pathogenesis^[28]. Blood glucose and insulin levels often fluctuate in diabetic patients with poor metabolic control, thereby promoting hepatic glycogen accumulation. High plasma glucose levels cause an insulin-independent glucose influx into hepatocytes by facilitated diffusion. In the cytoplasm of hepatocytes, glucose is irreversibly converted into glucose-6-phosphate by glucokinase, an enzyme regulated by glucose and insulin. Then, glucose-6-phosphate is converted into glycogen by glycogen synthase, which is converted from the inactive form into the activated form by a phosphatase. This phosphatase plays a key role in regulating this step in the pathway: its concentration is maintained by insulin, and its activity depends on the presence of glucose (Figure 1). Therefore, the synthesis of hepatic glycogen is the consequence of the combination of high blood glucose levels (which promote the flow of glucose into hepatocytes) and hyperinsulinemia (which stimulates the conversion of glucose to glycogen)^[2,5,11,12]. This situation is frequently observed in patients with unstable diabetes who are treated with insulin for marked or prolonged hyperglycemia.

CLINICAL PRESENTATION

The clinical presentation is not specific and can include abdominal pain that is sometimes associated with nausea, vomiting and anorexia^[2,11,12]. The key clinical features are hepatomegaly and a mild to moderate increase in transaminases, although in some cases, the transaminase levels can be dramatically elevated^[2,5]. Alkaline phosphatase levels can be elevated, and liver synthetic function is usually normal. Ascites has rarely been reported^[2,11]. The clinical and pathological features are similar in adults and children.

Torbenson *et al.*^[2] reported 14 patients (range, 8 to 25 years old) with HG. All had T1D with poor glycemic control. The clinical presentations included hepatomegaly, abdominal pain and elevated transaminases. Ascites was present in 1 patient. In 3 cases, the transaminase levels were markedly elevated to 10 times greater than the upper limit of normal. All the biopsies revealed

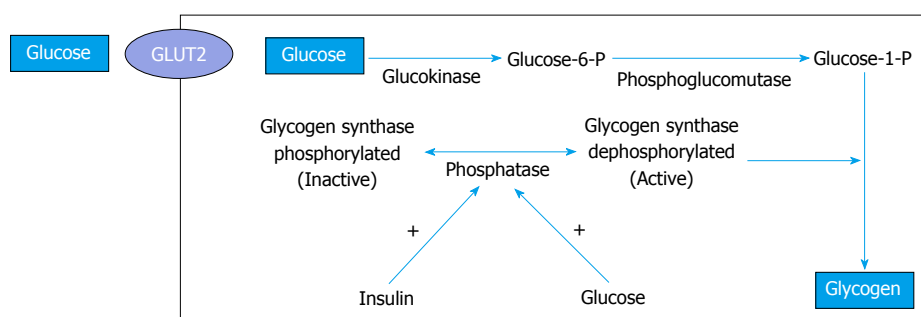


Figure 1 Pathogenesis of hepatic glycogenosis. Glucose from the blood enters hepatocytes by facilitated diffusion independent of insulin and is converted into glycogen via glucose-6-phosphate. Glycogen synthesis depends on insulin and glucose (modified from Munns *et al*^[12]). GLUT2: Glucose transporter 2.

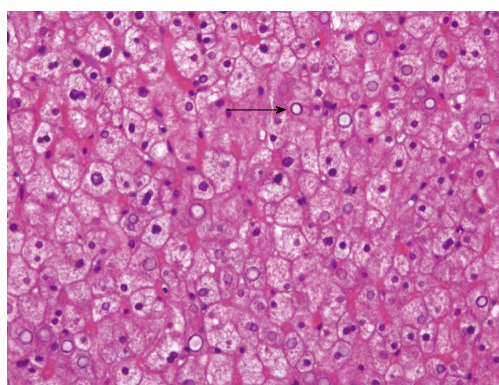


Figure 2 Liver biopsy, haematoxylin and eosin staining. The hepatocytes are swollen with pale cytoplasm and accentuation of the cell membranes. Sinusoids appear compressed by the swollen hepatocytes. Glycogen nuclei are present (black arrow).

excessive glycogen accumulation. Another large cohort was reported that included 11 patients (8 adults between 19 and 70 years of age and 3 children) with poorly controlled insulin-dependent diabetes (T1D or T2D was not specified)^[11]. Nine patients (6 adults and 3 children) had hepatomegaly as evidenced by physical examination or ultrasonography. Ascites was present in 1 patient, and serum transaminases were markedly elevated in 4 patients.

DIAGNOSIS AND HISTOLOGY FINDINGS

HG cannot be distinguished from NAFLD by history, physical examination or laboratory blood tests. In addition, ultrasound cannot distinguish fatty liver from glycogen accumulation^[2,12]. The usefulness of abdominal computed tomography (CT) in distinguishing HG from NAFLD was reported by Sweetser *et al*^[19]. A low density liver is usually observed by CT in patients with fatty liver, whereas the liver density by CT is typically increased in patients with HG. However, a low liver density by CT may be not observed in the early stages of NAFLD^[28], and CT only provides qualitative information. Recently, it has been reported that gradient dual-echo magnetic resonance imaging (MRI) can effectively discriminate glycogen from fat in the liver^[28,29]. A gradient dual-echo MRI sequence, as well as magnetic resonance

spectroscopy, can quantify the intrahepatic lipid content and detect even small amounts of fat accumulation^[30].

The differential diagnosis of HG, as opposed to NAFLD, must consider several other potential causes of liver damage, such as infection (*e.g.*, viral hepatitis), metabolic disorders (*e.g.*, α 1-antitrypsin deficiency and Wilson's disease), obstruction, autoimmune liver disease and celiac disease^[12]. On the other hand, there is an increasing evidence that focal, but sometimes also diffuse, HG is a potential preneoplastic lesion^[31-33]. Investigations in animal's models of chemical, viral and hormonal hepatocarcinogenesis and some observations in humans suggest that focal HG, represents a critical early event in the pathogenesis of benign and malignant hepatocellular neoplasm^[34,35]. Although the exact mechanism remains elusive, recent data suggest that oncogenic agents have an early insulin-like effect^[35-37]. It is noteworthy that a number of epidemiology studies have shown that DM is a risk factor for the development of hepatocellular carcinoma^[38,39]. However, no relationship has been described between diabetes-related HG and hepatocellular neoplasms, but further studies are warranted in order to clarify this point.

HG is only diagnosed by liver biopsy. In general, HG is characterized by several histological features: (1) marked glycogen accumulation. After conventional tissue preparation (fixation by formaldehyde-solution and staining with haematoxylin and eosin) the glycogen is usually removed from the hepatocytes. Thus, the hepatocytes are diffusely swollen with a pale cytoplasm and accentuation of the cell membranes, frequently with displacement of the nuclei to the cell periphery (Figure 2), the sinusoids are compressed by swollen hepatocytes, and glycogenated nuclei and giant mitochondria are present; glycogen accumulation within hepatocytes is demonstrated by periodic acid-Schiff staining (Figure 3A) which disappeared after digestion with diastase (Figure 3B); (2) no or a minimal change in fat content; (3) the absence of or minimal inflammation; (4) the absence or minimal presence of spotty lobular necrosis; and (5) intact liver architecture without or with minimal fibrosis^[2,5,11].

In contrast to NAFLD, which can progress to cirrhosis, HG is potentially reversible with optimal diabetes control. The abnormal transaminase levels and hepatomegaly

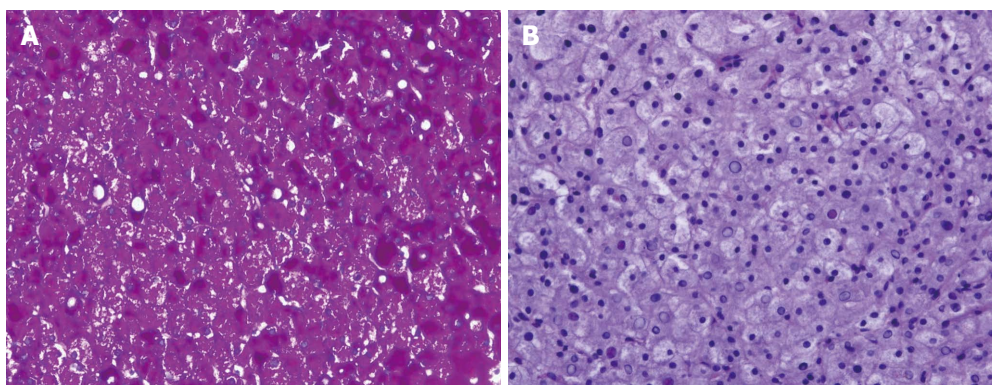


Figure 3 Liver biopsy, periodic acid-Schiff staining. A: Periodic acid-Schiff (PAS) demonstrates abundant cytoplasmic deposits. An intense reaction is also found in the nucleus; B: The hepatocyte cytoplasm is not stained with PAS after diastase treatment confirming the present of glycogen.

have been described to be reversible after improving glycemic control with insulin treatment, usually within 2 to 14 wk^[11,12,16]. In a large series published by Chatila *et al.*^[11], hepatomegaly was resolved in all the patients within 2 wk of stabilizing the blood sugar levels. Aminotransferases rapidly decreased, but remained moderately elevated in some patients during the 14-wk follow-up. In 2011, our group published a case of HG in a 31-year-old woman with poorly controlled T1D. During admission for acute ketoacidosis, she presented with hepatomegaly and markedly elevated transaminases. Liver glycogen storage was diagnosed by biopsy. After optimal glycemic control, transaminase levels rapidly decreased, but the hepatomegaly remained after 6 mo^[20]. In two previously published severe cases, pancreatic transplantation was reported to be effective^[40].

CONCLUSION

HG most likely represents an underdiagnosed hepatic complication of diabetes that is difficult to distinguish from NAFLD. For this reason, a diagnosis of HG should be considered in diabetic patients, especially in those with T1D, who exhibit poor metabolic control and present with a transient elevation of liver transaminases and hepatomegaly. Although HG is definitively diagnosed histologically, a gradient dual-echo magnetic resonance imaging sequence combined with CT of the liver is a powerful methodology for distinguishing HG from NAFLD. The correct diagnosis of this disease is important given its potential resolution after improved glycemic control.

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Role of oxidative stress in endothelial insulin resistance

Francesco Paneni, Sarah Costantino, Francesco Cosentino

Francesco Paneni, Sarah Costantino, Francesco Cosentino, Cardiology Unit, Department of Medicine, Karolinska University Hospital, 17176 Stockholm, Sweden

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Correspondence to: Francesco Paneni, MD, PhD, FESC, Cardiology Unit, Department of Medicine, Karolinska University Hospital, Solna, 17176 Stockholm, Sweden. francesco.paneni@ki.se
 Telephone: +46-8-51779413

Fax: +46-8-344964

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Abstract

The International Diabetes Federation estimates that 316 million people are currently affected by impaired glucose tolerance (IGT). Most importantly, recent forecasts anticipate a dramatic IGT increase with more than 470 million people affected by the year 2035. Impaired insulin sensitivity is major feature of obesity and diabetes and is strongly linked with adverse cardiometabolic phenotypes. However, the etiologic pathway linking impaired glucose tolerance and cardiovascular disease remains to be deciphered. Although insulin resistance has been attributed to inflammatory programs starting

in adipose tissue, emerging evidence indicates that endothelial dysfunction may represent the upstream event preceding peripheral impairment of insulin sensitivity. Indeed, suppression of reactive oxygen species-dependent pathways in the endothelium has shown to restore insulin delivery to peripheral organs by preserving nitric oxide (NO) availability. Here we describe emerging theories concerning endothelial insulin resistance, with particular emphasis on the role of oxidative stress. Complex molecular circuits including endothelial nitric oxide synthase, prostacyclin synthase, mitochondrial adaptor p66^{Shc}, nicotinamide adenine dinucleotide phosphate-oxidase and nuclear factor kappa-B are discussed. Moreover, the review provides insights on the effectiveness of available compounds (*i.e.*, ruboxistaurin, sildenafil, endothelin receptor antagonists, NO donors) in restoring endothelial insulin signalling. Taken together, these aspects may significantly contribute to design novel therapeutic approaches to restore glucose homeostasis in patients with obesity and diabetes.

Key words: Endothelium; Insulin resistance; Oxidative stress; Obesity; Cardiometabolic risk; Vascular disease

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Core tip: We present here the most recent advances in the understanding of endothelial insulin resistance, with a particular focus on the role of oxidative stress. The molecular pathways described may be instrumental for the development of mechanism-based therapeutic strategies to prevent maladaptive endothelial insulin signalling in patients with cardiometabolic disturbances.

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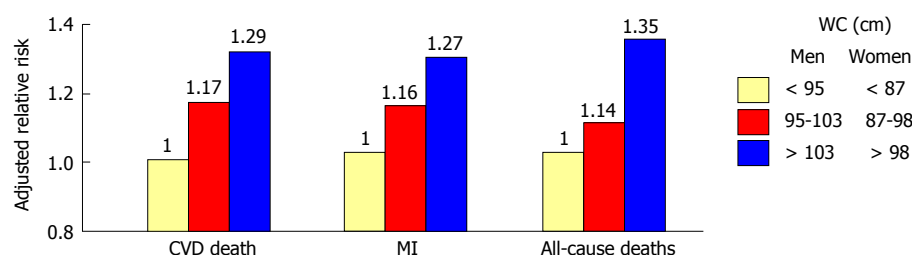


Figure 1 Association between abdominal obesity and cardiovascular disease. Subjects in the upper tertile of waist circumference had an increased adjusted relative risk of 29% for cardiovascular (CV) death, 27% for myocardial infarction (MI), and 35% for total mortality, suggesting a strong association between abdominal obesity and CV events. Adapted from Dagenais *et al*^[9]. WC: Waist circumference; CVD: Cardiovascular disease death.

PREVALENCE OF IMPAIRED GLUCOSE TOLERANCE

The most recent update of the International Diabetes Federation shows that 6.9% of the global population (316 million people) is currently affected by impaired glucose tolerance (IGT) and, most importantly, forecasts anticipate a dramatic IGT increase with more than 470 million people affected by the year 2035^[1]. Such pandemic of metabolic syndromes and obesity-related disorders hints a proportional increase in the prevalence of type 2 diabetes (T2D), a major driver of morbidity and mortality worldwide^[2]. Currently, 382 million people are affected by T2D, with a global age-adjusted prevalence of 10%. If these trends continue, 592 million people, or one adult in 10, will have diabetes by 2035^[1]. The link between environmental factors (pollution, caloric intake, sedentary lifestyles), obesity and subsequent dysglycemia indicates that the progression to diabetes is not linear and involves different cellular mechanisms including alterations of insulin signalling, changes in glucose metabolism, free fatty acids oxidation as well as dysregulation of genes relevant to endothelial integrity^[3,4]. The progression from obesity to T2D may take many years to occur, leading to different intermediate phenotypes with progressive changes in glucose parameters and shifts in glucose tolerance category. Yet, the etiologic pathway linking increased body weight, altered insulin signaling and subsequent hyperglycemia remained to be understood. Novel insights in this area may be instrumental to identify novel mechanism-based therapeutic strategies for the preservation of insulin signaling and, hence, diabetes development.

IMPACT OF INSULIN RESISTANCE ON CARDIOVASCULAR OUTCOME

Mortality from cardiovascular disease (CVD) is significantly higher in subjects with T2D than in those without^[5]. Notably, the risk of macrovascular complications seems to be proportional to the impairment of glucose homeostasis^[6]. Among different diabetes-related

conditions, insulin resistance (IR) and hyperglycemia are major precursors of atherothrombotic events and poor CV outcome^[7]. Waist circumference, an hallmark of IR, is a strong independent predictor of CVD^[8]. Dagenais *et al*^[9] showed that subjects in the upper tertile of waist circumference had an increased adjusted relative risk of 29% for CV death, 27% for myocardial infarction, and 35% for total mortality, suggesting a strong association between abdominal obesity and CV events (Figure 1). Along this line, elevated insulin and glucose concentrations are associated with increased CVD risk, regardless of diabetes^[10,11]. Impaired insulin signalling is a key feature of the metabolic syndrome (MetS), defined by the presence of hyperglycemia, central obesity, low high density lipoprotein cholesterol level, high triglyceride level and elevated blood pressure or antihypertensive drugs use. MetS is highly represented in patients with type 1 diabetes (T1DM) (38% in men and 40% in women) and is an important predictor of CV events^[12]. Indeed, MetS was associated with a 2.1-fold increased risk of CV events and a 2.5-fold increased risk of CV-related mortality after 5.5 years follow-up in 3783 patients with T1DM^[13]. The main issue when it comes to insulin resistance is how to measure it in clinical practice. The Homeostasis Model Assessment IR (HOMA-IR) is emerging as well-established marker of IR with a high predictive value for incident coronary events and stroke^[14-16]. This is likely due to the fact that HOMA-IR includes in its formula both fasting glucose and insulin levels thus showing a stronger association with cardiovascular disease than glucose or insulin alone^[14]. Despite many investigations confirmed a potential predictive value of IR, the mechanisms underlying this phenomenon still remain poorly understood. First, it is not clear whether IR is an active process or rather the consequence of the inflammatory milieu observed in obese and diabetic patients. Second, it remains unknown if the impairment of insulin signaling occurs simultaneously in all insulin-sensitive organs or whether tissue-specific IR has a primary role in triggering maladaptive insulin responses in other tissues. In order to answer these complex questions, many researchers are now exploring the pathophysiology of IR in different organs as well as its impact on metabolic features and

longevity.

ENDOTHELIAL INSULIN RESISTANCE: AN EMERGING CONCEPT

Evidence accumulated over the last decade has shown that loss of insulin signaling in the endothelium accelerates atherosclerotic lesions and vascular dysfunction in mice^[17-21]. Noteworthy, these effects occur regardless of concomitant CV risk factors, suggesting a central role of endothelial IR^[4]. Although IR has been attributed to inflammation in adipocytes, recent work has overturned such "adipocentric paradigm"^[22]. The novel concept that IR may primarily starts in the endothelium squares with the notion that endothelial cells are highly represented within the entire vascular system and, hence, within different organs^[18,20,23]. Recent experimental work has demonstrated that the transcription factor nuclear factor kappa-B (NF- κ B) is a key determinant of endothelial insulin resistance in mice^[17]. NF- κ B is a well known molecular complex involved in inflammatory programs enabling transcription of cytokines, activation of stress kinases and dysregulation of insulin-related pathways^[24,25]. Of note, genetic disruption of I κ B prevents inflammation and insulin resistance in obesity and T2D^[26]. Hasegawa *et al.*^[17] have shown that mice with endothelium-specific suppression of NF- κ B signaling (E-DNI κ B) were protected against IR in adipose tissue and skeletal muscle. These mice displayed reduced oxidative stress markers, decreased macrophage infiltration of adipose tissue as well as increased blood flow and muscle mitochondrial content. Of note, capillary recruitment and subsequent insulin delivery were explained by restoration of nitric oxide (NO) levels in E-DNI κ B animals^[17]. This latter observation is important since endothelial nitric oxide synthase (eNOS) dysfunction may lead to a reduction in microcirculatory blood flow and, hence, reduced delivery of insulin within hormone-sensitive organs. Indeed, insulin-mediated glucose uptake is reduced in eNOS^{-/-} as compared with WT mice^[27]. In other words, microvascular dysfunction occurring in liver, adipose tissue and skeletal muscle explains the progressive decline of peripheral insulin distribution^[28,29]. Of note, restoration of eNOS functionality due to suppression of endothelial NF- κ B signaling was capable to rescue aging-associated insulin resistance and, most importantly, to prolong lifespan in mice^[17]. In line with these experimental data, studies in humans demonstrated that insulin-dependent vasodilation may represent a significant contributor to insulin-stimulated glucose uptake^[30-32]. Muris *et al.*^[33] proposed that approximately 40% of insulin-mediated glucose uptake by skeletal muscle can be attributed to capillary recruitment; according to this hypothesis, microvascular dysfunction not only precedes and predicts the development of T2D but also constitutes one of the links between IR and hypertension in MetS. Consistently, improvement of insulin sensitivity in

patients with cardiometabolic disturbances is associated with restoration of flow-mediated vasodilation^[34,35]. Another study showed that disruption of endothelial insulin signaling by genetic deletion of insulin receptor substrate-2 (IRS-2) alters insulin delivery in muscle thus affecting glucose tolerance in mice^[18]. Of interest, endothelial IRS-2 and ApoE knockout mice showed a more severe atherosclerotic disease progression as compared to controls^[18]. Further work demonstrated that knockout of three major FoxO isoforms in endothelial cells attenuates endothelial IR and atherosclerosis in low density lipoprotein receptor knockout mice, suggesting that FoxO inhibition may represent a potential therapeutic approach to prevent CVD and IR in patients with diabetes^[36]. Accordingly, activity of protein kinase C (PKC) β 2 and NF- κ B in endothelial cells isolated from insulin resistant subjects was markedly enhanced and this finding was associated with blunted eNOS phosphorylation, reduced nitric oxide availability and impaired endothelial function^[23].

Research discussed so far implies that reprogramming detrimental pathways in the vascular endothelium may be considered a novel approach to prevent metabolic disease.

ROLE OF OXIDATIVE STRESS IN MALADAPTIVE INSULIN SIGNALING

In obese subjects, exposure to environmental cues triggers many pathological processes including reprogramming of oxidant genes and subsequent redox changes in different tissues^[37,38]. The importance of reactive oxygen species (ROS) has been claimed over the last 50 years since these mediators are likely the most pervasive precursors of maladaptive intracellular signalling^[39]. Previous seminal work carried out in conditions of hyperglycemia has elegantly demonstrated how ROS accumulation can easily boost activation of detrimental downstream pathways such as advanced glycation end products (AGEs), polyol and hexosamine pathways as well as proinflammatory transcriptional programs initiated by NF- κ B^[40]. These ROS-sensitive molecular events are being translated to endothelial dysfunction and, hence, micro and macrovascular complications^[7]. While the relation between hyperglycemia and oxidative stress has been clearly delineated, the etiologic path linking insulin resistance to ROS generation remains to be deciphered. Recent evidence suggests that oxidative stress may contribute to alter insulin sensitivity in the vascular endothelium. Du *et al.*^[19] have shown that enhanced oxidation of free fatty acids (FFAs) in aortic endothelial cells increases the production of superoxide by the mitochondrial electron transport chain thus triggering molecular pathways of maladaptive insulin signalling. Indeed, FFAs-induced overproduction of superoxide was able to activate an array of proinflammatory signals while hampering the activity of key anti-atherogenic enzymes such as prostacyclin synthase (PGIS) and

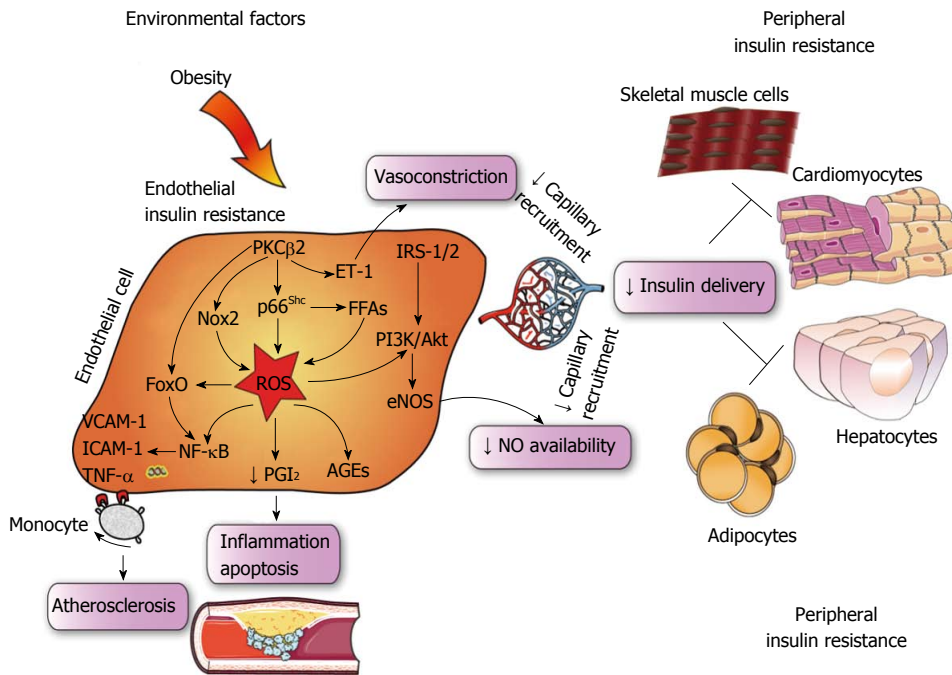


Figure 2 Central role of endothelial insulin resistance. Schematic representing intricate inflammatory and ROS-sensitive pathways responsible for maladaptive insulin signalling in the vascular endothelium. In obese subjects, environmental stimuli favour progressive impairment of endothelial cell function due to ROS accumulation and reduced NO bioavailability, leading to defective capillary recruitment and hampered insulin delivery to hormone sensitive organs. PKC: Protein kinase C; NF- κ B: Nuclear factor kappa-B; IRS: Insulin receptor substrate; ROS: Reactive oxygen species; AGEs: Advanced glycation end products; PGI₂: Prostacyclin; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular cell adhesion molecule-1; NO: Nitric oxide; TNF- α : Tumor necrosis factor α ; FFA: Free fatty acid; ET-1: Endothelin-1; Nox2: NADPH oxidase 2; FoxO: Forkhead box O; eNOS: Endothelial nitric oxide synthase. NADPH: Nicotinamide adenine dinucleotide phosphate.

eNOS. The importance of ROS in this setting was outlined by experiments in obese mice showing that inactivation of PGIS and eNOS was prevented by inhibition of FFAs release from the adipose tissue^[19]. For the first time, this study demonstrated that ROS may actively participate to impaired endothelial signalling thus favouring a pro-atherosclerotic phenotype in subjects with IR. Consistently, in *ApoE*^{-/-} mice with endothelium-specific IR, generation of superoxide was strongly linked to hampered insulin sensitivity, vasorelaxation and atherosclerotic lesions^[41]. A further study recently showed that nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase 2 (Nox2) may also be implicated in maladaptive insulin response by inducing a detrimental rearrangement of insulin receptors with subsequent deregulation of downstream kinase effectors, and eNOS dysfunction. Interestingly, obese mice with genetic disruption of Nox2 were protected against ROS accumulation and endothelial IR, suggesting that targeting Nox2 could represent a valuable therapeutic strategy in the context of prediabetes^[42] (Figure 2). On such a background, we have recently explored the possibility that the mitochondrial adaptor p66^{Shc} might participate to ROS-driven IR in the endothelium. The adaptor p66^{Shc} is a pivotal modulator of mitochondrial ROS through oxidation of cytochrome c^[43,44]. We have previously reported that genetic deletion of p66^{Shc} protects against vascular dysfunction and oxidative stress in diabetic mice^[45]. Moreover, p66^{Shc} expression is increased in patients with T2D and correlates with

plasma isoprostane levels, a reliable *in vivo* marker of oxidative stress^[46]. We have recently found that *in vivo* gene silencing of p66^{Shc} restored endothelial insulin response by affecting the IRS-1/Akt/eNOS pathway^[47]. Furthermore, p66^{Shc} knockdown in endothelial cells isolated from obese mice attenuated ROS production, FFAs oxidation and prevented dysregulation of redox-sensitive pathways such as NF- κ B, AGE precursor methylglyoxal and PGI₂ synthase. Collectively, our results show that p66^{Shc} may contribute to the pathogenesis of IR and increased vascular risk in the context of obesity and T2D. Selective targeting of p66^{Shc} may restore endothelial insulin sensitivity thus preventing adverse cardiometabolic phenotypes. In line with our findings, a recent work has shown that endothelium-specific overexpression of PKC β 2, a key molecular event eliciting ROS production, suppressed insulin-dependent pathways in *APOE*^{-/-} mice^[21]. Interestingly, expression of the potent vasoconstrictor endothelin-1 was highly increased in vessels isolated from *APOE*^{-/-} animals with PKC β 2 overexpression (Figure 2). Taken together, these results indicate that p66^{Shc} stands along a detrimental signalling cascade involved in ROS generation, microvascular dysfunction and, hence, peripheral insulin resistance. The clinical relevance of these experimental findings is supported by the notion that oxidative stress is significantly increased in cardiometabolic disorders. A cross-sectional study from the LIPGENE cohort revealed that levels of total nitrite, lipid peroxidation products, hydrogen peroxide (H₂O₂), superoxide dismutase

and glutathione peroxidase activities were all strongly associated with metabolic syndrome traits^[48]. Despite such evidence brings enthusiasms toward the possibility of targeting oxidative stress in humans, we are still far from having achieved satisfactory results in term of intermediate endpoints such as endothelial function and atherosclerotic lesions. Indeed, available antioxidants may not fully scavenge cellular ROS since they are unable to target intracellular enzymes involved in redox signalling. This notion is confirmed by the negative results of major trials with oral supplementation of high-dose vitamins^[49].

FUTURE PERSPECTIVES

The possibility to target specific machineries in the vascular endothelium may represent an attractive challenge to prevent or delay systemic features of IR favouring adiposity and related comorbidities. There are several examples suggesting that mechanism-based therapeutic approaches might be tested over the next decades. High doses of salicylates have been shown to ameliorate IR and improve glucose tolerance by suppressing NF- κ B activity in patients with T2D^[50]. Moreover, selective pharmacological inhibition of PKC β with LY379196 in freshly isolated endothelial cells from T2D patients reduced basal eNOS phosphorylation and improved insulin-mediated eNOS activation^[23]. Consistently, the Food and drug administration-approved PKC inhibitor ruboxistaurin ameliorates functional endothelial IR and smooth muscle cell hypersensitivity to insulin in experimental obesity and diabetes^[51]. In conditions of IR also the phosphodiesterase 5 inhibitor sildenafil has shown to improve NOS activity in human endothelial cells, thus suggesting the potential therapeutic use of this compound to warrant glucose homeostasis^[52]. Furthermore, preclinical work demonstrated that dual ET(A)/ET(B) receptor blockade enhanced endothelium-dependent vasodilatation in individuals with IR, thus restoring vascular recruitment and insulin delivery to peripheral organs^[53]. Yet, strategies to drive compounds specifically to the vascular endothelium are still far to be applied in humans. The main problem when it comes to tissue-specific treatment is represented by drug delivery. It is clear that selective rearrangement of maladaptive pathways in the endothelium would provide invaluable to restore microvascular dysfunction and insulin distribution to the liver, adipose tissue and skeletal muscle. An alternative option may be represented by NO donors or administration of eNOS cofactors in order to improve tissue capillary recruitment. Unfortunately this approach has failed many times due to the high oxidative burden in patients with metabolic disease which rapidly inactivates NO, thus favouring accumulation of peroxynitrite (ONOO⁻), protein nitrosylation and cellular dysfunction. In this respect, an example is provided by a recent clinical trial where oral treatment with eNOS cofactor tetrahydrobiopterin (BH4) has shown

limited effectiveness on endothelial function due to systemic oxidation and poor uptake into the vascular wall^[54]. These latter results highlight the need for more mechanistic understanding and alternative strategies to counteract pathways triggering eNOS dysfunction in patients with IR. We have recently showed that *in vivo* RNA interference may represent a valid approach to target specific ROS-generating enzymes in the endothelium^[55]. Distribution studies showed that *in vivo* delivery of small interfering RNA together with a cationic transfection reagent is able to target the vascular endothelium while sparing surrounding tissues. Indeed, we demonstrated that *in vivo* gene silencing of the adaptor p66^{Shc} restores insulin-dependent vasorelaxation in obese mice, suggesting that blunting endothelial oxidant pathways may be efficient for the maintenance of glucose homeostasis^[47]. This work will be instrumental to understand the efficacy and safety of such technology in humans, and whether other candidates may be considered for gene therapy in the setting of endothelial IR.

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Antioxidant role of zinc in diabetes mellitus

Kyria Jayanne Clímaco Cruz, Ana Raquel Soares de Oliveira, Dilina do Nascimento Marreiro

Kyria Jayanne Clímaco Cruz, Ana Raquel Soares de Oliveira, Dilina do Nascimento Marreiro, Department of Nutrition, Center of Health Sciences, Federal University of Piauí, Neighborhood Ininga, Teresina PI 64049-550, Brazil

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Correspondence to: Dr. Dilina do Nascimento Marreiro, Associate Professor, Department of Nutrition, Center of Health Sciences, Federal University of Piauí, Neighborhood Ininga, Teresina PI 64049-550, Brazil. dilina.marreiro@gmail.com

Telephone: +55-86-88459778

Fax: +55-86-32371812

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Abstract

Chronic hyperglycemia statue noticed in diabetes mellitus favors the manifestation of oxidative stress by increasing the production of reactive oxygen species and/or by reducing the antioxidant defense system activity. Zinc plays an important role in antioxidant defense in type 2 diabetic patients by notably acting as a cofactor of the superoxide dismutase enzyme, by modulating the glutathione metabolism and metallothionein expression, by competing with iron and copper in the cell membrane

and by inhibiting nicotinamide adenine dinucleotide phosphate-oxidase enzyme. Zinc also improves the oxidative stress in these patients by reducing chronic hyperglycemia. It indeed promotes phosphorylation of insulin receptors by enhancing transport of glucose into cells. However, several studies reveal changes in zinc metabolism in individuals with type 2 diabetes mellitus and controversies remain regarding the effect of zinc supplementation in the improvement of oxidative stress in these patients. Faced with the serious challenge of the metabolic disorders related to oxidative stress in diabetes along with the importance of antioxidant nutrients in the control of this disease, new studies may contribute to improve our understanding of the role played by zinc against oxidative stress and its connection with type 2 diabetes mellitus prognosis. This could serve as a prelude to the development of prevention strategies and treatment of disorders associated with this chronic disease.

Key words: Diabetes mellitus; Type 2; Oxidative stress; Zinc; Superoxide dismutase; Metabolism

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Core tip: Type 2 diabetes mellitus is a metabolic disease characterized by the presence of chronic hyperglycemia which favors the manifestation of oxidative stress due to high production of reactive oxygen species and/or induced by the reduction of the antioxidant defense system activity. Zinc plays a relevant role in antioxidant defense in type 2 diabetic patients by acting through different protection mechanisms. Zinc for instance is an essential cofactor for superoxide dismutase enzyme. This mineral also facilitates reduction and neutralization of free radicals. The aim of the present review is to examine the antioxidant role of zinc in type 2 diabetic patients.

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INTRODUCTION

Type 2 diabetes mellitus is a metabolic disease characterized by the presence of glucose intolerance and hyperglycemia. The main pathophysiological effect is to induce a peripheral resistance to insulin action associated with a relative deficiency of secretion of this hormone in response to glucose^[1,2].

Chronic hyperglycemia status in diabetes favors the manifestation of oxidative stress due to high production of reactive oxygen species and/or a decrease of the antioxidant defense system activity linked to lipid peroxidation and oxidative cellular injury themselves resulting in damages in the metabolism of lipids, proteins and DNA and from changes in cells functions^[3-5].

Hormonal, biochemical and nutritional disorders present in type 2 diabetic individuals have been subject to researches with the aim of clarifying the mechanisms involved in the pathogenesis of this disease. Regarding both biochemical and nutritional disorders, studies show changes in the mineral metabolism and the activity of antioxidant enzymes such as zinc and superoxide dismutase^[6,7].

Zinc plays a relevant role in antioxidant defense in patients with type 2 diabetes mellitus. This mineral may act by different protection mechanisms by notably being an essential cofactor for more than 300 enzymes, such as superoxide dismutase. This mineral also facilitates reduction and neutralization of free radicals^[8,9].

Considering changes in zinc metabolism and in superoxide dismutase enzyme activity present in type 2 diabetic patients simultaneously with the importance of these compounds in antioxidant defense, the aim of this review is to examine the antioxidant role of zinc in this type of patients.

RESEARCH

The bibliographical survey was conducted in the data base of Pubmed, Scielo and Lilacs, without limit of year of publication, considering the following inclusion criteria: studies that evaluated the effect of zinc supplementation on markers of oxidative stress in type 2 diabetes mellitus. Articles were selected for their originality and relevance, considering both the accuracy and adequacy of the experimental design, sample size, type of physiological and the performance measures undertaken. Classic and recent works were preferentially used.

The search of literature references was performed using the following keywords: "diabetes mellitus type 2", "zinc", "oxidative stress", "superoxide dismutase". The bibliographical survey included the following types of studies: randomized controlled clinical trials, cohort, case

control study, being surveyed in 80 articles of which 36 were used, all of them related with this literature.

ZINC, OXIDATIVE STRESS AND TYPE 2 DIABETES MELLITUS

Recently, several researches have been conducted from the perspective of clarifying the connection between the metabolic and biochemical aspects involved in the pathogenesis of type 2 diabetes mellitus and the metabolism of minerals such as zinc. In this way, studies reveal changes in the metabolism of this nutrient and the results are still limited and controversial^[6,10,11]. The Table 1 shows studies that evaluate participation of zinc in diabetes mellitus.

Saharia *et al*^[6], Basaki *et al*^[7] and Jansen *et al*^[17] found reduced plasma concentration of zinc in type 2 diabetic patients. These results are associated with a high amount of the mineral lost in the urine. Such loss is influenced by glycemic control in these patients not compensated neither by an increase in its absorption by intestinal cells nor the concomitant reduction of intestinal excretion. Jayawardena^[18] affirms that hyperglycemia interferes in active transport of zinc into the renal tubular cells promoting hyperzincuria.

Agte *et al*^[10] found reduced zinc concentrations in the erythrocytes of type 2 diabetic patients compared to the control group, which seems to be related to the high osmotic fragility of erythrocytes resulting in oxidative stress. Percentage of hemolysis of these cells also showed significant negative correlation with values of glycated hemoglobin.

On the other hand, study of Lima *et al*^[11] found increased erythrocyte and plasma concentrations of zinc in type 2 diabetic patients compared to the control group. The authors suggest that plasma values observed are linked to the time of diagnosis of the disease, being higher at the beginning of its manifestation. About the erythrocyte concentration of the mineral, the authors have highlighted the role of metallothionein as a regulator of homeostasis of zinc. The oxidative stress present in type 2 diabetic patients indeed favors both the release of the mineral of this protein and the increase in intracellular zinc content.

Another factor that may favor the increase in zinc concentration in erythrocytes is the fact that oxidative damages induced by type 2 diabetes mellitus seem to be more prominent in erythrocytes, favoring increased concentration of antioxidants as a compensatory mechanism to protect these cells^[11,19].

It is appropriate to draw attention to the antioxidant role of zinc. This mineral acts as a cofactor for superoxide dismutase enzyme, regulates the glutathione metabolism and the metallothionein expression, competes with iron and copper in the cell membrane and also inhibits the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase) enzyme^[20,21].

Another important point is the action of a group of

Table 1 Studies that evaluate participation of zinc in diabetes mellitus

Ref.	Samples	Results
Aly <i>et al</i> ^[12]	Diabetics rats	Zinc chloride supplementation (5 mg/kg) during one month, helped maintain serum concentration of glucose; preserved hepatic tissue; diminished NO, MDA, and PEPCK and increased SOD, GSH, LDH, pyruvate kinase and hexokinase
Zhang <i>et al</i> ^[13]	Diabetics mice (<i>n</i> = 12) and control groups (<i>n</i> = 14)	Reduced hepatic zinc concentration were found in diabetics mice Zinc deficiency has contributed to increase serum concentrations of ALT and deposit of lipids in the liver of the mice. Furthermore, this deficiency stimulated expression of inflammatory cytokines PAI-1, TNF- α and ICAM-1 and the oxidative damage markers (3-NT e 4-HNE)
Gunasekara <i>et al</i> ^[14]	Diabetics adults: (<i>n</i> = 96) Group A (<i>n</i> = 29): Zinc and multivitamin/mineral complex supplementation Group B (<i>n</i> = 31): Multivitamin/mineral complex supplementation Group C (<i>n</i> = 36): Placebo	Zinc and multivitamin/mineral complex supplementation decreased serum concentrations of HbA1c, fasting glucose, postprandial glucose and serum cholesterol. This supplementation also decreased cholesterol/HDL ratio
Yoshikawa <i>et al</i> ^[15]	Diabetics mice (<i>n</i> = 8)	Bis(aspirinato)Zn complex supplementation improved glycemia, insulin resistance, leptin resistance, hypoadiponectinemia and arterial hypertension
Foster <i>et al</i> ^[16]	Women with type 2 diabetes mellitus (<i>n</i> = 48)	Zinc supplementation (40 mg/d) during 12 wk did not alter HbA1c, insulin and HOMA-IR values. Also, this supplementation did not change metallothionein and zinc transporters gene expression

ALT: Alanine aminotransferase; GSH: Reduced glutathione; HbA1c: Glycosylated haemoglobin; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model of assessment-insulin resistance; LDH: Lactate dehydrogenase; MDA: Malondialdehyde; NO: Nitric oxide; PAI-1: Plasminogen activator inhibitor type 1; PEPCK: Phosphoenol pyruvate carboxykinase; SOD: Superoxide dismutase; TNF- α : Tumor necrosis factor- α ; 3-NT: 3-nitrotyrosine; 4-HNE: 4-hydroxynonenal; ICAM-1: Intercellular adhesion molecule-1.

antioxidants enzymes called superoxide dismutase, which regulates the detoxification of reactive oxygen species and catalyzes the dismutation of superoxide anion into hydrogen peroxide and oxygen^[22,23]. Mammals have three isoforms of this enzyme, but only isoforms 1 (CuZnSOD) and 3 (SOD extracellular) need zinc as a cofactor for its enzymatic activity and to act predominantly and respectively in the intracellular space and extracellular fluids^[20,24,25].

A study by Zhu *et al*^[22] with diabetic mice shows that the zinc supplementation increased the activity of superoxide dismutase and reduced malondialdehyde concentrations in both serum and pancreas. According to the authors, low levels of zinc in the organism impair the action of the antioxidant defense system. Corroborating previous findings, Li *et al*^[13] verified that zinc supplementation increased the activity of superoxide dismutase and decreased lipid peroxidation in the liver of diabetic rats, emphasizing that zinc can protect the liver from oxidative damage.

However, Anderson *et al*^[26] did not find any increase in superoxide dismutase activity after supplementation with 30 mg of zinc for 6 mo in type 2 diabetic patients. Roussel *et al*^[27] supplemented type 2 diabetic patients with 30 mg of zinc gluconate over 6 mo and noticed a reduction in the production of reactive substances to the thiobarbituric acid, but did not find any increase in the activity of superóxido dismutase.

Action of zinc on glutathione metabolism is significant and as such must be mentioned. Zinc indeed influences the expression of glutamate-cysteine ligase enzyme involved in the synthesis of glutathione, which directly

acts on the neutralization of free radicals and indirectly as a cofactor of glutathione peroxidase^[20,28].

Karaturg *et al*^[29] performed zinc sulfate supplementation in diabetic rats and found both an increased concentration of glutathione and a diminution of the lipid peroxidation. The non-enzymatic glycosylation in renal tissue substantiate the relevant antioxidant properties of this mineral in reducing the risk of renal complications associated with type 2 diabetes mellitus.

In terms of zinc action on metallothionein expression, numerous studies indicate that zinc supplementation increases both mRNA levels and the activity of such enzyme in type 2 diabetic individuals. The induction of metalloprotein being one of the explanations for the protective effect of supplementation with zinc in these patients^[30,31].

Wang *et al*^[32] evaluated the effects of zinc supplementation in diabetic rats and found reduced concentrations of blood glucose and malondialdehyde, as well as an increased expression of metallothionein in the liver. No changes in serum zinc levels were observed, implying a beneficial effect of supplementation in the reduction of oxidative stress.

A study by Özcelik *et al*^[31] showed that zinc supplementation increased the concentrations of both metallothionein and zinc, and decreased the lipid peroxidation in renal tissue of diabetic rats, showing the performance of the mineral acting as an antioxidant nutrient and its role in the prevention of renal damages in type 2 diabetes mellitus.

On the other hand, Seet *et al*^[33] evaluated the effect of an intake of 240 mg zinc/d in type 2 diabetic

patients with normozincemia and observed that the supplementation with this nutrient did not change the concentration of markers of oxidative stress and vascular function, suggesting that high doses of zinc have no beneficial effect on diabetics who do not have hypozincemia.

Another mechanism that explains the antioxidant role of zinc in type 2 diabetes mellitus, refers to its ability to compete with iron and copper for binding sites on the cell membrane. The iron and copper ions can catalyze the production of lipid peroxides, and the replacement of these metals for zinc in the plasma membrane could prevent lipid peroxidation in diabetic patients^[28].

The literature has shown that zinc also regulates the production of free radicals in neuronal cells in type 2 diabetic individuals. This mineral is known for its inhibiting effect on N-methyl-D-aspartate (NMDA) receptors involved in calcium transportation from the extracellular medium to the cytosol. Therefore, in case of zinc deficiency, NMDA receptors activation promotes and increase intracellular calcium concentration. In return, NADPH-oxidase and nitric oxide synthase enzymes are activated, favoring the production of reactive oxygen and nitrogen species^[28].

Liu *et al.*^[34] noticed that zinc supplementation decreased malondialdehyde concentration and stimulated the transcription of metallothionein genes in peripheral nerves of diabetic mice. This suggests that this mineral may improve peripheral neuropathy associated with type 2 diabetes. Such protective effect seems to be mediated by the reduction of oxidative stress.

Zhu *et al.*^[22] observed that zinc supplementation in diabetic rats caused an increase in glutathione peroxidase enzyme activity as well as a drop in concentrations of malondialdehyde and nitric oxide. The nitric oxide synthase activity in both pancreas and serum of these rats also demonstrates the protective action of zinc against oxidative stress present in type 2 diabetes mellitus. Moreover, the authors observed that the intake of this mineral improved liver functions and also prevent damage to pancreatic tissue induced by the diabetes.

Oxidative stress found in type 2 diabetes is improved by the action of zinc because it also reduces chronic hyperglycemia. It is important to point out that this oligoelement takes part in insulin inventory, secretion and action processes for being a catalytic cofactor for carboxypeptidase H enzyme which catalyzes the conversion from proinsulin (inactive) into insulin (active). Zinc also promotes phosphorylation of insulin receptor by enhancing glucose transport into cells^[30,35]. In this perspective, Vashum *et al.*^[36] demonstrated the role of zinc in reducing chronic hyperglycemia in type 2 diabetes mellitus by considering that patients with higher serum concentration of the mineral improved their insulin sensitivity.

Considering the biochemical and nutritional aspects presents in type 2 diabetes mellitus pathophysiology, important is the participation of zinc in mechanisms

involved in this process, for instance, its relevant role as an antioxidant nutrient that improve metabolic control in these patients.

CONCLUSION

Scientific evidences highlighted in this review point out changes in zinc metabolism which contributes to an oxidative stress manifestation in patients with type 2 diabetes mellitus. Several researches have found controversial results regarding zinc supplementation and its positive impact on oxidative stress in these patients. Faced with the serious challenge of the metabolic disorders related to oxidative stress in diabetes in addition to the importance of antioxidant nutrients in the control of this disease, the carrying out of studies may contribute to improve our understanding of the role played by zinc against oxidative stress and its connection with type 2 diabetes mellitus prognosis. This could serve as a prelude to the development of prevention strategies and treatments of disorders associated with this chronic disease.

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Effects of maternal diabetes on trophoblast cells

Marlúcia Bastos Aires, Anne Caroline Veríssimo dos Santos

Marlúcia Bastos Aires, Anne Caroline Veríssimo dos Santos, Department of Morphology, Federal University of Sergipe, São Cristóvão 49100-000, Sergipe, Brazil

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Correspondence to: Marlúcia Bastos Aires, PhD, Department of Morphology, Federal University of Sergipe, Av. Marechal Rondon s/n Cidade Universitária Professor José Aloísio de Campos, São Cristóvão 49100-000, Sergipe, Brazil. marlucia_aires@yahoo.com.br

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control in diabetes. A proper coordination of trophoblast proliferation, differentiation and invasion is required for placental development. Initially, increased expression of proliferative markers in junctional and labyrinth zones of rat placentas and villous cytotrophoblast, syncytiotrophoblast, stromal cells and fetal endothelial cells in human placentas is reported among diabetics. Moreover, reduced apoptotic index and expression of some apoptotic genes are described in placentas of GDM women. In addition, cell cycle regulators including cyclins and cyclin-dependent kinase inhibitors seem to be affected by the hyperglycemic environment. More studies are necessary to check the balance between proliferation, apoptosis and differentiation in trophoblast cells during maternal diabetes.

Key words: Diabetes; Placenta; Proliferation; Apoptosis; Differentiation; Trophoblast

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Core tip: This review article focuses on current knowledge about the effects of diabetes on trophoblast function such as proliferation, apoptosis and cell cycle control during placental development in human and rodent animal models. It also briefly discusses some placental pathological findings as a consequence of altered metabolic environment during diabetes.

Abstract

Diabetes mellitus (DM) is a health condition characterized by hyperglycemia over a prolonged period. There are three main types of DM: DM type 1 (DM1), DM2 and gestational DM (GDM). Maternal diabetes, which includes the occurrence of DM1 and DM2 during pregnancy or GDM, increases the occurrence of gestational complications and adverse fetal outcomes. The hyperglycemic intrauterine environment affects not only the fetus but also the placental development and function in humans and experimental rodents. The underlying mechanisms are still unclear, but some evidence indicates alterations in trophoblast proliferation, apoptosis and cell cycle

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both^[1]. DM is a

public health problem worldwide which is increasing mainly because of the high prevalence of obesity and sedentarism. In 2000, a previous study including all age groups, estimated the global prevalence of DM at 2.8% expecting to rise up to 4.4% by 2030^[2].

There are three main types of DM: DM type 1 (DM1), DM2 and gestational DM (GDM). About 10% of all diabetes cases are DM1: an autoimmune disease in which an absolute deficiency of insulin resulting from the pancreatic β cells destruction occurs. This destruction can be caused by an autoimmune process (type 1a) or it can be idiopathic (type 1b). The former represents the vast majority of DM1 cases and usually manifests itself before 30 years of age, being more common in individuals of European origin. On the other hand, the vast majority of cases of diabetes existing in the population is DM2, characterized by peripheral resistance to insulin action and relative insulin deficiency^[3].

In general, the overall prevalence of DM has increased in recent years due to the aging population and lifestyle changes. Parallel to this trend, the number of pregnant women with pre-existing diabetes (DM1 and DM2) has been increasing worldwide, and in some countries, this numbers are even doubling^[4,5]. This increase is closely related to the high number of diabetic patients in reproductive age as well as to advances in clinical care available for pregnant diabetic women. Until the mid-20th century, DM1 women either had not reached the child-bearing age or had had serious health problems that contraindicated pregnancy. The discovery and commercial availability of insulin changed this scenario and a better glycemic control for women with diabetes led to a considerable reduction in the rates of maternal and fetal complications^[6].

GDM is the principal metabolic disorder that occurs during pregnancy and can affect 3% to 30% of pregnant women depending on the population studied and the diagnostic criteria used^[7]. It is also defined as any degree of glucose intolerance of variable severity which arises or is diagnosed during pregnancy. Besides, it is characterized by the maternal pancreas inability to meet the growing demand of insulin as from the second trimester of gestation^[8].

Maternal diabetes, which includes the occurrence of either DM1 or DM2 in pregnancy and GDM, creates an unfavorable environment for embryonic and fetoplacental development. Despite the several developmental and morphological differences between rodents and women placenta, the alterations induced by maternal diabetes are similar in diabetic patients and diabetic experimental models^[9]. Several works have been published addressing the impact of diabetes on placental weight and growth and materno-placental oxygen supply^[10,11].

As it is known, the placenta is a highly specialized organ in the interface between maternal and fetal circulation with fundamental functions for pregnancy. It permits the fetus anchorage to the uterus, O_2/CO_2

exchange, the nutrition and the waste products removal during embryonic and fetal development^[12]. Also, it acts as a protective barrier against xenobiotics and releases a variety of steroids, hormones and cytokines^[13]. Therefore, placental dysfunction has deleterious effects on adequate pregnancy support. Among placental cells, trophoblasts permit the embryo implantation and nutrition in the early pregnancy and thereafter they will contribute considerably to the development and function of the placenta. The underlying mechanisms of placental pathology during diabetes are still unclear, but some evidence indicates changes in trophoblast proliferation, apoptosis and cell cycle control.

MATERNAL DIABETES EFFECTS ON TROPHOBLAST PROLIFERATION

A proper coordination of trophoblast proliferation, differentiation and invasion is required for placental development. Initially, cell proliferation should be tightly controlled for proper tissue growth and differentiation. Throughout gestation, growth factors such as epidermal growth factor, vascular endothelial growth factor, platelet-derived growth factor, placental growth factor, colony stimulating factor 1, insulin-like growth factor I (IGF- I), or IGF- II are abundantly secreted from diverse cell types of the fetal-maternal interface and have been to promote proliferation, adhesion and/or invasion^[14-17].

In diabetes, the enlargement of the junctional zone (JZ) in the diabetic rat placenta is described as the increased number of glycogen and giant trophoblast cells^[10]. Indeed, our previous stereological study confirmed a greater volume of spongiotrophoblast/glycogen cells in diabetic rats compared with controls^[18]. Also, other works have shown changes in the size and organization of the spongiotrophoblast and glycogen cells in rat models of diabetes^[10,19,20], suggesting the JZ as the placental compartment most sensitive to the diabetic condition^[18,21].

Studies on the effects of maternal diabetes on placental development have solely reported increased expression of proliferative markers in the JZ and labyrinth zone (LZ) of rat placentas^[22,23]. Zorn *et al.*^[23] showed that diabetes promotes an increased cell proliferation rate, detected by Ki67 immunostain, especially of spongiotrophoblast cells at gestational day 14 (gd 14), and of labyrinth cells, spongiotrophoblast and trophoblast giant cells at gd 17. Also, intense proliferating cell nuclear antigen (PCNA) immunostain in labyrinth and spongiotrophoblast cells on 17 d and in spongiotrophoblast and trophoblast giant cells on 21 d of pregnancy were noted in diabetic groups than in control groups, indicating deregulated cell proliferation in hyperglycemic condition which may explain the placentomegaly observed in diabetic animals at gd 20^[22].

In humans, the placentas of GDM pregnancies are heavier than those of control patients^[24,25] and the

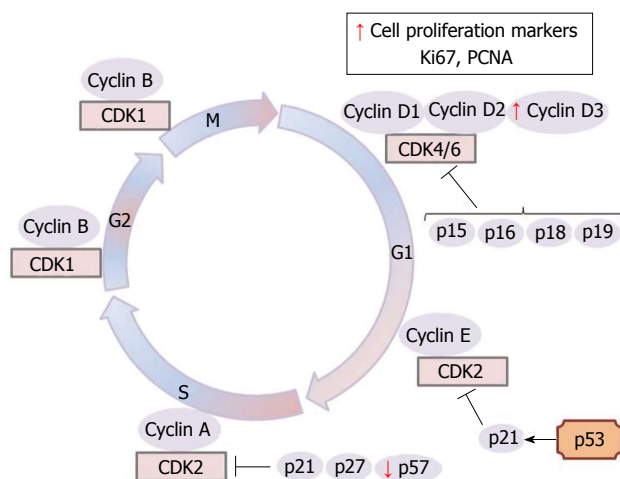


Figure 1 Schematic representation of eukaryotic cell cycle and key regulatory proteins which allow the transition from one cell cycle phase to another. CDKs inhibitors, such as p15, p16, p18, p19 (INK4 group), p21, p27 and p57 (CIP/KIP class) and proliferative markers are also showed. The arrows in red indicate increased or decreased expression of some trophoblast key regulatory proteins, CDKs inhibitors and proliferative markers in maternal diabetes. G1: Gap 1 phase; S: synthesis phase; G2: Gap 2 phase; M: Mitosis; CDKs: Cyclin-dependent kinases; PCNA: Proliferating cell nuclear antigen; p53: Tumor protein p53.

mechanism accounting for this increased placental mass is unknown. Enlargement of the capillary surface area with capillary proliferation and penetration of newly formed vessels have also been shown in DM^[26]. Villous immaturity is present in 60% of diabetic placentas and is characterized by an increase in the number of mature and immature intermediate villi^[27]. At the same time, a higher number of villous cytotrophoblast, villous stromal fibroblasts, macrophages, endothelial cells and syncytiotrophoblast nuclei in diabetes were noted^[28-30]. As in diabetic animals, it was also reported, increased proliferative activity in villous cytotrophoblasts compared to normal placentas^[27,30,31]. Leach *et al.*^[32] reported higher PCNA immunoreactivity in endothelial cells of diabetic placentas. In addition to PCNA staining, Ki67 and cyclin D3 (Figure 1) staining of villous cytotrophoblast, syncytiotrophoblast, villous stromal cells and fetal endothelial cells increased in diabetic placentas compared to controls^[33].

TROPHOBLAST APOPTOSIS IN MATERNAL DIABETES

The occurrence of apoptosis is shown during normal placental development and in morbid states^[34-44]. In the normal human placenta, the presence of apoptotic cells could be associated with many events like trophoblast attachment and invasion^[45,46], spiral artery remodelling^[47,48], trophoblast differentiation^[47-49] and labor^[34,50]. However, the rates of placental apoptosis, even in normal human gestations, are still controversial. A predominance of apoptosis during early gestation,

diminishing after the second trimester^[41,51] and a significant increase in apoptosis as pregnancy progresses were reported^[34,38,44].

Some works showed reduced apoptotic index, by TUNEL assay, in placentas from GDM^[52,53] and DM1^[36] patients compared to control placentas. Increased placental weight in GDM was associated with significantly reduced trophoblast apoptosis^[52,53]. On the other hand, some authors reported increased apoptosis of villous cytotrophoblasts and syncytiotrophoblast nuclei in diabetic placentas *in vivo*^[54] or *in vitro*^[55]. Some technical differences such as the mode of delivery, the placental sampling or differences in gestational age could be responsible for study discrepancies^[52]. Therefore, more studies are necessary to check the balance between proliferation and apoptosis in human diabetic placentas.

In the placenta, conditions like low oxygen and oxidative stress could induce to apoptosis that may be initiated by intrinsic or extrinsic pathways resulting in the activation of central apoptotic effectors, the caspases^[56]. The extrinsic pathway involves members of the tumor necrosis factor (TNF) death receptor family, whose ligands include TNF- α , Fas ligand, Apo3 ligand (Apo3L) and Apo2L^[44]. The activation of death receptors results in receptor aggregation and recruitment of adaptors molecules Fas-associated death domain or TNF-R-associated death domain^[57]. As a consequence, procaspase-8 and procaspase-10 are recruited and become activated, initiating the cleavage of downstream effector caspases^[58-60]. Caspase-8 could occasionally cleavage BH3-interacting domain death agonist that activates the intrinsic pathway^[61].

The intrinsic pathway could be initiated by toxins, radiation, DNA damage and reactive oxygen species that lead to cellular stress and deficiency of growth factors^[62]. This pathway induces mitochondrial membrane permeability modification by changes in the association of pro- and anti-apoptotic B-cell lymphoma 2 (BCL2) proteins^[63]. The outer membrane permeabilization leads to the release of cytochrome c from the mitochondrial intermembrane space to the cytosol^[64]. Then, the cytochrome c binds to the protease activating factor-1 forming the apoptosome^[65]. The apoptosome cleaves procaspase-9, activating the terminal pathway of apoptosis. Additionally, the Smac (Second mitochondria-derived activator of caspase) is released from the mitochondria and eliminates the inhibitory effect of inhibitor of apoptosis proteins on caspases^[66,67]. Both pathways culminate in a terminal pathway involving the cleavage and activation of caspase-3, -6, and -7, initiating cell destruction by activating DNases and cleaving DNA repair enzymes such as poly (ADP-ribose) polymerase (PARP)^[68,69].

Concerning proteins associated with cell death pathways reduced expression of BCL2 has been reported in placentas from diabetic patients compared to normoglycemic women^[54]. Furthermore reduced

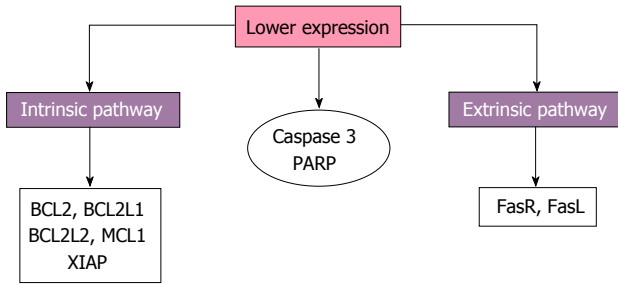


Figure 2 Schematic representation of trophoblast apoptosis findings in maternal diabetes. Reduced expression of apoptotic components from both intrinsic and extrinsic pathways, caspase-3 and poly (ADP-ribose) polymerase (PARP) are reported by some works. FasR: Fas receptor; FasL: Fas ligand; MCL1: Myeloid cell leukemia 1; BCL2: B-cell lymphoma 2; BCL2L1: BCL2-like 1; XIAP: X-linked inhibitor of apoptosis; BCL2L2: BCL2-like 2.

gene expression of *BCL2*, *BCL2L1*, *BCL2L2*, myeloid cell leukemia 1 and X-linked inhibitor of apoptosis and reduced protein expression of the Fas receptor (FasR), FasL, caspase-3 and its PARP has been reported, indicating extrinsic and intrinsic pathways downregulation in placentas with GDM^[50,53] (Figure 2).

According to Rudge *et al.*^[70] (2012), severe diabetes in mice decrease placental TUNEL index from day 18 to 21 of pregnancy, at the same time that small for pregnancy age fetus and increased placental weight are also found. A GDM animal model (*db/+* mice), when treated with TNF- α at gd 11.5, a pro-apoptotic peptide, there was an increased number of apoptotic cells, detected by cleaved caspase-3 immunostaining, in both labyrinth and trophospongium, at gd 18.5^[71]. Unfortunately, little is known about the cleaved caspase-3 placental activity from other's models of animal diabetes or even human diabetic pregnancies.

CELL CYCLE CONTROL OF TROPHOBLAST IN MATERNAL DIABETES

The appropriate development of an organism depends on the balance between cell cycle exit and the differentiation process in all tissues. The cell cycle exit is required for terminal differentiation of many cell types and cell cycle progression is regulated by a series of cyclin-dependent kinases (CDKs) that consist of catalytic subunits, designated CDKs, and activating subunits, designated cyclins^[72,73]. The activation and inactivation of different cyclin-CDKs at adequate moments is necessary for precise progression into the cell cycle^[74,75].

Although placental growth is essentially a result of the coordination of trophoblast proliferation and differentiation, there is little information about the mitotic regulators that provide the synchronization of trophoblast proliferation and differentiation^[76]. In the rat term placenta, cyclin D1 and cyclin D3 are expressed in placental fetal cells, whereas the G1/S cyclin E are present only in the spongiotrophoblast and labyrinthine trophoblast cells^[77]. The D-type cyclins serve as growth factor sensors that integrate extracellular signals with

the cell cycle machinery. Together with their partner kinases, CDK4 and CDK6, they operate in early-to-mid G1 to promote progression through the G1-S restriction point^[73]. The nuclei expression of cyclins D1 and D3 in mesenchymal and labyrinthine trophoblast cells could infer a role in the differentiated state maintenance in late gestation^[77]. In the human placenta, cyclins D1 and D3 have been observed in endothelial cells^[78]. However, cyclin activity during diabetes has been little explored in placental cells. Only one work reported cyclin D3 staining intensities significantly increasing in villous parts, basal plates and chorionic plate of a diabetic group when compared to control placentas^[33]; perhaps prominent cyclin D expression could contribute to the increased cell proliferation observed in diabetic placentas (Figure 1).

There are two families of CDK inhibitors that act to inhibit cell cycle progression. The INK4 family (p15ink4b, p16ink4a, p18ink4c, p19ink4d) inhibits the CDK4, CDK6 and cyclin D activities in the G1 phase and G1/S transition of cell cycle. In turn, the CIP/KIP family (p21waf/cip1, p27kip1 and p57kip2) inhibits the cell cycle at many checkpoints by acting on multiple cyclin-CDK complexes^[74,75].

The altered metabolic environment in maternal diabetes could affect the expression of genes that control the cell cycle events as was observed for reduced p57 expression in diabetic rat placentas on days 17 and 21 of pregnancy^[79] (Figure 1). In the normal rat placenta, immunostaining intensities of cell cycle inhibitors p27, and p57 were observed to be higher in the JZ compared to the LZ close to term^[77]. Accordingly, since p57 is a cell cycle inhibitor and tumor suppressor, lack of p57 activity can lead to a loss of cell cycle control and hyperproliferation^[33]. Therefore, less p57 expression may explain the reason why diabetic placentas are heavier and bigger^[33].

In fact, abnormal placental development is present in p27 and p57 knockout mice^[80]. The LZ is less vascularized and contains more trophoblasts than those from wild type placentas. Moreover, Takahashi *et al.*^[81] demonstrated that placentomegaly was observed in p57 deficient mice in which the numbers of placental cells in the LZ and spongiotrophoblasts were twice the number of those of the wild type. In addition to placentomegaly, deregulation of the cell cycle can result in the development or progression of some trophoblastic diseases like preeclampsia that can occur in diabetic women. In humans, p57 staining index of villous parts decreases significantly in diabetes^[33]. Another CIP/KIP family member, p27, has no difference in staining intensity in the villous part between diabetic and control groups of human patients, but has different staining patterns in different placental cell types^[33]. Some studies indicated that p27 and p57 have different functions in human placental development^[82,83].

In summary, this article reviewed the current knowledge about the effects of hyperglycemia on trophoblast proliferation, apoptosis and cell cycle control

during pregnancy. More detailed studies are required to check the balance between proliferation, apoptosis and differentiation in trophoblast cells during maternal diabetes.

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Impact of glucose level on morbidity and mortality in elderly with diabetes and pre-diabetes

Orit Twito, Meir Frankel, Dan Nabriski

Orit Twito, Meir Frankel, Dan Nabriski, Endocrinology, Diabetes and Metabolism Institute, Meir Medical Center, Kfar Saba 44281, Israel

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Correspondence to: Dr. Orit Twito, Endocrinology, Diabetes and Metabolism Institute, Meir Medical Center, 59 Tschernihovsky, St., Kfar Saba 44281, Israel. orit.twito@clalit.org.il

Telephone: +972-9-7472788

Fax: +972-9-7471644

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Abstract

The prevalence of type 2 diabetes mellitus (DM) increases with age and reaches 25% in those older than age 65 years. Pre-diabetes status is also very common in the elderly, and is present in about half of those age 75 years and older. Many physicians care for elderly patients with diabetes and pre-diabetes, dealing with the challenge of controlling glucose levels and improving health with minimal adverse events. Over the

last decade, research on diabetes among the elderly population has proliferated, adding new information on this topic. This review summarizes the updated medical literature on diabetes and pre-diabetes in the elderly, including the significance of pre-diabetic conditions, new-onset DM in the elderly and long-standing DM. The role of therapeutic intervention and the level of glycemic control for this population are discussed in particular.

Key words: Diabetes mellitus; Elderly; Old age; Pre-diabetes; Glycemic control

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Core tip: The prevalence of diabetes mellitus (DM) and pre-diabetes in old age is very high. However, clinical guidelines do not provide complete information to the clinician managing patients with these conditions. Pre-diabetes status in the elderly increases the risk for DM, but probably does not increase the risk of cardiovascular morbidity and mortality. The role of therapeutic interventions in elderly patients with pre-diabetes is not yet proven. New-onset DM in older age is associated with better glycemic control and better prognosis compared to long standing DM in this population. Nevertheless, higher glucose levels in elderly with new-onset DM are associated with increased all-cause mortality. The benefits of tight glycemic control in elderly with long standing DM are doubtful and may cause more harm than good. To conclude, more research in this field is needed. Currently, the clinical approach for DM and pre-diabetes in the elderly should be tailored to meet individual needs.

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INTRODUCTION

The concept of individualized treatment for type 2 diabetes mellitus (DM) is becoming established and replaces previous recommendations for tight glucose control for all diabetic patients. One of the main criteria in constructing personalized care for the patient is the chronologic and biologic age.

The incidence and prevalence of DM increase with age (www.cdc.gov/diabetes/statistics). Pre-diabetes states, including impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and elevated HbA1c are even more prevalent among the elderly^[1-3].

However, the clinical impact of glucose levels on microvascular and macrovascular complications, and mortality is not well established. The commonly used clinical guidelines do not provide separate recommendations for elderly individuals with pre-diabetes, and do not differentiate between elderly with long-standing or new-onset DM^[4].

This review summarizes the data in the literature regarding the effect of glycemia in different stages on morbidity and mortality in the elderly population. It will address the aspects of the clinical impact of glucose levels in pre-diabetes, diabetes that was first diagnosed in old age and long-standing diabetes separately.

PRE-DIABETES IN THE ELDERLY

According to American Diabetes Association (ADA) guidelines, pre-diabetes may be diagnosed as IFG, IGT and/or by elevated HbA1c values of 5.7%-6.4%^[4]. The rates of pre-diabetes states, including IFG and IGT, are very common in the general population, and increase with age^[1-3].

It is well-established that pre-diabetes states are a significant risk factor for developing type 2 DM, as well as for diabetic complications and mortality in younger adults^[5,6]. It is also well known that lifestyle changes, including loss of at least 7% of body weight and ≥ 150 min/wk of activity, delay or even prevent development of DM, and may potentially reduce its complications among persons with pre-diabetes^[7]. Thus, pre-diabetic patients are an important target group for primary prevention interventions.

Understanding the clinical impact of pre-diabetes in older adults is very important, as the prevalence of pre-diabetes increases with age and reaches about 50% in those age 75 years and older^[2]. Moreover, lifestyle interventions are more successful in decreasing hyperglycemia in the elderly than in younger adults. This was demonstrated in the Diabetes Prevention Program (DPP) trial, which included subjects with

combinations of IGT and IFG, considered to be at high risk for developing DM. The oldest age group, 60-85 years at enrollment, had the greatest benefit from the program, both in terms of weight loss and decreased incidence of DM over time^[7,8].

Yet, there are some important, unanswered questions for the clinician. First, what is the clinical impact of pre-diabetes state in the elderly? Second, do glucose lowering interventions improve morbidity and mortality in this population?

The answer to the first question, regarding the clinical significance of pre-diabetes in elderly subjects, is based on a small number of studies (Table 1). A prospective, observational study followed 1466 elderly subjects with IGT and compared their mortality rate to subjects with normal glucose levels and overt diabetes. The age of enrolled participants was 55-74 years and median follow-up was 8.8 years. Mortality rates were almost equal in the pre-diabetes and normal glucose groups. Nevertheless, within the non-diabetic range (*i.e.*, normal and pre-diabetic glucose levels), a J-shaped association was demonstrated between glycemia and all-cause mortality, even after adjustment for multiple risk factors. The lowest mortality rates were documented in subjects with fasting plasma glucose 88-93 mg/dL and HbA1c 5.4%-5.5%. Participants with glucose levels at the upper pre-diabetes range had a higher mortality rate^[9].

On the other hand, a recent prospective cohort study of 8365 older subjects, 50-74 years old, revealed that the increased cardiovascular risk in pre-diabetes (defined as IFG or HbA1c 5.7%-6.4%) can mainly be explained by other concurrent cardiovascular risk factors and not by the hyperglycemia itself^[10]. Similar results arose from the Cardiovascular Health Study of 4602 community-dwelling elderly participants, 65 years-of-age and older. This study found no evidence that pre-diabetes is an independent risk factor for a variety of cardiovascular outcomes, including heart failure, myocardial infarction, stroke and all-cause mortality^[11]. Pre-diabetes increased the risk of developing DM, but the absolute rate was low and not related to increased cardiovascular risk.

An interesting pooled analysis examined the age specific effect of different metabolic risk factors on cardiovascular diseases. The analysis for plasma glucose included 372000 participants in 116 cohorts. The authors calculated the impact of mildly elevated glucose on the relative risk of ischemic heart disease and stroke. They concluded that the proportional effect of elevated fasting glucose declined with age^[12].

For the second question - whether glucose lowering intervention would improve morbidity and mortality outcomes in elderly population - there is still no satisfactory answer. The DPP study, mentioned above, found a better response of elderly subjects to lifestyle interventions, in particular weight loss and DM prevention. However, clinical outcome data were not reported^[7,8]. The effect of lifestyle

Table 1 Studies comparing cardiovascular morbidity and mortality in elderly with pre-diabetes and normal glucose tolerance

Ref.	No. of participants	Age at inclusion	Length of follow up (yr)	Population	Results
Kowall <i>et al</i> ^[9]	1466	55-74	8.8 (median)	German	Mortality rates were almost equal in the pre-diabetes and NGT groups
Schöttker <i>et al</i> ^[10]	8365	50-74	7.9 (median)	German	Major CV event ¹ rates were almost equal in the pre-diabetes and NGT groups
Deedwania <i>et al</i> ^[11]	4602	≥ 65	13 (median)	United States: 87% Caucasians, 13% African American	Major CV event ² rates were almost equal in the pre-diabetes and NGT groups

¹Major cardiovascular events including non-fatal stroke, non-fatal MI and cardiovascular mortality; ²Major cardiovascular events including heart failure, MI, angina pectoris, stroke and all-cause mortality. CV: Cardiovascular; NGT: Normal glucose tolerance; MI: Myocardial infarction.

interventions on mortality and cardiovascular disease in pre-diabetes patients is questionable, even among younger adults^[13].

In summary, existing data regarding the clinical impact of pre-diabetes on morbidity and mortality among elderly individuals are limited and study results are conflicting. There is an association between pre-diabetes and mortality, which increases with higher glucose levels within the pre-diabetic range. However, it is unclear whether pre-diabetes is a marker of poor metabolic condition or an independent risk factor. There is almost no information regarding the influence of lifestyle or medical interventions on morbidity and mortality in this population.

The ADA does not have separate clinical guidelines for pre-diabetes in the elderly population^[4]. The 2012 consensus report of the ADA and the American Geriatrics Society provided vague advice on this issue. They recommended screening for pre-diabetes in elderly patients who are likely to benefit from diagnosis of pre-diabetes and from subsequent intervention. Similarly, they recommended lifestyle interventions for elderly individuals with pre-diabetes who are able to participate and are likely to benefit from DM prevention^[14].

NEW-ONSET DIABETES IN THE ELDERLY

The pathophysiology of the appearance of DM in the elderly is a combination of age-related changes in carbohydrate metabolism, pancreatic endocrine dysfunction and adverse lifestyle factors^[15,16]. The epidemiology of incident DM in relation to population age is interesting. The incidence of new-onset DM increases with age until age 65 years, after which both incidence and prevalence of DM seem to level off (www.cdc.gov/diabetes/statistics).

The natural history of new-onset DM in the elderly seems to have a benign course in comparison to that of long-standing DM. Interesting information comes up from a study of centenarian subjects, ages 100-109, compared to elderly subjects aged 65-84 years. The centenarians had relatively low prevalence of DM (7.64%), and almost exclusively had senile DM, that is DM diagnosed after 65 years of age. The authors

suggest that long-standing DM is not compatible with extreme longevity, while senile DM does not change the clinical outcomes significantly^[17]. New onset DM in older age is associated with better glycemic control^[18] and with less frequent microvascular complications compared to long standing DM^[19]. Data from the National Health and Nutrition Examination Survey database found that although elderly with new-onset DM were 5 years older in average, they had much lower prevalence of retinopathy and a similar burden of macrovascular disease compared with long-standing DM. The difference in retinopathy rate may reflect the difference in DM duration between the two groups^[20].

The few studies that compared elderly subjects with new-onset DM to non-diabetic patients demonstrated short term elevation in all-cause and cardiovascular mortality and long term elevation of microvascular and macrovascular complications^[21-25]. None of these studies checked the association of glucose levels with mortality or diabetic complications and did not consider the influence of other cardiovascular risk factors on morbidity and mortality.

A large, observational study focused on the association between glycemic control and mortality in elderly patients with new-onset DM. This study followed almost 3000 elderly patients with new-onset DM for 7 years. A J-shaped relationship was found between HbA1c level and mortality rate. A HbA1c level above 7.5% was associated with significantly higher all-cause mortality, while the lowest mortality rate was found in subjects with HbA1c levels from 6.5% to 6.99%. This association remained statistically significant after adjustment for other conventional cardiovascular risk factors^[26].

In summary, the existing data suggest that new-onset DM in the elderly is associated with better glycemic control and better prognosis compared to long-standing DM in this population. However, when compared to elderly people with normal glucose levels, the new-onset DM patients have higher rates of morbidity and mortality (Table 2). There is some evidence that higher glucose levels within the diabetic range are associated with increased mortality.

The ADA guidelines do not deal separately with new-onset DM in elderly individuals, but mention the

Table 2 Studies comparing cardiovascular morbidity and mortality in elderly subjects with new-onset diabetes mellitus and subjects with normal glucose tolerance and long-standing diabetes mellitus

Ref.	No. of participants	Glycemic status	Age at inclusion(yr)	Length of follow up (yr)	Population	Results
Wang <i>et al</i> ^[19]	155	New-onset DM and long-standing DM	≥ 65	-	China	Microvascular complication rate was higher in long-standing DM
Selvin <i>et al</i> ^[20]	2809	New-onset DM and long-standing DM	≥ 65	-	United States	Microvascular complication rate was higher in long-standing DM
Smith <i>et al</i> ^[21]	1119	NGT and new-onset DM	≥ 65	5.9 (median)	United States	Mortality rate was higher in new-onset DM
Bethel <i>et al</i> ^[22]	59335	NGT and new-onset DM	≥ 65	10 (median)	United States	Microvascular and macrovascular complication rates were higher in new-onset-DM
Panzram <i>et al</i> ^[23]	2381	New-onset DM	All	10 (median)	German	Mortality rate was related to age of onset of DM and was higher in men
Croxson <i>et al</i> ^[24]	861	NGT, IGT, new-onset DM and long-standing DM	65-85	4.5 (median)	United Kingdom	New onset DM was associated with increased mortality
Tan <i>et al</i> ^[25]	10782	NGT and new-onset DM	≥ 65	4.6 (median)	Scotland	New onset DM was associated with increased mortality in females
Twito <i>et al</i> ^[26]	2994	New-onset DM	≥ 65	5.5 (mean)	Israel	Mortality rates in new-onset DM were associated with HbA1c levels

NGT: Normal glucose tolerance; IGT: Impaired glucose tolerance; DM: Diabetes mellitus.

duration of the disease as a parameter that should be considered when choosing HbA1c target levels^[4]. Vacante *et al*^[27] suggested combining the current age of the patient with the duration of DM.

LONG-STANDING DIABETES IN THE ELDERLY

As mentioned above, long-standing DM in the elderly has higher morbidity rates compared to new-onset DM. Therefore, the question is whether good glycemic control in elderly people with long-standing DM will influence the course of the disease.

In young and middle-aged diabetic patients, the role of tight glycemic control is crucial, as was proven in the Diabetes Control and Complications Study (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) in type 1 and type 2 DM, respectively^[28-30]. These two studies confirmed the benefit of intensive glycemic control in reducing microvascular and macrovascular complications^[31]. Nonetheless, these studies included only new-onset diabetic patients and excluded patients ages 65 years and above at the time of enrollment.

Almost 10 years after the publication of the UKPDS study, and 15 years after the DCCT study, 3 large randomized controlled trials examined the influence of intensive glycemic control on microvascular and macrovascular complications in older subjects with long standing DM. The Action to Control Cardiovascular Risk in Diabetes trial^[32] enrolled diabetic patients (mean

age 62.2 ± 6.8 years) 10 years after diagnosis, 35% with previous cardiovascular disease. This trial was terminated after 3 years because of excessive deaths in the intensive glycemic control arm. The Action in Diabetes and Vascular Disease trial^[33], which had also enrolled people with advanced disease (mean age 66 ± 6 years, average duration of diabetes 8 years, 32% with previous major macrovascular disease), showed no significant effect of tight glycemic control on major macrovascular events or death from any cause, but there was significant reduction in nephropathy incidence and as a result, reduction in the incidence of combined microvascular and macrovascular events. This reduction was proven only for patients younger than 65 years, according to a sub-group analysis published as part of the trial. The Veterans Affairs Diabetes Trial trial^[34] enrolled similar diabetic patients (mean age 60.5 ± 9 years, 11.5 years after diagnosis, 41% with major macrovascular disease) and also showed no significant effect of tight glycemic control on major macrovascular events or death from any cause. As one would expect, adverse events related to intensive glycemic control, such as hypoglycemia, were more common in the elderly^[35].

Few studies are directed to glycemic control in the old age. For example, the retrospective diabetes and aging study^[36] enrolled participants above 60 years of age (38% between 70-79 years and 15% age 80 or older); 57% had diabetes for more than 4 years at enrollment. There was a U-shaped relationship between

Table 3 Studies on the role of tight glycemic control in long-standing diabetes mellitus

Study	No. participants	Years since diagnosis	Mean age at enrollment (yr)	Length of follow up (yr)	Population	Results
ACCORD trial ^[32]	10251	10 (median)	62.2 ± 6.8	3.4 (median)	United States and Canada	Terminated after 3.5 yr; excessive deaths in the intensive glycemic control arm
ADVANCE trial ^[33]	11140	8 (mean)	66 ± 6	5 (median)	Asia, Australasia, Europe, and North America (20 countries)	No significant effect of tight glycemic control on major macrovascular events or death; significant reduction in nephropathy incidence
VADT trial ^[34]	1791	11.5 (mean)	60.5 ± 9	5.6 (median)	United States military veterans; 97% males	No significant effect of tight glycemic control on major macrovascular events or death
Diabetes and aging study ^[35]	71092	8.3 (mean)	71 ± 7.4	3.1 (mean)	California, United States	U-shape relationship between mortality and HbA1c, with the lowest mortality rate at HbA1c 6%-8%

ACCORD: Action to Control Cardiovascular Risk in Diabetes; ADVANCE: Action in Diabetes and Vascular Disease; VADT: Veterans Affairs Diabetes Trial.

mortality and HbA1c, with the lowest mortality rate at HbA1c 6%-8%, in all age-groups (Table 3).

The benefit of tight glycemic control for prevention of microvascular complications is not immediate. As proven in the UKPDS studies, the difference in outcome between the tight glycemic control group and the control group only appeared 9 years after randomization^[14,31]. In other words, for patients with life expectancy of 7 years or less, the benefit in this area is doubtful.

The complexities of diabetes care in old age, with benefits alongside potentially serious adverse events, led researchers to quality-adjusted life year (QALY) trials. Vijan *et al.*^[37] compared the QALY gained with intensive glycemic control versus moderate glycemic control, in different age-groups. They concluded that older patients, age 75 years and older, experience smaller benefit from glycemic control compared to younger patients, and their expected gain in QALYs for a 1-point change in HbA1c was minimal, even with the favorable assumption that the benefits of glycemic control extend to the elderly.

The results of the aforementioned studies resulted in changes in the clinical guidelines regarding treatment goals for elderly with long-standing DM. However, the guidelines offer general instructions and leave a large margin for clinical judgment.

The 2014 ADA guidelines^[4] recommend a standard glycemic goal of HbA1c below 7% for adults, and a less stringent goal, such as < 8% for patients with a more complex status, which is defined according to disease duration, life expectancy, important comorbidities, risk for adverse events and existing vascular complications. The International Association of Gerontology and Geriatrics and the European Diabetes Working Party for Older People published similar recommendations^[38].

The consensus report of the ADA and the American Geriatrics Society from 2012^[14], offered 3 levels of glycemic control for the old patient: HbA1c < 7.5% for healthy patients, < 8% for patients with intermediate health status (multiple chronic illnesses, 2+ instrumental impairments or mild cognitive impairment), and < 8.5% for patients with poor health status (end-stage chronic illnesses or moderate-to-severe cognitive impairment or 2+ activities of daily living dependencies). The International Diabetes Federation 2013 guidelines^[39], suggest a similar categorization. They also emphasize that for end-of-life situations, the goal should be merely to avoid symptomatic hyperglycemia (Table 4).

CONCLUSION

This review summarizes the current evidence about glycemic control in the elderly. Similar to young and middle-aged adults with DM, it seems that the elderly patient with diabetes has higher risks for morbidity and mortality compared to a non-diabetic person of the same age. Even so, new-onset DM is less severe in elderly patients compared to young adults and easier to control. The impact of pre-diabetes state on morbidity and mortality risk in the elderly is doubtful, and the role of screening and treatment in these patients is questionable. Finally, the importance of tight glycemic control on long-standing DM in the elderly in not well-established and the preferred level of glycemic control should be considered in the overall context of the patient's health status. The optimal level of control among elderly patient subgroups requires

Table 4 Guideline recommendations for glycemic control in the elderly

Article	Age	HbA1c target	Population
The 2014 ADA guidelines ^[4]	Any age	< 7% < 8%	Adult patients without serious comorbidities Patients with a more complex status (see text)
The consensus report of the ADA and the American Geriatrics Society 2012 ^[14]	> 65 yr	< 7.5% < 8% < 8.5%	Age itself not a criteria Healthy old patients Patients with intermediate health status (multiple chronic illnesses or 2+ instrumental impairments or mild cognitive impairment) Patients with poor health status (end-stage chronic illnesses or moderate to severe cognitive impairment or 2+ ADL dependencies)
The 2013 IDF guidelines ^[39]	> 70 yr	7%-7.5% 7%-8% < 8.5% Any HbA1c; just to avoid hypoglycemia	Functionally independent old patients Functionally dependent old patients Frail elderly or dementia Patients at end-of-life

ADA: American Diabetes Association; ADL: Activities of daily living; IDF: International Diabetes Federation.

further evaluation.

Beyond all the above, the heterogeneity of the elderly population presents a significant challenge in clinical decision making. Old diabetic patients can be healthy or with much comorbidity and the risks of adverse events from medications increases with age. The decision regarding an individual patient's glycemic goal should be made, ideally with the patient himself, after considering all the comorbidities, together with current cognitive state, risk of adverse events, quality of life aspects and life expectancy.

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Statin use and risk of diabetes mellitus

Bharti Chogtu, Rahul Magazine, KL Baiyy

Bharti Chogtu, KL Baiyy, Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka 576104, India

Rahul Magazine, Department of Pulmonary Medicine, Kasturba Medical College, Manipal University, Manipal, Karnataka 576104, India

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Correspondence to: Dr. Bharti Chogtu, Associate Professor, Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka 576104, India. bhartimagazine@gmail.com

Telephone: +91-820-2922365

Fax: +91-820-2922083

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Abstract

The 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors, statins, are widely used in the primary and secondary prevention of cardiovascular diseases to lower serum cholesterol levels. As type 2 diabetes mellitus is accompanied by dyslipidemia, statins have a major role in preventing the long term complications in diabetes and are recommended for diabetics with

normal low density lipoprotein levels as well. In 2012, United States Food and Drug Administration released changes to statin safety label to include that statins have been found to increase glycosylated haemoglobin and fasting serum glucose levels. Many studies done on patients with cardiovascular risk factors have shown that statins have diabetogenic potential and the effect varies as per the dosage and type used. The various mechanisms for this effect have been proposed and one of them is downregulation of glucose transporters by the statins. The recommendations by the investigators are that though statins can have diabetogenic risk, they have more long term benefits which can outweigh the risk. In elderly patients and those with metabolic syndrome, as the risk of diabetes increase, the statins should be used cautiously. Other than a subset of population with risk for diabetes; statins still have long term survival benefits in most of the patients.

Key words: New onset diabetes mellitus; Statins; Hyperglycemia; Cardiovascular risk; Dyslipidemia

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Core tip: The use of statins in diabetics has long term benefits in terms of decreasing morbidity and mortality. Recent studies have shown that statins increase the incidence of new onset diabetes. The issue became debatable after Food and Drug Administration released changes to statin safety that they increase glycosylated haemoglobin and blood glucose levels. At the same time statins are beneficial in preventing cardiovascular events. Most of the investigators are of the opinion that the risk of diabetes with statins can be outweighed by the long term benefits in preventing complications. In patients with high risk of diabetes, statins should be cautiously used.

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INTRODUCTION

Statins, the 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, inhibit the rate limiting step of conversion of HMG-CoA to mevalonate and thus limit cholesterol synthesis. Lower hepatic cholesterol levels subsequently increase expression of low density lipoprotein (LDL)-receptors in liver cells. This in turn leads to enhanced clearance of LDL-particles from blood. Lowering of plasma LDL-cholesterol by statins reduce production and increase catabolism of apo B 100^[1]. A range of products like coenzyme Q10, heme-A, and isoprenylated proteins are generated by mevalonate pathway^[2] which have an important role in cell biology and human physiology. The role of statins has been hypothesized to be widespread as in inflammatory markers and nitric oxide (NO)^[3], polyunsaturated fatty acids^[4], immunomodulation^[5], neuroprotection^[6], cellular senescence^[7], *etc.*

STATINS IN DIABETES

Statins are used for primary and secondary prevention of cardiovascular diseases. Other benefits due to statins are not mediated by their lipid lowering properties^[8] but due to its pleiotropic effects. In conditions like heart failure, cardiac arrhythmias, vascular disease and hypertension the non-lipid lowering pleiotropic benefits of statins have been observed^[9]. These pleiotropic effects mediated by statins can be due to inhibition of isoprenoid synthesis which in turn inhibits intracellular signaling molecules Rho, Rac and Cdc42. The predominant mechanism that has been postulated is inhibition of Rho and its activation to Rho kinase^[10].

Type 2 diabetes is characterized by hyperglycemia, insulin resistance and insulin deficiency. The insulin resistance contributes to the abnormal lipid profile associated with type 2 diabetes^[11]. Dyslipidemia contributes to increased cardiovascular events in patients with type 2 diabetes^[12]. A linear relationship exists between cholesterol levels and cardiovascular diseases in diabetics even if we ignore the baseline LDL^[13]. By predominantly lowering LDL-Cholesterol and due to minor effects on other lipoproteins, statins appear to be beneficial^[12]. In Heart Protection Study which was done in diabetics, the decrease in cardiovascular events like first major coronary event, stroke were to the tune of 22% as compared to placebo^[14]. It was recommended by American Diabetes Association that statin therapy should be initiated in individuals with diabetes and other cardiovascular risk factors with target LDL cholesterol of 100 mg/dL^[15]. Investigators are also of the opinion that statin therapy should depend not on the LDL levels but the cardiovascular complications accompanying diabetes^[16]. Other studies which showed

reduced coronary events with statins in patients with diabetes mellitus are Cholesterol and Recurrent Events and Long-term Intervention with Pravastatin in Ischaemic Disease studies of pravastatin^[17,18]. In The Collaborative Atorvastatin Diabetes Study, statins significantly reduced acute coronary events by 36% and stroke by 48%. The beneficial effects of statins were so clear in this study that it was halted two years in advance^[19]. West of Scotland Coronary Prevention Study also showed that the risk of diabetes was reduced by 30% in patients on pravastatin 40 mg/d^[20].

STATINS AND DIABETES RISK

In February 2012, Food and Drug Administration released changes to statin safety label to include that statins have been found to increase haemoglobin (HbA1C) and fasting serum glucose levels^[21]. This release has brought in the debate of using statins in patients with cardiovascular risk factors like diabetes.

In Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin study participants with LDL cholesterol levels of less than 130 mg/dL and elevated high-sensitivity C-reactive protein levels were included. They received rosuvastatin or placebo for a period of two years. It was observed that rosuvastatin significantly reduced the rates of a first major cardiovascular event and death from any cause as compared to placebo. A 54% lower risk of heart attack, 20% lower risk of stroke and 20% lower risk of death from any cause was noted in statin group^[22]. An increase in new onset diabetes, *i.e.*, 3% in statin arm and 2.4% in placebo arm was reported. This was accompanied by increase in median value of glycated haemoglobin and was one of the earlier studies to report the increase in new onset diabetes in patients on statins. Women's Health Initiative trial was a post hoc analysis and included 153840 postmenopausal women without diabetes mellitus. Even after adjustment for potential confounders, statin therapy was associated with an increased risk of new-onset diabetes mellitus^[23]. There was no difference between women with and without overt cardiovascular disease, which could have influenced the risk-benefit ratio of statins^[23]. Authors suggest that statin-induced diabetes mellitus is a medication class effect^[23]. Another study also reported that as compared to placebo, statin group showed a higher risk of physician reported incident diabetes and it was also observed that risk was higher in women as compared to men^[24].

Meta-analysis of randomized controlled trials by Sattar *et al.*^[25] involving 91140 non-diabetic patients showed that statin therapy was associated with 9% increased risk of incident diabetes. After a period of four years during which 255 patients were treated, there was one extra case of diabetes mellitus^[25]. Authors did not find any apparent difference between lipophilic and hydrophilic statins in association with

diabetes risk^[25].

Though some studies put forth this as a class effect, others showed different effects with different statins and at different doses. A number of studies showed dose dependent association between statin administration and incident diabetes. A meta-analysis with 32752 participants was done in which the risk of intensive dose statin therapy was compared with moderate dose statin therapy on incident diabetes. It revealed that intensive dose of statins was associated with high incidence of new - onset diabetes, though it decreased cardiovascular events as well^[26]. In those receiving intensive dose of statins, 18.9 ± 5.2 diabetic cases per 1000 patient years were observed vs 16.9 ± 5.5 cases per 1000 patient years with moderate doses of statin therapy^[26].

In PROVE-IT TIMI 22 trial 3382 patients without pre-existing type 2 diabetes mellitus were included. The levels of HbA1C increased by 0.12% in patients treated with pravastatin 40 mg, while in those receiving atorvastatin 80 mg showed a significant difference and the levels increased by 0.30%^[27]. Another study comparing glycaemic control between diabetic patients receiving atorvastatin 10 mg, pravastatin 10 mg or pitavastatin 2 mg/d showed that it was only the atorvastatin-treated patients in which the blood glucose and HbA1C levels increased^[28]. Treatment with atorvastatin and simvastatin may be associated with an increased risk of new onset diabetes as compared to pravastatin^[29]. Pitavastatin has shown favourable profile in patients with diabetes by improving insulin resistance and minimally impairing glucose metabolism^[30]. Increased incidence of diabetes was seen with atorvastatin in the Anglo-Scandinavian Cardiac Outcomes Trial^[31] and impaired glucose metabolism in some cases of type 2 diabetes^[32]. Increased insulin resistance secondary to statins was demonstrated in a prospective non randomised study in patients with coronary bypass surgery^[33]. So, many studies have reported an increase in new onset diabetes and there is a variation in response depending on the statin administered and the dose of statin.

MECHANISM OF STATINS IN HYPERGLYCEMIA

Statin-induced insulin resistance can be due to inhibition of isoprenoid biosynthesis and downregulating C/EBP α production^[34]. Decreased synthesis of isoprenoids can produce downregulation of GLUT4 expression on adipocyte cells^[35]. It can lead to decrease in insulin-mediated cellular glucose uptake and possibly manifest as intolerance to glucose^[36]. Acceleration of type 2 diabetes can be seen secondary to downregulation of GLUT4/SLC2A4 in adipocytes^[37]. Over-production of NO by inducing cytokines can cause β -cell apoptosis^[38].

On incubating rat pancreatic cells with statin, there was a decrease in insulin secretion due to inhibition of glucose stimulated increase in free cytoplasmic

calcium^[39]. Statins also inhibit the insulin secretion due to reduced production of ATP by suppressing the synthesis of ubiquinone (CoQ10)^[39]. Clinical doses of atorvastatin in animal model of type 2 diabetes led to inhibition of adipocyte differentiation, decreased SLC2A4 expression in both differentiating and mature adipocytes, and impaired insulin sensitivity and post-challenge glucose tolerance^[34]. Animal models have also shown that there is an association between development of insulin resistance and statin-induced myopathy^[40].

Other mechanisms hypothesized for the possible effect of statins on new-onset diabetes are that statins by inhibiting phosphorylation interfere with intracellular signal transduction pathways of insulin, reduce action of small GTPase, decrease peroxisome proliferator activated receptor gamma by inhibiting the differentiation of adipocytes, inhibit β -cell proliferation and insulin secretion by inhibiting leptins^[41]. Atorvastatin, a lipophilic statin, may decrease insulin secretion due to increased HMG-CoA inhibition or cytotoxicity^[42]. The actions of statins on beta cells of pancreas can be summarised^[43] as shown in Figure 1.

RECOMMENDATIONS FOR USE OF STATINS IN DIABETES

Authors have put forth the recommendations from time to time regarding the use of statins in patients with cardiovascular diseases. For patients with cardiovascular risk factors, statins prevent cardiovascular event 8 times more likely than they can cause a case of incident diabetes^[44] shifting the risk-benefit ratio in favour of statin therapy^[44]. Modest increase in blood glucose levels by statins will not be an issue of concern if they decrease morbidity and mortality due to macrovascular and microvascular complications^[45]. In patients with low cardiovascular risk factors, statins should be cautiously used, less aggressive LDL-C-lowering targets should be kept and monitoring of fasting blood glucose levels should be done routinely^[46].

In high risk patients with impaired glucose tolerance and established cardiac risk factors, statins and diuretics increased the risk of new onset diabetes. As both the drugs have a propensity to increase blood glucose levels, there is a need of regular monitoring^[47]. As compared to other cardiovascular medications like thiazide diuretics and beta blockers, statins can three times less likely cause diabetes^[48].

When statins are being used in primary prevention patients at high risk of diabetes, pravastatin should be preferred over other statins^[29]. One of the meta-analysis comparing high-dose statin therapy with moderate dose found that the former is associated with improved cardiovascular outcomes, though at the same time there was a 12% increased risk of new-onset diabetes mellitus^[49]. Such studies have stimulated the controversy about the treatment of patients not attaining target lipid profile on moderate dose of statins^[49].

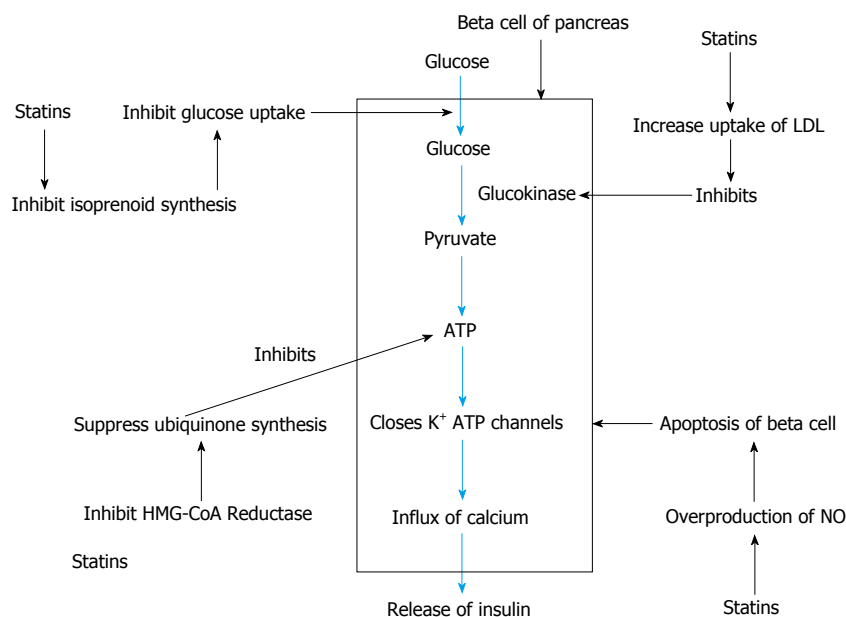


Figure 1 Actions of statins on beta cell of pancreas^[43]. HMG-CoA: 3-hydroxy-methylglutaryl coenzyme A; NO: Nitric oxide; LDL: Low density lipoprotein.

Increasing age increases the risk of diabetes and benefits secondary to statins can decrease. There is a need to be vigilant in these patients^[50]. Factors like older age, increased weight, and higher blood sugar levels before the use of statins predict that whether a patient will develop diabetes mellitus. The use of statins can unmask diabetes mellitus in patients with other risk factors^[51]. So in obese patients and those with metabolic syndrome these findings may be relevant^[52]. While analysing one of the initial studies which suggested the link between statins and diabetes, it was found that rate of reduction of cardiovascular events outbalanced the risk of incident diabetes even in patients at highest risk for diabetes though the absolute risk increase was small (placebo 1.2%, rosuvastatin 1.5% developed diabetes^[53]). Meta-analysis of individual data of over 170000 persons from 27 randomized trials also put forth the risk benefit ratio in favour of statins^[54]. In patients on statins, there was an improved outcome after cardiac surgery^[55]. Current guidelines recommend use of statins in patients undergoing coronary artery bypass graft^[56]. Statins can reduce cardiovascular complications like atrial fibrillation and MI after cardiac surgery, but at the same time poor glycemic control may lead to deterioration of non-cardiovascular complications like infections and renal complications^[33].

Elevated triglycerides and low HDL-C are associated with type 2 diabetes mellitus. The evidence for drugs targeting this type of dyslipidemia is not as strong as those targeting LDL-C^[57].

To conclude, physicians should be cautious about development of diabetes in patients on intensive statin therapy^[26]. Lifestyle management should be considered in patients with low risk of cardiovascular diseases^[58] and the use of statins should be reconsidered^[58]. In patients with cardiovascular risk factors, the benefits of statins supersede the risk of diabetes^[59]. There is a need

of randomized clinical trials to find the role of statins on microvascular complications as the existing evidence only shows a benefit on macrovascular complications^[60]. Overall evidence at present shows that the risk of new onset diabetes is less as compared to the long term benefits of statins in patients with cardiovascular risk factors. But there is a small subgroup of population in whom a more careful use of statins is mandatory.

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Case Control Study

Association of gene variants with susceptibility to type 2 diabetes among Omanis

Sawsan Al-Sinani, Nicolas Woodhouse, Ali Al-Mamari, Omaima Al-Shafie, Mohammed Al-Shafae, Said Al-Yahyaee, Mohammed Hassan, Deepali Jaju, Khamis Al-Hashmi, Mohammed Al-Abri, Khalid Al-Rassadi, Syed Rizvi, Yengo Loic, Philippe Froguel, Riad Bayoumi

Sawsan Al-Sinani, Nicolas Woodhouse, Ali Al-Mamari, Omaima Al-Shafie, Mohammed Al-Shafae, Said Al-Yahyaee, Mohammed Hassan, Deepali Jaju, Khamis Al-Hashmi, Mohammed Al-Abri, Khalid Al-Rassadi, Syed Rizvi, Riad Bayoumi, Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Sultanate of Oman

Yengo Loic, Philippe Froguel, Genomics and Metabolic Disease, CNRS UMR8199, 59045 Lille Cedex, France

Yengo Loic, Philippe Froguel, Institute Pasteur of Lille, Lille2 University, 59000 Lille, France

Author contributions: Al-Sinani S and Bayoumi R performed the majority of design and experiments; Al-Sinani S, Woodhouse N, Al-Mamari A, Al-Shafie O and Al-Shafae M helped in patients selection and samples collection; Al-Sinani S, Woodhouse N, Al-Mamari A, Al-Shafie O, Al-Shafae M, Al-Yahyaee S, Hassan M, Jaju D, Al-Hashmi K, Al-Abri M, Al-Rassadi K and Bayoumi R designed the study and helped in writing the manuscript; Al-Sinani S, Rizvi S, Loic Y and Froguel P helped in statistical analysis.

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Correspondence to: Sawsan Al-Sinani, PhD Biochemistry, Department of Biochemistry, College of Medicine and Health

Sciences, Sultan Qaboos University, PO Box-35, Muscat 123, Sultanate of Oman. sawsan.alsinani@gmail.com

Telephone: +968-24-141113

Fax: +968-24-141114

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Abstract

AIM: To investigate the association of 10 known common gene variants with susceptibility to type 2 diabetes mellitus (T2D) among Omanis.

METHODS: Using case-control design, a total of 992 diabetic patients and 294 normoglycemic Omani Arabs were genotyped, by an allelic discrimination assay-by-design TaqMan method on fast real time polymerase chain reaction system, for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634) *CAPN10* (rs3792267) and *HHEX* (rs1111875). T2D patients were recruited from the Diabetes Clinic ($n = 243$) and inpatients ($n = 749$) at Sultan Qaboos University Hospital (SQUH), Muscat, Oman. Adult control participants ($n = 294$) were volunteers from the community and from those visiting Family Medicine Clinic at SQU, for regular medical checkup. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study. Almost all volunteers questioned had a relative

with diabetes mellitus. In spite of the small number of normoglycemic controls in this study, this sample was sufficient for detection of genes and loci for common alleles influencing T2D with an odds ratio of ≥ 1.3 reaching at least 80% power. Data was collected from June 2010 to February 2012.

RESULTS: Using binary logistic regression analysis, four gene variants showed significant association with T2D risk: *KCNJ11* (rs5219, $P = 5.8 \times 10^{-6}$, OR = 1.74), *TCF7L2* (rs7903146, $P = 0.001$, OR = 1.46), *CDKAL1* (rs10946398, $P = 0.002$, OR = 1.44) and *CDKN2A/B* (rs10811661, $P = 0.020$, OR = 1.40). The fixation index analysis of these four gene variants indicated significant genetic differentiation between diabetics and controls {[*KCNJ11* (rs5219), $P < 0.001$], [*TCF7L2* (rs7903146), $P < 0.001$], [*CDKAL1* (rs10946398), $P < 0.05$], [*CDKN2A/B* (rs10811661), $P < 0.05$]}. The highest genotype variation % between diabetics and controls was found at *KCNJ11* (2.07%) and *TCF7L2* (1.62%). This study was not able to detect an association of T2D risk with gene variants of *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Moreover, no association was found between *FTO* gene variants (rs9939609 and rs8050136) and T2D risk. However, T2D risk was found to be significantly associated with obesity ($P = 0.002$, OR = 2.22); and with the Waist-to-Hip ratio ($n = 532$, $P = 1.9 \times 10^{-7}$, OR = 2.4), [among males ($n = 234$, $P = 1.2 \times 10^{-4}$, OR = 2.0) and females ($n = 298$, $P = 0.001$, OR = 6.3)].

CONCLUSION: Results confirmed the association of *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661) gene variants with susceptibility to T2D among Omani Arabs.

Key words: Type 2 diabetes; Genetics; Oman; Case-control; Association; Gene; Variants

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Core tip: To investigate the association of 10 known common gene variants with susceptibility to type 2 diabetes mellitus (T2D) among Omani Arabs using case-control design. A total of 992 diabetic patients and 294 normoglycemic Omani Arabs were genotyped for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Four gene variants showed significant association with T2D risk: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). The highest genotype variation % between diabetics and controls was found at *KCNJ11* and *TCF7L2* gene variants.

Shafae M, Al-Yahyaee S, Hassan M, Jaju D, Al-Hashmi K, Al-Abri M, Al-Rassadi K, Rizvi S, Loic Y, Froguel P, Bayoumi R. Association of gene variants with susceptibility to type 2 diabetes among Omanis. *World J Diabetes* 2015; 6(2): 358-366 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i2/358.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i2.358>

INTRODUCTION

Type 2 diabetes mellitus (T2D) is one of the most common non-communicable diseases globally. Insufficient compensatory insulin secretion due to insulin resistance causes T2D. Insulin resistance is, mostly, an early event due to environmental factors, such as obesity. Decline in β -cell function is gradual but generally a late event^[1]. In addition to the environmental factors, there is strong evidence that genetic factors play an important role in the pathogenesis of T2D^[2].

Candidate gene approach identified few T2D susceptibility gene variants: *Pro12Ala* (rs1801282) in the coding region of peroxisome proliferator-activated receptor γ gene and it is the more common proline allele that is associated with T2D^[3]; *E23K* (rs5219) in the coding region of the subunit kir6.2 of the ATP-sensitive potassium channel gene of β -cells (*KCNJ11*)^[4] and a series of polymorphisms and haplotypes (UCSNP-43 or rs3792267; UCSNP-19 or rs3842570 and UCSNP-63 or rs5030952) in the coding region of the cysteine protease calpain 10 (*CAPN10*)^[5].

Genome-wide association studies (GWAS) of T2D susceptibility genes and loci and their meta-analysis identified a large number of gene variants and confirmed the previously discovered ones^[6]. The common intronic variants within the transcription factor 7-like 2 (*TCF7L2*) gene was reported as the strongest genetic risk factor for T2D^[7]. Other loci most consistently associated with T2D risk include variants within or near the solute carrier family 30/zinc transporter (*SLC30A8*), hematopoietically expressed homeobox (*HHEX*), cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*), insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*), a genomic region between cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) and fat mass and obesity associated protein (*FTO*)^[6,8].

In total, approximately, forty four common T2D susceptibility gene variants and loci have been identified to-date, but all these variants could only explain approximately 10%-15% of the heritability of T2D; which suggests that more variants remain to be discovered^[9]. Rare large-effect mutations have recently been recognized as causes of many complex diseases^[10-12].

According to the international diabetes federation, six out of the world's top ten countries with the highest prevalence (%) of T2D among adults aged 20-79 years, in 2011, are in the Middle East and North Africa region; Kuwait (21.1%), Lebanon (20.2%), Qatar (20.2%), Saudi Arabia (20.0%), Bahrain (19.9%) and

United Arab Emirates (19.2%). Small studies were conducted among Arabs, with a limited number of participants, to investigate genetic susceptibility for T2D^[7,13-24].

The prevalence of diabetes in Oman, in 2011, was estimated to be 10.8%; with a further 9.7% of the population at high risk of diabetes with impaired glucose tolerance, (<http://www.idf.org/diabetesatlas/content/what-is-diabetes>). Oman has a high inbred population and consanguineous marriages are about half of all marriages^[25]. Therefore, genetic factors might play an important role in the pathogenesis of T2D among Omanis.

In the present study, 10 known common gene variants, described previously, were examined for their association with susceptibility to T2D among Omani Arabs using case-control study design. Selection of variants was predominantly based on earlier GWAS studies, which extensively investigated T2D and showed a significant association of those variants with the highest odds ratios (ORs) among all the genes/loci discovered^[4-8,26].

MATERIALS AND METHODS

Sample size

For determining the sample size, we employed the Power Calculator for Genetic Studies developed by Skol *et al.*^[27] (2006) in their Website <http://www.sph.umich.edu/csg/abecasis/CaTS/index.html>. We used a T2D prevalence of 10% in the adult population of the Region as reported previously^[28,29]. We also anticipated disease allele frequencies of ≥ 0.25 , and assumed a multiplicative disease model^[30]. An optimum one-stage sample was deduced from the Power Calculator: 1000 cases and 1000 controls, will guarantee detection of genes and loci for common alleles influencing T2D with an OR of ≥ 1.2 reaching at least 80% power. However, we could not collect the required sample size, and only 992 cases and 294 controls were collected. This sample will guarantee detection of genes and loci for common alleles influencing T2D with an OR of ≥ 1.3 reaching at least 80% power.

Study population

A total of 992 T2D Omani Arab patients and 294 normoglycemic Omani Arab controls were included in this study. T2D patients were recruited from the Diabetes Clinic ($n = 243$) and inpatients ($n = 749$) at Sultan Qaboos University Hospital (SQUH), Muscat, Oman. A history of T2D among patients was ascertained from the diagnosis and medical history deposited in the electronic records of the hospital information system. Exclusion criteria for T2D patients included: patients diagnosed with type 1 diabetes; maturity onset diabetes of the young; positive diabetic antibodies (islet cell antibodies and glutamic acid decarboxylase antibodies) or patients diagnosed with any type of cancer. Adult

control participants ($n = 294$) were volunteers from the community and from those visiting Family Medicine Clinic at SQU, for regular medical checkup. The inclusion criteria for controls were: Omani, age ≥ 35 years, no family history of diabetes (first degree relatives) and with fasting glucose value of < 6.1 mmol/L, according to the World Health Organization 2006 criteria. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study. Almost all volunteers questioned had a relative with diabetes mellitus (DM). Data was collected from June 2010 to February 2012. Participants were informed about the project and written consents were obtained. The study was approved by the Ethics and Research Committee of the College of Medicine, Sultan Qaboos University, Muscat, Oman.

Anthropometric and biochemical parameters

T2D patients and normoglycemic control participants underwent demographic, anthropometric and biochemical investigations, summarized in Table 1. Anthropometric variables measured were: weight, height, waist and hip circumference. Obesity status was defined according to the international classification of an adult's weight (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html), [normal body mass index (BMI): 18.5-24.99 kg/m², overweight: 25.00-29.99 kg/m² and obese ≥ 30.00 kg/m²]. The biochemical investigations included: fasting glucose level and HbA_{1c}. To compare T2D patients and normoglycemic control participants' obesity status, we selected 294 T2D patients; age and sex matched with the normoglycemic control participants ($n = 294$). Waist-to-Hip ratio (WHR) was also calculated among T2D patients and control participants. Health risk based solely on the WHR, was identified according to the ranges specified at waist-to-hip ratio chart (<http://www.bmi-calculator.net/waist-to-hip-ratio-calculator/waist-to-hip-ratio-chart.php>) for males (low risk = 0.95 or below, moderate risk = 0.96-1.0, high risk = over 1.0) and females (low risk = 0.80 or below, moderate risk = 0.81-0.85, high risk = over 0.85).

Genotyping

All participants ($n = 1286$) were genotyped for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Genotyping was done by an allelic discrimination assay-by-design TaqMan method on 7500HT fast real time polymerase chain reaction system (Applied Biosystems, United States). Accuracy was achieved by duplicating approximately 10% of the samples.

Statistical analysis

The SPSS statistical package software (v20.0) was

Table 1 Anthropometric and biochemical characteristics of Omani type 2 diabetes mellitus patients and controls

	T2D patients			Controls		
	Mean \pm SD or median (range) ¹			Mean \pm SD or median (range) ¹		
	Males	Females	Total	Males	Females	Total
Total number (n)	473	519	992	121	173	294
Age (yr)	56 \pm 11	56 \pm 10	56 \pm 11	41 (35-80) ¹	44 (32-79) ¹	43 (32-80) ¹
Weight (kg)	78.8 \pm 15.4 ^{NS}	75.1 \pm 17.0	76.8 \pm 16.4	76.8 \pm 14.2	70.3 \pm 13	73.7 \pm 14.7
Height (cm)	165 \pm 9 ^a	152 \pm 9	157 (106-182) ^{INS}	167 \pm 8	154 \pm 6	159 \pm 9
BMI (kg/m ²)	29.1 \pm 4.8 ^b	32.8 \pm 10.3	30 (15-58) ¹	27.6 \pm 4.3	29.5 \pm 5.2	29.3 \pm 5.8
Waist circumference (cm)	100 \pm 12	100 \pm 13	100 \pm 13	95 \pm 13	91 \pm 11	92 \pm 12
Fasting blood glucose (mmol/L)	8.7 (3-24) ¹	9.0 (3-25) ¹	8.8 (3-25) ¹	5.1 \pm 0.31	4.9 \pm 0.36	5.1 \pm 0.41
HbA _{1c} (%)	8.3 \pm 1.8	8.3 (4.9-15.5) ¹	8.2 (4.1-18.6) ¹	5.7 \pm 0.46	5.7 (4.0-7.9) ¹	5.7 \pm 0.44
Obesity status (%)						
Underweight	-	0.5	0.3	2.1	1	1.4
Normal weight	16.8	12.5	14.5	22.2	18.2	19.1
Overweight	45	30.2	36.1	43.8	34.5	38.9
Obese	38.2	56.8	49.1	29.9	45.8	39.6
Missing	-	-	-	2.1	0.5	1

¹Median (range = minimum-maximum) displayed in the table when the variable does not follow a normal distribution pattern. In all parameters, $P < 0.001$ between diabetics and controls, except: NS; ^a $P < 0.05$ between diabetics and controls; ^b $P < 0.01$ between diabetics and controls. NS: No significant difference between diabetics and controls; T2D: Type 2 diabetes mellitus; BMI: Body mass index.

used for statistical analysis of measured parameters. The measured anthropometric and biochemical parameters were tested for normal distribution using one sample Kolmogorov-Smirnov test. Independent sample *t*-test was used to test the significance of the difference in the mean values for the measured anthropometric and biochemical parameters between T2D patients and control participants with a normal distribution, while the Mann-Whitney *U* test was used for variables with skewed distribution.

The frequencies of the risk allele for each gene variant were calculated for T2D patients and normoglycemic control participants. The proportions of the genotypes of the gene variants were tested for departures from Hardy-Weinberg equilibrium (HWE) for both groups using population genetics software GenAEx 6.3 (Genetic analysis in Excel, version 6.3)^[31]. However, in case-control studies, HWE should be applied only to controls because a deviation from HWE in cases may indicate a genetic association^[32,33].

Genotyping data were further analyzed using GenAEx 6.3^[31] and Arlequin 3.1 software^[34]. For each polymorphism, GenAEx was used to calculate fixation index (*F*), heterozygosity (*He*) and *F*_{st}, which provides a measure of genetic differentiation among subpopulations (T2D patients and control populations). Arlequin was used to calculate genotype's % variation among the subpopulations and its level of significance.

Binary logistic regression analysis on the SPSS statistical package was used to test the association between each gene variant and susceptibility to T2D, adjusted for age, sex and BMI. Bonferroni correction was applied for multiple testing and adjusted *P* values were calculated to be 0.005. Beta coefficients, ORs and 95%CI were also estimated. An OR is a measure of association between an exposure and an outcome. It is measured as an exponential function of the regression

beta coefficient value ($e^{\text{beta coefficient}}$).

The association between obesity status of participants and *FTO* gene variants (rs9939609 and rs8050136) was also tested. In addition, obesity and health risk status, based on the WHR, were tested for their association with T2D risk.

RESULTS

Anthropometric and biochemical characteristics of all participants are summarized in Table 1. About 48% of the T2D patients and 41% of the control participants were males. The mean age of T2D patients (56 years) was higher than that of the normoglycemic control participants (45 years). T2D patients had significantly higher weight, BMI, waist circumference, fasting glucose values and HbA_{1c} % levels compared with control subjects (Table 1). Eighty five percent of the T2D Omani patients were overweight to obese in comparison to 78.5% of the control Omani participants. Half of the T2D patients and 39.6% of the control participants were obese. T2D risk was found to be significantly associated with obesity ($P = 0.002$, OR = 2.22); and with the WHR ($n = 532$, $P = 1.9 \times 10^{-7}$, OR = 2.4), [among males ($n = 234$, $P = 1.2 \times 10^{-4}$, OR = 2.0) and females ($n = 298$, $P = 0.001$, OR = 6.3)].

Among control participants, there were no significant deviation in the proportions of gene variant frequencies from HWE except in *SLC30A8* (rs13266634) ($P = 2.35 \times 10^{-4}$, $\chi^2 = 13.5$) gene variant. However, among T2D patients, there were significant deviations in: *KCNJ11* (rs5219) ($P = 4.14 \times 10^{-9}$, $\chi^2 = 34.6$), *CDKAL1* (rs10946398) ($P = 0.008$, $\chi^2 = 6.9$) and *IGF2BP2* (rs4402960) ($P = 0.038$, $\chi^2 = 4.3$) gene variants.

The risk allele frequencies of the tested variants for diabetic and control participants are summarized in Table 2. Using binary logistic regression analysis,

Table 2 Risk allele frequencies for the tested gene variants among Omani type 2 diabetes mellitus patients and control participants

Gene	Gene variant (SNPs)	Risk/non-risk allele	Risk allele frequency (f)		¹ P value	OR	95%CI for OR
			T2D patients (n = 992)	Controls (n = 294)			
<i>KCNJ11</i>	rs5219	T/C	0.320	0.222	² 5.8 × 10 ⁻⁶	1.74	1.37-2.22
<i>TCF7L2</i>	rs7903146	T/C	0.445	0.354	² 0.001	1.46	1.16-1.83
<i>CDKAL1</i>	rs10946398	C/A	0.364	0.311	² 0.002	1.44	1.15-1.80
<i>CDKN2A/B</i>	rs10811661	T/C	0.836	0.799	0.020	1.40	1.06-1.84
<i>FTO</i>	rs9939609	A/T	0.480	0.435	0.358	1.11	0.899-1.37
<i>FTO</i>	rs8050136	A/C	0.458	0.425	0.770	1.03	0.829-1.29
<i>IGF2BP2</i>	rs4402960	T/G	0.400	0.357	0.286	1.13	0.904-1.41
<i>SLC30A8</i>	rs13266634	C/T	0.857	0.855	0.329	1.16	0.859-1.57
<i>CAPN10</i>	rs3792267 (-43)	G/A	0.802	0.790	0.445	1.11	0.850-1.45
<i>HHEX</i>	rs1111875	T/C	0.301	0.280	0.636	1.06	0.839-1.33

¹P value: Level of significance; ²P value remained significant after correction for multiple testing (< 0.005). The P value, OR and 95%CI were calculated for the association between each gene variant with T2D risk. f: Frequency; T2D: Type 2 diabetes mellitus; SNPs: Single nucleotide polymorphisms.

Table 3 Fixation index, heterozygosity, Fst and % variation among Omani type 2 diabetes mellitus patients and control participants

Gene	Gene variants	T2D patients		Controls		Fst	% variation among T2D and controls	¹ P value
		F	He	F	He			
<i>KCNJ11</i>	rs5219	0.190	0.352	0.091	0.314	0.012	2.07	0.000
<i>TCF7L2</i>	rs7903146	0.017	0.486	0.000	0.476	0.009	1.62	0.000
<i>CDKAL1</i>	rs10946398	0.084	0.424	0.056	0.405	0.003	0.48	0.020
<i>CDKN2A/B</i>	rs10811661	0.000	0.275	0.003	0.320	0.002	0.37	0.042
<i>FTO</i>	rs9939609	0.000	0.502	0.000	0.497	0.002	0.31	0.050
<i>FTO</i>	rs8050136	0.006	0.493	0.000	0.503	0.001	0.12	0.147
<i>IGF2BP2</i>	rs4402960	0.066	0.448	0.000	0.476	0.002	0.23	0.089
<i>SLC30A8</i>	rs13266634	0.061	0.230	0.188	0.201	0.000	0.00	1.000
<i>CAPN10</i>	rs3792267	0.057	0.300	0.000	0.335	0.000	0.00	0.517
<i>HHEX</i>	rs1111875	0.055	0.398	0.052	0.382	0.001	0.00	0.341

This provides a measure of genetic differentiation among population; ¹P value: Level of significance; F: Fixation index; He: Heterozygosity; Fst: The inbreeding coefficient within subpopulation, relative to total; T2D: Type 2 diabetes mellitus.

four gene variants out of 10 showed statistically significant association ($P < 0.05$) with susceptibility to T2D: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661), Table 2. After correction for multiple testing, *KCNJ11*, *TCF7L2* and *CDKAL1* gene variants still showed a significant association ($P < 0.005$) with T2D.

Fst values, showed statistically significant genetic differentiation between T2D patients and controls (Table 3), in the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). These findings confirmed the results obtained using binary logistic regression analysis.

This study was not able to detect an association of T2D risk with gene variants of *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Moreover, no association was found between *FTO* gene variants (rs9939609 and rs8050136) and T2D risk.

DISCUSSION

In this study, four gene variants showed significant association with T2D risk using binary logistic regression analysis after adjustment for confounding factors

of age, sex and BMI: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). The association of *KCNJ11* (rs5219), *TCF7L2* (rs7903146) and *CDKAL1* (rs10946398) gene variants with T2D risk remained significant after correction for multiple testing. Fst, a measure of genetic differentiation among subpopulations (diabetics and controls), confirmed the significant risk difference between diabetics and controls at the four gene loci [*KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661)]. The highest genetic variation between diabetics and controls was found in *KCNJ11* and *TCF7L2* gene variants (Table 3). However, none of the other gene variants previously reported in GWAS were found to be associated with risk to T2D in Omanis.

KCNJ11 (rs5219) gene variant was found to be associated with T2D risk among Omani Arabs with an OR of 1.74, which is higher than that reported in previous European studies. Our findings were consistent with what was reported among Saudi Arabs (OR = 1.7), although the risk allele frequency was found to be lower among Saudis compared to Omani Arabs^[16]. However, in both Arab studies the association may be overestimated due to the small number of participants included or due to a high-inbred population.

In contrast, no association of this gene variant with susceptibility to T2D was found among Tunisian Arabs^[14]. Large scale studies and meta-analysis of the *KCNJ11* gene variants have shown that the lysine variant of the rs5219 gene loci resulting in a 1.15 times higher risk of developing T2D^[4,35] and GWAS studies confirmed this association^[26,36,37]. The rs5219 variant is located within the N-terminal of the subunit kir6.2 of the ATP-sensitive potassium channel gene of β -cells and can cause spontaneous hyperactivity of pancreatic beta-cells and reduced sensitivity of KATP channels to ATP, resulting in impaired insulin secretion^[38].

Although this study is relatively small, the association of *TCF7L2* (rs7903146) gene variant with T2D risk among Omani Arabs is consistent with previous large GWAS studies^[14,15,22,36,37,39-44]. The association of *TCF7L2* gene variants with susceptibility to T2D was marginal between the rs12255372 gene variant and T2D risk among Emirati Arabs; but not in Saudi Arabs^[13,17]. In contrast, subsequent studies among North African Arabs (Tunisians and Moroccans), Palestinians and Iranians, confirmed the association of *TCF7L2* (rs7903146) gene variant with susceptibility to T2D^[7,14,15,22]. Comprehensive genotyping studies across the *TCF7L2* gene showed that the rs7903146 variant to be consistently associated with T2D among European with an OR of 1.37 (1.28 to 1.47)^[45,46]. Meta-analysis of 27 different studies found a global OR of 1.46 (95%CI: 1.42-1.51)^[7]. The common variants in the *TCF7L2* gene predispose to T2D by reducing beta-cell function and insulin secretion. *TCF7L2* mRNA levels in human pancreatic islets increase with the number of risk alleles and are fivefold higher in T2D patients than in controls pancreatic islets, and over-expression of *TCF7L2* leads to reduced glucose stimulated insulin secretion^[47].

CDKAL1 (rs10946398) gene variant's OR was found to be 1.44 among Omani Arabs, which may be overestimated due to the small number of participants. Variants at *CDKAL1* gene loci showed an association with T2D risk among Caucasians^[26,36,37], Asians^[48,49], African Americans^[50] and Arabs^[22,24]. A recent meta-analysis of *CDKAL1* (rs10946398) gene variant showed a significant association of this variant with susceptibility to T2D risk (OR = 1.12)^[51]. Another study among Russian population showed a significant association of the C allele with higher risk of T2D^[52]. The *CDKAL1* gene encodes cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1. The CDK5 is a serine/threonine enzyme that inhibits both Ca^{2+} efflux into the beta-cell and insulin secretion, while inhibition of this enzyme results in enhanced insulin secretion^[53].

The association of *CDKN2A/B* (rs10811661) gene variant with susceptibility to T2D risk among Omanis was also confirmed in this study (OR = 1.40), in agreement with a recent large study among North African Arabs^[22]. In contrast, this gene variant was not found to be associated with T2D risk in other Arab studies^[24,54]. Previous studies among European populations have

shown an association with an OR of 1.20^[26,36,37]. A recent meta-analysis concluded that the T allele of rs10811661 is a risk factor of T2D both in Asians and Europeans^[55]. *CDKN2A* and *CDKN2B* genes encode p16^{INK4a} and p15^{INK4b}, which inhibit CDK4 and CDK5, respectively. CDK4 and CDK5 play an important role in β cell function and regeneration^[55].

FTO gene variants (rs9939609 and rs8050136) have been shown to associate with BMI and obesity^[8,56] and GWAS studies of T2D have also suggested the involvement of *FTO* gene in T2D pathogenesis through obesity^[8,26]. It is surprising, therefore, that we could not detect an association of T2D risk with *FTO* gene variants (rs9939609 and rs8050136). This could be attributed to the fact that both diabetics and controls have similar distributions of body weight. The impact of the *FTO* gene variants on T2D risk through obesity, seen in other populations, was not observed here. Hennig *et al.*^[57] tested the effect of *FTO* variants on measures of BMI in a population of lean Gambians (Africans) and also found no association. However, this study showed a significant association between obesity and T2D risk; and between health risk, based on the WHR, and T2D risk among males and females.

In spite of the small number of participants examined in this case-control study, we were able to confirm the effect of four common gene variants on T2D risk among Omani Arabs. Oman has a homogeneous population due to a high level of inbreeding and the tradition of consanguineous marriages. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study, where, almost everybody has a relative with DM. This might have raised risk allele frequencies of *T2D* gene variants and made it easier to detect.

All previous GWAS identified common gene variants, which could only explain 10%-15% of the heritability of T2D. Large studies with new strategies, other than the classic case-control study design, are required to find the hidden heritability due to rare variants behind developing T2D among Omani and other Arab populations.

Limitation of this study was the lack of oral glucose tolerance test, where we could not run the test among the control group. However, the strength of this study was that the control participants were with no family history of diabetes.

This study confirmed the effect of four common gene variants on T2D risk among Omani Arabs: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). However, we could not detect the association of other known common gene variants with susceptibility to T2D.

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COMMENTS

Background

Type 2 diabetes mellitus (T2D) is one of the most common non-communicable diseases globally. Insufficient compensatory insulin secretion due to insulin resistance causes T2D. In addition to the environmental factors, there is strong evidence that genetic factors play an important role in the pathogenesis of T2D.

Research frontiers

Oman has a high inbred population and consanguineous marriages are about half of all marriages. Therefore, genetic factors might play an important role in the pathogenesis of T2D among Omanis.

Innovations and breakthroughs

In the present study, 10 known common gene variants were examined for their association with susceptibility to T2D among Omani Arabs using case-control study design. Selection of variants was predominantly based on earlier Genome-wide association studies, which extensively investigated T2D and showed a significant association of those variants with the highest odds ratios among all the genes/loci discovered.

Applications

Large studies with new strategies, other than the classic case-control study design, are required to find the hidden heritability due to rare variants behind developing T2D among Omani and other Arab populations.

Terminology

T2D is one of the most common non-communicable diseases globally, and it is a result of insufficient compensatory insulin secretion due to insulin resistance. Candidate gene approach focuses on associations between genetic variation within pre-specified genes of interest and phenotypes or disease states. Genome-wide association studies scan the entire genome for common genetic variation.

Peer-review

This is a well-written and interesting paper evaluating the association between a variety of gene polymorphisms and the risk for type 2 diabetes mellitus.

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Editorial Board Member of *World Journal of Diabetes*, Shan-Dong Ye, MD, Professor, Department of Endocrinology, Anhui Provincial Hospital, No. 17, Lujiang Road, Hefei 230001, Anhui Province, China

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Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
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Ménage-à-trois of bariatric surgery, bile acids and the gut microbiome

Rajendra Raghov

Rajendra Raghov, Department of Veterans Affairs Medical Center, Memphis, TN 38104, United States

Rajendra Raghov, Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN 38163, United States

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Correspondence to: Rajendra Raghov, PhD, Professor, Department of Veterans Affairs Medical Center, 1030 Jefferson Avenue, Memphis, TN 38104, United States. r.ghov@uthsc.edu
 Telephone: +1-901-5238990

Fax: +1-901-5237274

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causing weight loss dependent glucose intolerance, bariatric surgery induces other physiological changes that contribute to the alleviation of diabetes. However, the putative post-surgical neuro-hormonal pathways that underpin the therapeutic benefits of bariatric surgery remain undefined. In a recent report, Ryan and colleagues shed new light on the potential mechanisms that determine the salutary effects of bariatric surgery in mice. The authors demonstrated that the improved glucose tolerance and weight loss in mice after vertical sleeve gastrectomy (VSG) surgery were likely to be caused by post-surgical changes in circulating bile acids and farnesoid-X receptor (FXR) signaling, both of which were also mechanistically linked to changes in the microbial ecology of the gut. The authors arrived at this conclusion from a comparison of genome-wide, metabolic consequences of VSG surgery in obese wild type (WT) and FXR knockout mice. Gene expression in the distal small intestines of WT and FXR knockout mice revealed that the pathways regulating bile acid composition, nutrient metabolism and anti-oxidant defense were differentially altered by VSG surgery in WT and FXR^{-/-} mice. Based on these data Ryan *et al*, hypothesized that bile acid homeostasis and FXR signaling were mechanistically linked to the gut microbiota that played a role in modulating post-surgical changes in total body mass and glucose tolerance. The authors' data provide a plausible explanation for putative weight loss-independent benefits of bariatric surgery and its relationship with metabolism of bile acids.

Abstract

Bariatric surgeries have emerged as highly effective treatments for obesity associated type-2 diabetes mellitus. Evidently, the desired therapeutic endpoints such as rates of weight loss, lower levels of glycated hemoglobin and remission of diabetes are achieved more rapidly and last longer following bariatric surgery, as opposed to drug therapies alone. In light of these findings, it has been suspected that in addition to

Key words: Vertical sleeve gastrectomy; Bile acids; Farnesoid-X-receptor; Type-2 diabetes mellitus; Gut microbiome; Bariatric surgery

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Core tip: The report of Ryan *et al*, raises a number of questions with regard to the prevailing notion that

mechanical restriction of the stomach and weight loss are the sole mechanisms that mediate the therapeutic effects of bariatric surgery. The authors showed that both lowering of body mass index and improved glucose tolerance after vertical sleeve gastrectomy (VSG) surgery were mechanistically linked to an altered composition of circulating bile acids and their ability to modulate farnesoid-X receptor (FXR) mediated signaling mechanisms. Additionally, it was observed that the wild type and FXR knockout mice, after receiving VSG surgery, were significantly different with respect to the make up of their gut microbiomes. Finally, the experiments revealed that the composition of gut microbiota in wild type VSG and FXR^{-/-} VSG mice were highly correlated with their differential abilities to lose weight and acquire glucose tolerance.

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COMMENTARY ON HOT TOPICS

The provocative mechanism proposed by Ryan *et al*^[1], that mechanistically links bile acids, farnesoid-X receptor (FXR) nuclear receptor signaling and microbial ecology of the gut to therapeutic superiority of bariatric surgery over intensive drug therapies, opens up new avenues of clinical research that deserves serious consideration.

According to the World Health Organization (WHO), obesity associated type-2 diabetes mellitus (T2DM) and cardiovascular diseases represent a looming health-care crisis of the 21st century [WHO Global Infobase: data on overweight and obesity mean body mass index (BMI), healthy diets and physical inactivity; www.who.int/mediacentre/]. Traditionally, obesity, T2DM and their co-morbidities have been treated with anti-diabetic drugs combined with behavioral modification therapies (e.g., better nutrition and regular exercise). However, in recent years, a number of surgical interventions, that include Roux-en Y gastric bypass, vertical sleeve gastrectomy (VSG), laparoscopic adjustable gastric banding and bilio-pancreatic diversion, have emerged as highly effective treatments for obesity-associated diabetes^[2].

There is a growing body of evidence to indicate that patients undergoing bariatric surgery, achieve desirable clinical endpoints more rapidly and for a longer duration compared with therapeutic benefits obtained with anti-diabetic drug therapy alone^[2]. A recently completed, randomized clinical trial revealed that the superior post-surgical therapeutic benefits (e.g., improved glucose tolerance and lower BMI) of bariatric surgery over intensive drug treatment persisted for at least three years, when the study ended. Additionally, patients

receiving bariatric surgery not only achieved their main therapeutic goal of weight loss and alleviation of diabetes but also enjoyed a better quality of life, with fewer obesity-associated complications^[3]. Similar beneficial effects of bariatric surgery have also been observed in rodent models of obesity^[4,5]. The strikingly more effective therapeutic outcomes of surgical procedures over pharmacological and behavioral interventions in the treatment of obesity-associated diabetes represent an unsolved medical puzzle^[6,7].

The hormonal and metabolic underpinnings of surgical interventions and how they differ from conventional drug therapy in shaping the anabolic and catabolic pathways are being actively pursued^[6-8]. Obviously, much of this research is prompted by a desire to define the basic mechanisms involved in the clinical outcome of bariatric surgery. An additional motivation for such studies is the fact that bariatric surgery poses definite risks, and thus there is an urgent need for safer, less invasive therapies to assuage obesity associated diabetes and its complications. Investigations into the putative mechanisms that differentially regulate the therapeutic outcomes of bariatric surgery vs conventional drug therapies have led to somewhat conflicting findings^[6,9,10]. According to the prevailing view, a reduced intake and absorption of macronutrients by surgically downsized stomach is responsible for the post-surgical insulin sensitivity and weight loss after bariatric surgery^[2]. Although this scenario is generally supported by the data gathered from rodent models of obesity and in humans, this mechanism fails to explain the paradoxical finding that a substantial fraction of patients experienced remission of their diabetes long before they exhibited a significant weight loss^[6,7]. This has led some investigators to posit that in addition to causing weight loss bariatric surgery also leads to other physiological changes in the gastrointestinal (GI) tract. One of the key pieces of evidence in support of this view includes post-surgical changes in circulating bile acids seen in both rodents and humans after bariatric surgery^[11,12]. It has been suggested that simultaneous changes in the composition of bile acids and gut microbiota may be causally related to obesity and a loss of BMI^[13,14]. A highly revealing study, carried out with human twins discordant for obesity, showed that obese and lean individuals had unique gut microbial ecology; transplantation of the fecal microbiota from the obese or lean patients conferred analogous BMI and adiposity to the germ-free mice^[15]. Differences in body composition were correlated with differences in the metabolism of short chain fatty acids, branched chain amino acids, and altered bile acids and FXR signaling^[15]. These tantalizing correlations notwithstanding, the underlying mechanism by which gut microbiota regulates total body weight and glucose tolerance remain to be fully elucidated.

The experiments of Ryan *et al*^[1], reported in a recent issue of *Nature*, bring us a step closer to defining the molecular interactions that mediate the metabolic effects of VSG surgery in mice. Since bariatric surgery is known

to enhance enterohepatic circulation of bile acids^[16] that signal *via* nuclear FXR, Ryan *et al*^[1] hypothesized that altered bile acid homeostasis and FXR signaling were mechanistically involved in the weight loss and glucose tolerance. To test this hypothesis, the authors compared the gene expression profiles in the distal small intestines of wild type (WT) and FXR knockout mice after VSG surgery. These analyses led to the discovery of "glutathione-mediated detoxification", "nuclear receptors in lipid metabolism and toxicity", "meta-pathway bio-transformation" and "type II interferon signaling" pathways that were highly suggestive of a role of the gut microbiome in the metabolic changes elicited by VSG surgery.

The authors undertook an empirical investigation of this hypothesis. The WT and FXR^{-/-} mice, kept on high fat diet to induce obesity, showed similar rates of body weight loss in the first week after surgery. However, the FXR^{-/-} mice were compromised with respect to sustained (at 5 wk and after) losses of BMI and body fat. Interestingly, while the WT and FXR^{-/-} mice consumed lower calories in the first week after surgery only the former continued to maintain their hypophagic behavior. The cumulative caloric intake by sham-operated and VSG FXR^{-/-} mice were similar; both cohorts of animals consumed 15% higher than VSG WT mice. Thus, the authors concluded that the inability of FXR^{-/-} animals for sustained loss of body weight and total body fat were a result of their feeding behavior. Since FXR^{-/-} mice were also refractory to the metabolic benefits of VSG surgery, it became apparent that the metabolic outcomes of surgery were also influenced by the genotype of the mice. Nevertheless, VSG surgery had a more pronounced effect on the bacterial ecology of the gut than the absence of a functional FXR gene. Un-weighted UniFrac-based comparison of bacterial 16S rRNA data in sham-operated and VSG mice revealed that the population of *Bacterioides* was significantly reduced after VSG surgery in WT mice whose guts also showed greater abundance of *Porphyromonadaceae* and *Roseburia*. Genotype independent post-surgical changes in the populations of *Lactobacilli* and *Lactococci* in the guts were also observed. The abundance of *Roseburia* in the WT VSG correlated negatively with glucose intolerance. The abundance of *Roseburia* in the guts of sham operated and VSG FXR^{-/-} was similar and was significantly lower than in WT VSG guts. Thus, the authors posited that a functional FXR signaling was involved in the mechanisms of VSG-induced weight loss, feeding behavior and insulin sensitivity.

The current study did not directly address the molecular basis of how FXR contributes to the metabolic consequences of VSG surgery. The authors acknowledged however^[1], that in light of the inherent signaling complexity of bile acids that activate FXR as well as a G-protein coupled receptor, takeda G-protein-coupled receptor-5^[16], the explanation of these data is unlikely to be straightforward. This sentiment is supported by the contradictory phenotype of FXR^{-/-}

mice elicited in this study; thus, FXR knockout mice were resistant to some of the deleterious effects of high fat diet while remaining less responsive to the therapeutic benefits of VSG surgery. The apparently paradoxical phenotype of FXR^{-/-} mice is likely to be caused by (1) unique tissue-specific mechanisms involved in the activation of FXR; and (2) a possible induction of compensatory mechanisms that result from a global knockout of FXR. The data contained in this paper also did not directly address the key question of how post-surgical changes in bile acids modulate the gut microbial ecology, and vice versa. Finally, a role of the gut microbiota in differentially regulating the metabolism and energetics of WT and FXR^{-/-} mice could only be speculated upon in light of the limited information contained in this paper. It worth remembering however, that a number of earlier studies were driven by the hypothesis that obesity-associated microbiome was more efficient at extracting energy from lipids and carbohydrates^[13,14]. The observed differences in the abundance of common fatty acids and related metabolites among the WT and FXR^{-/-} mice following VSG surgery were not sufficiently clear to warrant an unequivocal explanation. Nevertheless, the mechanism involving more efficient extraction of energy from macronutrients by the microbiota appears to be too simplistic in light of some recent data showing that changes in gut microbial ecology impinged on the signaling mechanisms underlying satiety and motility of the GI tract^[17,18]. Despite these caveats, the experiments of Ryan *et al*^[1], have broken a new ground in elucidating a functional connection of FXR signaling and microbial ecology with the metabolic consequences of bariatric surgery. These exciting findings deserve to be systematically validated and extended in humans with an aim to discover less invasive therapies to treat obesity and its complications.

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Telehealth interventions to reduce management complications in type 1 diabetes: A review

Amanda M Balkhi, Adam M Reid, Sarah C Westen, Brian Olsen, David M Janicke, Gary R Geffken

Amanda M Balkhi, Adam M Reid, Sarah C Westen, David M Janicke, Gary R Geffken, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL 32611, United States

Amanda M Balkhi, Adam M Reid, Brian Olsen, Gary R Geffken, Division of Medical Psychology, Department of Psychiatry, University of Florida, Gainesville, FL 32611, United States

Gary R Geffken, Department of Pediatrics, University of Florida, Gainesville, FL 32611, United States

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Correspondence to: Amanda M Balkhi, MS, Department of Clinical and Health Psychology, University of Florida, PO Box 100165, 1600 S Archer Rd, Gainesville, FL 32611, United States. amanda.m.roberts@phhp.ufl.edu
Telephone: +1-352-2658873

Fax: +1-352-3766270

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especially true in children and adolescents as they have unique psychosocial and diabetes needs. Despite the development of effective in-person interventions targeting improving self-management and ameliorating psychosocial difficulties there are still a number of barriers to implementing these interventions, namely time, cost, and access. Telehealth interventions allow for the dissemination of these interventions to a broader audience. Self-management and psychosocial telehealth interventions are reviewed with a special emphasis on mobile phone and internet based technology use. While efficacy has been demonstrated in a number of telehealth interventions with improved cost effectiveness over in-person interventions, many challenges remain including high participant attrition and difficulties with receiving reimbursement for services rendered. These and other challenges are discussed with recommendations for researchers and telehealth providers provided.

Key words: Telehealth; Disease management; E-health interventions; Type 1 diabetes management; Type 1 diabetes

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Core tip: Type 1 diabetes is a chronic illness with a high burden of care. Despite the development of effective in-person interventions, telehealth interventions are necessary to improve access to and engagement in interventions to improve diabetes management. Mobile phone and internet based interventions appear to have the most potential to enact change. Challenges and recommendations for these telehealth interventions are provided.

Abstract

Type 1 diabetes is a chronic illness with a high burden of care. While effective interventions and recommendations for diabetes care exist, the intensive nature of diabetes management makes compliance difficult. This is

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INTRODUCTION

The prevalence of chronic diseases, such as diabetes, cancer, cardiometabolic and respiratory conditions continues to pose a challenge for often overtaxed health care systems, requiring fundamental changes in the delivery and maintenance of patient care^[1-4]. Telehealth (TH), defined as any medical activity involving an element of distance and use of a telecommunications strategy^[5], represents an approach which may enable patients with chronic medical conditions to seek disease specific information and support^[6-9], to be followed by clinicians more frequently and away from hospital settings^[10-12], reduce healthcare costs^[13], and to ultimately promote improved adherence to medical regimens resulting in improvement in health outcomes^[14].

BRIEF HISTORY OF TH

While TH interventions began more than 50 years ago with closed-circuit television, research into TH interventions did not truly begin to accelerate until the 1990s with a dramatic increase in TH publications through the 2000s^[15,16]. Initial TH interventions primarily emphasized providing the same care that would be provided in-person through an intermediary such as a closed circuit television or telephone. A majority of interventions that subsequently developed relied on direct telephone contact by nurses or skilled health care professionals or transmission of simple self-management data *via* a modem^[15]. The primary strength of these early TH interventions was in providing care coordination with more frequent feedback and without an in-person visit, which resulted in cost savings and improved patient health^[14]. As technology advanced, TH interventions did as well, moving to material presented through video phones, home computers, pre-programmed interactive problem solving programs, and mobile phone and internet based interventions^[17,18]. The flexibility and cost effectiveness of TH makes it well suited to be used in the treatment of chronic illnesses such as diabetes.

Previous interventions have shown efficacy implementing TH for a variety of chronic conditions, including cancer, transplant recipients, heart failure, and chronic pulmonary disease^[3,7-10,12]. These interventions have shown support for TH in providing condition specific education, social support, and self management assistance. In addition, this previous work has demonstrated the wide acceptability of TH and the ability for TH interventions to reach previously underserved populations.

NEED FOR TH IN TYPE 1 DIABETES

Type 1 diabetes (T1D) is one of the most common chronic diseases in pediatrics in the United States and affects

more than 151000 youth under 20 years of age^[19]. Poorly controlled diabetes poses many serious health complications thus optimal T1D management during childhood and adolescence is necessary to reduce negative health outcomes and improve life expectancy^[19,20]. The management of T1D is a complex and challenging task that involves integration of daily medical tasks and lifestyle modifications. While demanding, the successful intensive management of T1D is associated with improved health outcomes and protections against complications that maintain for as many as 6-10 years following intensive management^[21].

Children and adolescents with T1D have unique needs that dictate different standards of care than adults^[22]. Despite parental involvement in diabetes management being common, non-adherence is especially high in the transition to and within adolescence, increasing the risk of immediate and future microvascular complications^[23-28]. T1D management is further complicated by the social, emotional, and psychological demands of the disease^[23]. Poor psychosocial wellbeing (*e.g.*, depression, anxiety, stress) is related to poorer short and long term health outcomes due to suboptimal disease management^[29-34]. Family functioning, parent wellbeing, and family cohesion have also been identified as an important contributor to diabetes control^[35-44]. Therefore, when evaluating T1D management interventions, assessing and addressing the impact of patient and parent psychosocial wellbeing while being flexible and developmentally sensitive to the needs of the patient is essential to ensure that the intervention has a lasting impact.

TH IN T1D

While previous in-person diabetes interventions have successfully targeted increasing patient knowledge^[45-47], improving illness perception^[48,49], fostering family communication and relationships^[50,51], and advancing technological accuracy and ease of management devices^[52-54], there are several remaining challenges to in-person interventions. Primary limitations of in-person interventions include poor ease of access for rural or underserved families, increased healthcare utilization costs, and poor attendance^[55,56]. Additionally, individuals at greater risk for medical regimen nonadherence are likely to also be individuals who are at greater risk for not attending medical appointments^[57], making traditional clinic based recruitment and interventions potentially ineffective. TH addresses many of the limitations of previous T1D interventions by providing a unique avenue for improving the management of T1D that is engaging, cost effective, and accessible^[58].

SELF MONITORING AND EDUCATION INTERVENTIONS

A hallmark of many TH interventions are to focus on providing education, improving self-monitoring through

electronic check ins, and establishing more frequent communication with health care providers. While traditional phone interventions have demonstrated positive improvements in glycemic control and self-efficacy^[59,60], the increased availability of smartphones and the internet facilitated further innovation and development. Deploying interventions on a mobile device, especially those compatible with text messaging, also proved effective in improving glycemic control in both adults and children^[59,61-64]. These results suggest that text messaging and intervention through mobile phones are a substantial area for outreach and intervention. However, despite the increased ownership of mobile phones among adults (91%) and adolescents (78%), there is still a substantial portion of individuals without a mobile phone or texting ability, especially among younger adolescents^[65,66]. Additionally, some interventions using text messaging or smartphone applications in children or adolescents have not shown an ability to improve glycemic control, although secondary benefits such as increased adherence, communication or knowledge are generally noted^[41,67-69]. As such, while mobile based interventions are promising, continued research into maximizing desired outcomes and cost-effectiveness is necessary.

Internet based interventions have the potential to overcome this limitation of mobile phone based interventions, because of the wide spread availability of the internet for adults (85%) and teens (95%) in the United States^[66,70,71]. The internet may be especially appropriate for diabetes intervention, as one study suggests that 63.6% of parents of children with T1D use the internet to seek out diabetes information on their own^[72]. For a child or adolescent with T1D, diabetes psychoeducation^[73], problem solving vignettes^[74], and physician monitoring of HbA1c and intervention^[75-77], have all recently shown moderate to strong evidence of successfully improving glycemic control when implemented in an online environment. Similar results have been found with adults; however, most studies rely on adults with T2D^[78-80]. Despite their demonstrated efficacy and the wide spread availability of the internet, the primary challenge that continues to plague internet based interventions is the decreased engagement and participation of users over time, with participant attrition rates of 11.5%-37% reported^[74,78].

Notwithstanding the challenges in self-management interventions, these interventions have demonstrated effectiveness in multiple delivery modalities including voice calls, SMS/Text messaging, email, customized web portals, and video conferencing^[61,81,82]. A systematic review revealed that telemedicine solutions for diabetes care are also feasible and acceptable to patients and providers^[59]. Therefore, future research is necessary to integrate the previously proven delivery strategies with new technology that is engaging to users and cost-

effective.

PSYCHOSOCIAL AND SUPPORTIVE TH INTERVENTIONS

Other TH interventions have strived to improve adherence by providing psychosocial support and decreasing family conflict around T1D management. One such method of intervention developed by Grey *et al.*^[73] bundled effective psychoeducation intervention with Coping Skills Training which improved glycemic control, quality of life, social acceptance, and self-efficacy which maintained for a year after beginning the online program (which was only 5 wk in duration). Self-efficacy has also been targeted as a potential area of psychosocial intervention, with online interventions demonstrating a significant positive impact on self-care activities^[83]. Individual wellbeing has also been successfully addressed with web-based Cognitive Behavioral Therapy and peer mentoring^[84,85]. Taken together, the existing psychosocial interventions for patients with T1D have shown success in engaging patients and improving psychological wellbeing, but are mixed on their abilities to minimize attrition and improve objective measures of glycemic control (*i.e.*, HbA1c).

TH interventions have also been utilized to support the family and environment of patients with T1D. These interventions have successfully improved communication, improved HbA1c levels, and quality of life suggesting that targeting those supporting the individual with T1D (*i.e.*, nurses, physicians, and family) may also be an effective way to improve T1D health outcomes^[81]. There also appears to be awareness from family members and service providers of their need to find information and support regarding T1D care and a preference for online interventions^[86]. One way to reach supporting individuals and patients may be to extend interventions to build on pre-existing online networks and supports. Social networks and forums for T1D have been qualitatively examined; despite concerns regarding the quality of the information presented on these sources, it is clear that patients and family members actively use these online sources (such as Facebook and online message boards) for diabetes information and social support^[6,87-90]. Most notably, in one study 84.3% of caregivers that used online forums reported that their child's care was impacted by information they encountered online^[6]. Recent data also suggests that more than half of parents within a pediatric T1D clinic use the internet to seek out T1D information^[72]. This identifies pre-existing internet sources as a potentially strong source for information dissemination but also as a potential venue for the unintended spread of misinformation. While prospective studies are needed to understand the association between parents' use of online forums and their child's glycemic control, these may be an appropriate area for

future intervention.

ONGOING CHALLENGES IN TH

While research suggests that TH for patients with T1D can be a useful and effective method of improving glycemic control and overall adherence, there has been a significant delay in transitioning efficacious research interventions of T1D into community treatment settings. Chief among these issues are the financial feasibility and reimbursement for services delivered by skilled staff, creating and maintaining patient involvement in TH interventions while minimizing patient attrition, and ensuring patient safety, privacy, and legal accountability.

FINANCIAL CHALLENGES

A key challenge that permeates across the literature in TH is the difficulty in obtaining reimbursement for services. The literature suggests that providers' experience of receiving reimbursement varies significantly^[91-93]. As of this publication only 15 states in the United States mandate coverage for TH services with 39 states providing at least some reimbursement for TH, although dramatically less so for behavioral TH despite behavioral TH's appropriateness^[94,95]. Additionally, recent studies have suggested that private third party reimbursement is improving across the board, though the trajectory of these improvements continues to be slow^[93]. For example in 2005, 58% of TH programs received reimbursement for their services while in 2012, 45% of TH programs sought reimbursement and 81% of those reported receiving it^[92,93]. Obtaining grant funding to offset these costs increases the institution's ability to build a program^[92]; however, if program personnel lack information about how to obtain reimbursement for their services from third party payers, the program may be discontinued after the grant funding has remitted^[91,93]. This pattern of short term growth with long term discontinuation is concerning and hinders the growth of TH services.

The licensing and credentialing rules create another barrier to TH implementation. Similar to reimbursement regulations, there is a large discrepancy in state TH licensing laws. Current laws generally refer to the physical location of the patient as the place of service, regardless of the provider's location. Moreover, state laws generally require that providers be licensed and credentialed at the place of service, making state lines a finite barrier to service delivery^[91]. In 2011, Children's Medical Services (CMS) began to allow institutions to accept the credentialing of the provider's home institution instead of requiring the outside institution to put the provider through their credentialing process^[91]. The CMS regulations show promise for expanding the credentialing requirements and may facilitate providers' ability to reach patients who otherwise may not have been able to receive care. While efforts have been made to extend licensing adjustments by implementing

limited, federal, and reciprocal licenses, it is clear that the current system of licensure is hindering providers' ability to reach out to patients who live in other states and steps need to be made to resolve these concerns^[95].

PATIENT ENGAGEMENT AND ATTRITION

Attrition and noncompliance in TH interventions creates yet another barrier to successful establishment of TH interventions. As stated previously, attrition rates of 11.5%-37% among internet interventions have been reported^[74,78]. However, patients who complete TH interventions may also not adhere adequately to the intervention, given the lack of in-person oversight. In a study conducted by Wangberg^[83], participants were requested to repeatedly view and engage with online modules targeting self-efficacy and diabetes self-care, yet only 34% logged in more than twice to interact with the modules. Similarly, a review of TH adherence found a recurring theme in suboptimal frequency of uploading and submitting blood glucose values^[96]. Given these relatively high drop-out rates and problems with noncompliance, TH programs should incorporate measures to improve adherence and keep the patients engaged in treatment.

To this end, some studies have shown improved adherence when the TH interventions are tailored toward the patients unique needs by using customized messages, programs, or personalized functions within the program^[97]. For instance, a program may allow patients to use a data base of pictures of foods that have predetermined carbohydrate amounts instead of requiring the patient to estimate the carbohydrates for food they consume. Including features that communicated with patient's preexisting diabetes technology (*e.g.*, glucose monitors, insulin pumps) and automatically upload patients' blood glucose levels in order for parents or providers to review and provide feedback may also be helpful. Overall, these programs should be easy to access and provide immediate feedback^[98]. Programs should also take demographics into account. For instance, older individuals^[99], women^[100], and patients with higher self-efficacy^[101,102], are more likely to adhere to internet interventions. Thus, providers who are developing or implementing TH interventions should work to determine which patients are going to be appropriate for the TH intervention and how to address those groups with a history of poor adherence to TH interventions^[103].

PRIVACY AND SECURITY

Possibly the most common TH concern relates to the ability for TH interventions to maintain patient privacy and security in a mobile or online environment. Appropriately managing personal health information (PHI) is an important piece to maintaining patients' confidentiality. The federal laws provide regulations for protecting PHI under the Health Information Portability and Accountability Act^[104] (HIPAA). Each state also has

laws for managing PHI, which is not consistent from state to state and may be more or less stringent than HIPAA regulations^[95]. When state privacy laws conflict with HIPAA, the general rule is that the provider follows the more stringent law^[95]. TH providers who provide services across state lines must be aware of laws in both states and must work to resolve conflicts as they arise while being prepared for conflicting laws or regulations^[105]. While managing these challenges across providers, technology technicians, nurses, medical assistants, and billing personnel in two states may be manageable with practice, negotiating differences among state and federal privacy laws is likely to be increasingly more difficult with each additional state, thereby deterring providers from expanding their services and possibly hindering patients' access to specialized mental health care^[95]. In addition, though more recent technological advances and using secure or closed networks have improved security of online data transmission^[91,95] regulations are not clear regarding where or how the data should be stored (*e.g.*, online, at the providers' institution, or at the institution of the place of service). While providers should follow good safety procedures such as a personalized login, automatic time-out setting when not in use, encrypted data storage, and encrypted data transmission, they must also deliver informed consent and ensure a patient's understanding of the potential and ever-evolving risks of transmitting health information over the internet or mobile networks^[95,105,106]. These challenges require that a provider maintain understanding not only of the HIPAA and PHI laws but also of technological capabilities and challenges to technological safety, which is a daunting task for even the most informed provider.

CONCLUSION AND FUTURE DIRECTIONS IN TH

Taken together, research has shown the effectiveness and promise of TH in improving several primary (*i.e.*, glycemic control, adherence) and secondary (*i.e.*, social support, comorbidity, and knowledge) outcomes in T1D patients. Although a previous review suggested that phone interventions appear to have more promise than internet interventions in improving targeted T1D outcomes, the reviewed literature above suggests promise in both web based and mobile interventions. Broadly, TH interventions must strive to be theory driven, integrate multiple platforms, be secure, and be user-friendly^[87,107,108]. In doing so, TH interventions will expand to a broader audience and have an improved chance at reaching those most in need for TH, the underserved individuals who have difficulty accessing traditional services.

In order to improve current TH interventions, researchers and providers should invest in portability. As mobile computing and mobile phones reach a larger and larger share of the United States^[65] TH interventions must be optimized to provide an efficient, appealing, and

interactive environment on the smaller screen of mobile phones and tablets to reduce attrition and maintain participant engagement. This is especially true for adolescents and children, of whom a large portion (74%) access the internet from their phone and an increasing portion (25%) use their mobile phones as their primary device for navigating the web^[109]. Researchers who do not adjust their TH interventions to take advantage of the increased computing power and accessibility of portable technology will continue to struggle with participant engagement and attrition as fewer and fewer young individuals use a traditional desktop computer.

Researchers and clinicians should also seek to integrate their TH interventions into existing technological infrastructure both to increase participant familiarity and ease of use. By intentionally creating interventions that integrate with diabetes technology (*i.e.*, blood glucose monitors, insulin pumps), providers improve their ability to obtain objective health information and increase participant engagement through ease in integration. Integration, especially automated or hands free integration, with these technologies also has the benefit of providing a method for providers to easily view and provide feedback without relying on patients to physically produce their blood glucose meters at appointments. This improves the quality of information that providers have to deliver care, as well as decreases the burden of appointment preparation on the patient and potentially improving compliance.

In addition to technological integration, effective TH interventions should seek to involve family members, providers, and other supporting members of the patient's T1D management team. An effective way to do this may be by building on the preexisting support networks targeting these individuals and developing ways to improve how individuals find, interoperate, and communicate the information they find online. Recent research has demonstrated that parents of children with T1D are especially likely to use these online sources and actively incorporate them into how they care for their child's T1D^[6,72]. Providers may benefit from this predisposition of parents to search for information online by designing and implementing procedures to inform users of appropriate sources of information and increase awareness of effective TH interventions that may provide similar information. Building atop of pre-existing resources may also reduce the infrastructure cost that contributes to the short term growth and long term discontinuation of existing TH interventions.

Finally, clinical care providers are encouraged to advocate for improved legislation regarding TH. Notwithstanding substantial improvement over the last decade regarding TH reimbursement and rules, there continues to be a lack of guidelines regarding TH interventions delivered by the mental health profession and the associated technological, privacy, and security issues created by these interventions. In fact, many of the privacy and security concerns related to TH interventions may only be feasibly addressed by policy

makers and technology manufacturers. The development and national recognition of TH guidelines in conjunction with improved licensure recognition across state lines may provide increased support to mental health professionals who wish to pursue TH interventions. As a part of these guidelines, providers and researchers are encouraged to pair with technology consultants whom are informed and educated both on technological advances and advances in privacy and security laws. With proper support and the development of structured guidelines, TH interventions can grow to fit within the evolving scope of health care policy, reimbursement, and technological advancement while reducing the number of individuals who are underserved.

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Type 1 diabetes: A predictable disease

Kimber M Simmons, Aaron W Michels

Kimber M Simmons, Aaron W Michels, Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Aurora, CO 80045, United States

Author contributions: Simmons KS and Michels AW contributed to this paper.

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Correspondence to: Aaron W Michels, MD, Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Mail Stop A140, 1775 Aurora Court, Aurora, CO 80045, United States. aaron.michels@ucdenver.edu
 Telephone: +1-303-7241923
 Fax: +1-303-7246784

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease characterized by loss of insulin producing beta cells and reliance on exogenous insulin for survival. T1D is one of the most common chronic diseases in childhood and the incidence is increasing, especially in children less than 5 years of age. In individuals with a genetic predisposition, an unidentified trigger initiates an abnormal immune response and the development of islet autoantibodies

directed against proteins in insulin producing beta cells. There are currently four biochemical islet autoantibodies measured in the serum directed against insulin, glutamic decarboxylase, islet antigen 2, and zinc transporter 8. Development of islet autoantibodies occurs before clinical diagnosis of T1D, making T1D a predictable disease in an individual with 2 or more autoantibodies. Screening for islet autoantibodies is still predominantly done through research studies, but efforts are underway to screen the general population. The benefits of screening for islet autoantibodies include decreasing the incidence of diabetic ketoacidosis that can be life threatening, initiating insulin therapy sooner in the disease process, and evaluating safe and specific therapies in large randomized clinical intervention trials to delay or prevent progression to diabetes onset.

Key words: Autoimmunity; Autoantibodies; Diabetes prevention; Screening; Type 1 diabetes

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Core tip: Type 1 diabetes (T1D), the immune mediated form of diabetes, is now a predictable disease with the measurement of islet autoantibodies. The presence of two or more antibodies defines preclinical disease as nearly everyone with multiple antibodies progresses to clinical diabetes. With improved platforms to measure islet autoantibodies, screening the general population is now a goal. Early identification of preclinical diabetes allows for less diabetic ketoacidosis, early initiation of insulin therapy, and the potential to delay or prevent diabetes onset. Clinical trials using safe and specific therapies to block disease specific immune cells are underway in T1D.

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INTRODUCTION

Type 1 diabetes (T1D) is a chronic disease caused by immune-mediated destruction of insulin producing beta cells in the pancreas^[1]. The destruction of beta cells results in insulin insufficiency, and patients develop life-threatening hyperglycemia that clinically manifests with weight loss, polyuria, and polydipsia. The majority of patients who develop T1D have high-risk human leukocyte antigen (*HLA*) genes. Islet autoantibodies can be measured in the serum of these high-risk individuals years before the onset of any clinical symptoms, making T1D a predictable disease. Multiple prevention trials in patients with high-risk *HLA* genes or in patients who have measurable autoantibodies have been completed. To date, no trial has prevented the onset of T1D, but data indicates that the disease process may be delayed by administering oral insulin to induce insulin specific regulatory T-cells in the gut, resulting in decreased inflammation in the pancreas. This review summarizes the epidemiology, risk factors and pathogenesis of T1D. The review also examines the goal of screening the general population for T1D risk and preventing disease onset in individuals with preclinical disease.

EPIDEMIOLOGY

T1D is one of the most common chronic diseases in childhood and is diagnosed at an increasing rate in adults. The incidence rate varies significantly by geographical region. Sweden, Finland, Norway, United Kingdom, and Sardinia have the highest incidence of T1D at an age-adjusted rate of > 20/100000 patient years. For comparison, the United States has an incidence rate of 17.8/100000 patient years in a predominantly Caucasian population. China and South America have the lowest incidence of T1D, reported as < 1/100000 patient years^[2-5]. The rate of T1D diagnosis is increasing in most countries, with rates dramatically increasing in children less than 5 years of age^[6]. The annual incidence of T1D is increasing globally by 2.3% per year and is estimated to be increasing by 2.7%-2.8% in non-Hispanic white youth in the United States^[7]. Large registries in both Europe and the United States show that the incidence of T1D peaks between 5 to 7 years of age and again when children enter puberty^[8]. Unlike most autoimmune diseases, T1D is more common in males than females. The risk of T1D development in the general population is 1:300^[9]. In children who have a genetically related sibling, the risk is increased to 1:7 and is greatest in children under 5 years of age^[10,11]. Offspring of mothers with T1D carry approximately 3% risk and offspring of fathers with T1D carry approximately 5% risk^[12]. Genetics confer risk for development of T1D, as does seasonal variation and birth month suggesting an environmental influence on disease pathogenesis. Children born in the spring tend to be at a greater risk for developing T1D, while diagnosis is increased during climatically cold

seasons^[13-16]. This is an epidemiological association that requires further investigation.

RISK FACTORS

Genetic

T1D is a polygenic disorder with many genes contributing varying amounts of genetic risk for disease development. The genes conferring risk for diabetes are generally classified as *HLA* and *non-HLA* genes. Large genome wide association studies show that over 40 genes increase susceptibility to T1D^[17,18]. The major determinant of genetic susceptibility to T1D, contributing greater than 50% of the genetic risk, is conferred by genes in the *HLA* complex located on chromosome 6^[9]. The *HLA* complex is divided into 3 regions: classes I, II, and III. Alleles of the class II genes, DQ and DR (and to a lesser extent DP), are the most important determinants of T1D. These class II molecules are expressed on antigen-presenting cells (macrophages, dendritic cells, and B cells) and present antigens to CD4 T lymphocytes. DQ and DR genes are in close linkage disequilibrium on chromosome 6 with specific DQ and DR genes inherited together. The presence of the DR4/DQ8 haplotype increases the odds ratio for T1D development to approximately 11, indicating an individual with this haplotype is 11 times more likely to develop T1D than those without. Approximately 90% of all individuals with T1D have either or both the DR4/DQ8 or DR3/DQ2 haplotypes. Interestingly, *HLA* genes also confer protection from T1D development. Individuals who have the specific DQ6 allele (DQB1*06:02) are dominantly protected from T1D, with an odds ratio of 0.03 for disease development^[19].

Of the *non-HLA* genes, insulin and protein tyrosine phosphatase non-receptor type 22 (PTPN22) confer risk for T1D development but to lesser degrees than *HLA* genes^[20]. Similar to *HLA* class II genes, insulin gene polymorphisms can confer both susceptibility to and protection from T1D development. At the 5' end of the *insulin* gene, there are variable numbers of tandem repeats. Having more repeats correlates to more insulin message being expressed in the thymus. The thymus responds by developing central tolerance to insulin. In individuals with fewer repeats, autoreactive T-cells can persist, and the risk for T1D development is increased^[21]. PTPN22 helps regulate antigen receptor signaling and T cell activation, and a single nucleotide polymorphism (arginine to tryptophan at position 620) has been associated with a number of autoimmune disorders including T1D. A gain of function polymorphism decreases T cell receptor signaling which confers diabetes risk. It is unknown why decreased T cell activation leads to T1D risk, but it can be hypothesized that deficient negative selection of thymic cells may be involved^[22,23].

Environment

Genetics alone does not lead to T1D; the environment also plays a pivotal role. This is evidenced by the fact

that not all individuals with high-risk genes develop T1D. In fact, the majority of individuals with high-risk HLA class II genes (DR4/DQ8 and DR3/DQ2) do not develop T1D. There are likely one or more environmental factors that trigger and perpetuate the autoimmune disease process prior to hyperglycemia and a clinical diagnosis of hyperglycemia and T1D. Large natural history studies indicate that the development of islet autoantibodies (the first laboratory evidence of beta cell autoimmunity) in high-risk individuals often occurs between 9 mo and 2 years of age^[24]. This suggests that an environmental trigger is present early in life, possibly *in utero*.

One of the most extensively evaluated environmental triggers is viral infection. Many viruses are implicated in the development of T1D including enteroviruses such as coxsackie B virus, cytomegalovirus, congenital rubella syndrome, and rotavirus^[25-32]. Enterovirus is the leading candidate for contributing to T1D development. Epidemiologic studies in Finland show that the development of beta cell autoimmunity parallels the seasonal pattern of enterovirus infection and clinical symptoms of enteroviral infection^[33,34]. Enterovirus infection was strongly associated with the development of autoantibodies in the Diabetes Autoimmunity Study in the Young (DAISY) cohort^[35]. Laboratory evidence of enterovirus infection is reproducibly present in individuals with new onset T1D, pregnant women whose children develop T1D, and donor pancreata of individuals with T1D^[36,37]. The exact mechanism of how viruses induce autoimmunity is not clear. The molecular mimicry hypothesis proposes that because the P2-C protein sequence of enterovirus is similar to glutamic decarboxylase (GAD), which is expressed in islet cells, the immune system erroneously targets destruction of beta cells^[38]. The other leading hypothesis is that viral infection activates autoreactive T cells. As evidence, Cytomegalovirus B4 has tropism for pancreatic tissue and infection results in release of beta cell antigens that are phagocytized by macrophages and presented to autoreactive T cells^[39].

Another potential environmental influence relates to the north-south division of diabetes development in the world, with a higher incidence of T1D in northern climates compared to southern. The north-south hypothesis implicates that a lack of vitamin A and/or D exposure early in life predisposes individuals to the development of autoimmune diseases including T1D. Offspring of mothers supplemented with vitamin D during pregnancy and young children supplemented with vitamin D have shown a reduced risk of T1D development that may be dose responsive^[40,41]. However, an analysis from the DAISY Study found that vitamin D intake and 25(OH) vitamin D levels throughout childhood were not associated with the development of islet autoantibodies or T1D development^[42].

Early introduction of cow's milk and gluten have also been extensively studied. The introduction of gluten into an infant's diet prior to three months and after 7 mo has been associated with increased autoantibody development^[24,43]. Some studies also indicate

that breastfeeding or using elemental formula may be protective against T1D. Other environmental factors that continue to be explored include nitrosamine compounds, maternal age, pre-eclampsia, and childhood obesity. There is no evidence to suggest that vaccines increase the risk of T1D development^[44]. To date, there are no causal environmental factors that trigger the development of islet autoantibodies or increase the risk of progression to clinical T1D development. However, there is a large international prospective longitudinal study, The Environmental Determinants of Diabetes in the Young, currently underway to evaluate potential environmental factors in T1D^[45].

NATURAL HISTORY

Three decades ago, it was hypothesized that T1D is a chronic autoimmune disorder that develops in stages, and the model remains valid today (Figure 1). In genetically predisposed individuals (those with DR4/DQ8 and/or DR3/DQ2 haplotypes) there is an environmental trigger that leads to a break in immunologic tolerance and loss of beta cell mass. Over a period of time, usually years, there is autoimmune destruction of insulin producing beta cells that is marked by the presence of serum islet autoantibodies (Figure 2). As the process continues, very likely in a relapsing and remitting manner, there is a loss of glucose stimulated insulin release, and eventually insulin deficiency such that overt hyperglycemia results and clinical T1D is diagnosed^[4].

How an inciting event leads to an aberrant immune response is not completely understood. Most hypotheses focus on immunologic abnormalities in antigen presentation by HLA molecules to T cells in the thymus and peripheral lymph organs. T cells are educated in the thymus to self-antigens, such as insulin, and if there are dysregulated immune processes, self-reactive T cells can escape central tolerance and exist in the periphery^[46,47]. Once these cells encounter their target antigen or peptide in peripheral lymph organs, they become activated to target beta cells. Other hypotheses focus on environmental triggers leading to immune activation and targeting of beta cells. The molecular mimicry theory proposes that a viral or bacterial protein shares amino acid sequence homology with beta cells and induces immune system activation through targeting beta cell antigen that is molecularly similar to a foreign antigen^[38]. Finally an infectious triggering event may allow beta cells to become more sensitive to cytokine and free radical induced inflammation^[48].

Recently the network for pancreatic organ donors has been established to study the pancreata of deceased donors with islet autoantibodies (preclinical disease) or established T1D^[49]. The goal is to understand mechanisms of disease pathogenesis and interactions between beta cells and the immune system^[50]. What we have gleaned from the initial efforts is that islet infiltrates (insulinitis) are present in a lobular pattern in the pancreas, and there is a predominance of CD8 and CD4 T cells,

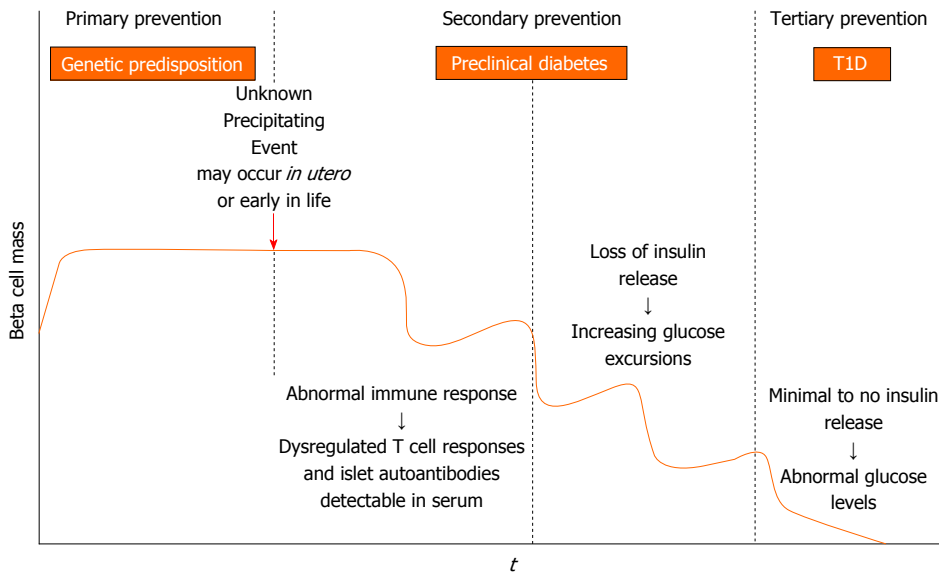


Figure 1 Stages in the development of type 1 diabetes adapted from the initial model proposed by George Eisenbarth. In genetically at risk individuals an unknown trigger, presumably environmental, initiates an autoimmune response that results in loss of beta cell mass. Before metabolic disturbances occur, islet autoantibodies (insulin, glutamic decarboxylase, islet antigen 2, zinc transporter 8) are measurable in serum. As beta cell mass decreases, potentially in a relapsing-remitting manner, there is loss of endogenous insulin release and ensuing hyperglycemia. Within this model, there are opportunities for type 1 diabetes (T1D) prevention in genotypically high risk individuals (primary prevention) and in autoantibody positive individuals (secondary prevention). Interventions to preserve remaining beta cell mass at diagnosis are also possible (tertiary prevention).

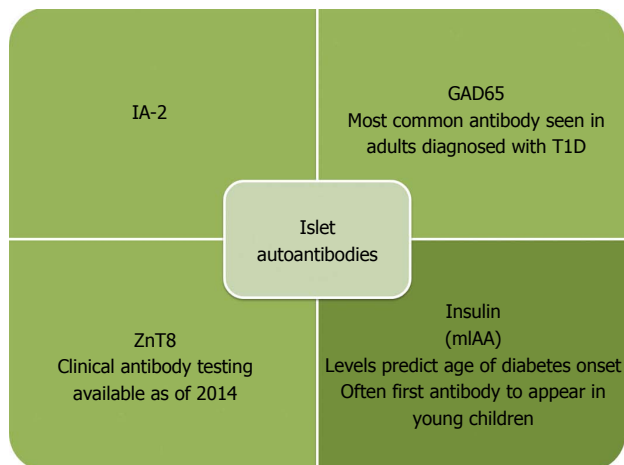


Figure 2 Insulin autoantibodies are often the first antibody to develop in young children. In contrast, adults most often are GAD65 and IA-2 autoantibody positive at diagnosis. The ZnT8 antibody is the most recently identified autoantibody with commercial testing now available. T1D: Type 1 diabetes; GAD65: Glutamic decarboxylase; IA-2: Islet antigen 2; ZnT8: Zinc transporter 8.

B-lymphocytes, and macrophages^[51,52]. Pancreata from established T1D patients also show an overall decrease in weight compared to age matched controls, potentially related to atrophy of the exocrine pancreas with the loss of beta cells^[52]. The first serological evidence of an autoimmune response to beta cells is the appearance of autoantibodies to insulin (IAA), GAD, islet antigen 2, and zinc transporter 8^[53]. Placental antibodies are no longer present after approximately 6 mo, so any antibodies in serum after that time reflect endogenous antibodies. If an individual develops two or more of these antibodies, they will eventually progress to clinical onset of T1D^[54].

Approximately 90% of individuals have two or more islet cell autoantibodies at diagnosis, and it is likely that the remaining 10% of individuals (islet autoantibody negative) have autoantibodies against antigens that have yet to be discovered. In children, IAA is usually the first antibody to develop, and the progression to T1D is 100% in children with a persistently high level of IAA^[55,56]. This is in contrast to adults who tend to have higher levels of GAD at diagnosis. Islet autoantibodies can be easily measured in the serum, with the gold standard method for detecting antibodies being fluid phase radioimmunoassays (RIA)^[57]. More recently, islet autoantibodies are now able to be measured from smaller volumes of serum and without the use of radioactivity using electrochemiluminescence as a detection method while maintaining similar sensitivity and specificity to RIA^[58,59]. The rate at which individuals with positive islet autoantibodies progress to clinical T1D is dependent upon the age of appearance, insulin autoantibody level, and the number of autoantibodies present^[55]. Hemoglobin A1c rises 1 to 1.5 years prior to diagnosis. Therefore, reduced insulin secretion and resultant hyperglycemia occur before T1D is clinically diagnosed^[60,61]. Once T1D is clinically diagnosed, individuals must commit to lifelong blood glucose monitoring and intensive insulin administration *via* multiple daily injections or an insulin pump to achieve good glycemic control. With improved diabetes management, the risk for long-term complications such as renal failure, myocardial infarctions, stroke, and lower extremity amputations has decreased over the last two decades^[62]. However despite the decreasing prevalence of complications in diabetes, the need still exists to understand the underlying pathogenesis of complications such as diabetic cardiomyopathy and

novel approaches for treating complications such as neovascularization in diabetic foot disease^[63,64].

SCREENING AND PREVENTION

The American Diabetes Association recently adapted their guidelines to recommend screening for islet autoantibodies in high-risk individuals^[65]. Highly sensitive serological assays are not widely available, and all screening is recommended to be done in the setting of a clinical research study. To date, general population screening has been done through large clinical trial networks such as the National Institutes of Health sponsored TrialNet, which enroll and screen first or second degree family members of individuals with T1D. By identifying individuals with positive islet autoantibodies, the rate of diabetic ketoacidosis (DKA) at diagnosis is reduced^[66]. Preventing DKA is important as altered mental status, coma, and even death can occur^[67]. In fact, DKA is the most common cause of death in children with T1D^[68]. Without screening, DKA at diagnosis is relatively common^[69]. In the EURODIAB study, 42% of children presented in DKA (pH < 7.3) at the time of diagnosis with T1D^[3]. By identifying individuals with positive autoantibodies, insulin therapy can be initiated early, and these children can enroll in studies aimed at preserving beta cell mass. In adults, maintaining endogenous insulin secretion reduces hemoglobin A1C, reduces the risk of severe hypoglycemia, decreases reliance on exogenous insulin, and decreases the rate of long-term complications^[70-77]. In children, there has been very little data collected regarding residual beta cell mass beyond the first year after diagnosis^[78-82]. A case-control study did show that children without severe hypoglycemia had increased residual beta cell mass compared to those children with severe hypoglycemia^[83]. An effective method of preserving beta cell mass is not yet available, and the benefit of increased residual beta cell mass in children remains to be confirmed.

According to the World Health Organization's principles of early disease detection, T1D is a condition that meets criteria for the establishment of a screening program. These principles include the condition is an important health problem, there is a recognizable latent stage of the disease, the natural history of the disease is understood, there is an adequate and accepted laboratory screening test, providers agree on who should receive treatment and there is a treatment available, there are adequate resources for diagnosis and treatment, and the cost of overall medical care would not increase^[84]. Islet autoantibodies can be reliably measured in serum, with each antibody assay having a specificity of 99% when measured by radioimmunoassay in tertiary referral centers such as the Barbara Davis Center for Diabetes. The sensitivity for each autoantibody assay ranges from 70%-80%. We view these radioimmunoassays as a confirmatory test for T1D. A desired screening test needs to be reliable with high sensitivity, cost effective, and technically feasible, likely as a multiplex assay in

which all four autoantibodies are measured in a single well of an assay plate. Currently, to measure islet autoantibodies a blood draw is required with subsequent shipping of venous or capillary blood samples to a reference laboratory. This is not feasible for population wide screening due to technical requirements of sample collection and high cost. Screening large populations of infants for metabolic diseases and other congenital disorders has been successfully done using dried blood spots^[85]. To establish an accepted screening program for T1D, the sensitivity and specificity of islet autoantibodies, specifically insulin autoantibody, needs to be established using a feasible collection method such as dried blood spots on filter paper, which would be a simplified collection method and more cost effective. Overall, T1D would not be over diagnosed with general population screening as diagnosis of the disorder requires both the presence of islet autoantibodies and metabolic abnormalities.

Ideally, individuals who screen positive for islet autoantibodies can be offered a treatment to prevent or delay the progression to T1D. Many secondary prevention trials have been completed with more currently underway^[86]. As population based screening may be feasible in the near future, it is important to continue secondary prevention trials with the goal of delaying or preventing progression to T1D in islet autoantibody positive individuals. Patients enrolled in clinical intervention trials benefit from close follow up by medical professionals, early diagnosis of T1D, decreased incidence of DKA, and early initiation of insulin therapy (Figure 3).

Secondary prevention trials

As T1D is a predictable disease with the measurement of islet autoantibodies, it logically follows that the disease should be preventable. To date, the majority of secondary prevention trials (enrolled individuals with preclinical disease) have administered different preparations of insulin to autoantibody positive individuals in an attempt to slow the progression to T1D onset^[87]. The first such trial was the diabetes prevention trial-type 1 in which at risk patients were either administered subcutaneous insulin or oral insulin in randomized, double-blinded, placebo controlled trials. Oral insulin has no metabolic effect; however, orally administered insulin does encounter mucosal gut-associated lymphoid tissue. The role of this lymphoid tissue is to provide protection from orally acquired pathogens and to keep individuals from developing reactions to ingested proteins. By administering low doses of oral insulin, insulin-specific T-regulatory cells are produced which may release cytokines that inhibit the inflammatory cascade that leads to β -cell destruction^[88-90]. Relatives of patients with T1D who were 3 to 45 years of age and had high-risk *HLA* genes and one or more positive autoantibodies were evaluated for abnormal glucose metabolism. Those individuals who had abnormal glucose tolerance ($n = 339$) were administered 0.25 units/kg per day of Ultralente insulin twice daily and received an intravenous insulin infusion for four

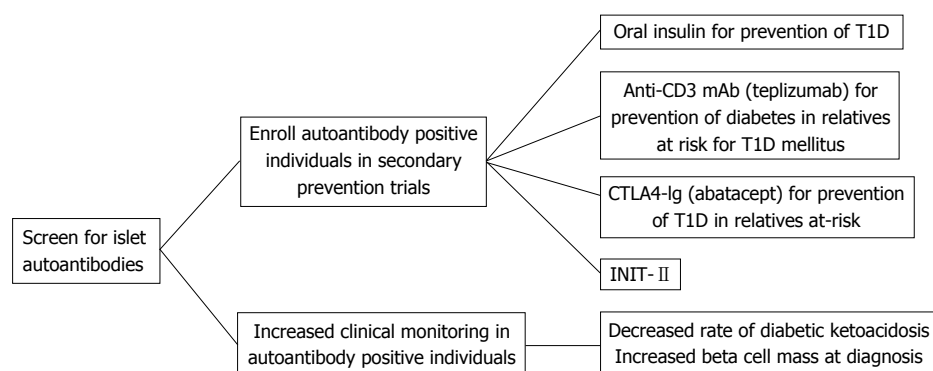


Figure 3 The measurement of serum islet autoantibodies has made type 1 diabetes a predictable disease. Early identification of islet autoantibody positive individuals leads to improved clinical outcomes by decreasing the risk for diabetic ketoacidosis and potentially preserving beta cell mass through clinical prevention trials. T1D: Type 1 diabetes; INIT- II : Intranasal Insulin Trial.

days at the beginning of the study and then annually. There was no effect of low-dose subcutaneous insulin on delaying the progression to T1D^[91]. Participants with normal glucose tolerance received 7.5 mg/kg per day of oral insulin ($n = 372$). An oral glucose tolerance test was completed every 6 mo during a 6-year follow up, and there was not a delay in progression to T1D. Of interest, a post-hoc analysis showed in participants with persistently high levels of IAA (≥ 80 nU/mL) there was a delay in disease onset of approximately five years^[92]. Also, the rate of progression to T1D onset after stopping insulin was more rapid^[93]. A follow-up oral insulin trial through TrialNet is currently enrolling participants in order to determine if oral insulin can delay the progression to T1D in individuals with high IAA levels (ClinicalTrials.gov Identifier: NCT00419562).

Another insulin intervention trial from the Belgian T1D Registry identified study participants who were insulin autoantibody positive and did not have a HLA haplotype conferring protection (DQB*0602). Study participants were given two subcutaneous injections of insulin daily for 3 years ($n = 25$) or observed and prospectively followed ($n = 25$). The participants who were treated with insulin and those who refused treatment or agreed to observation developed T1D at the same rate^[94].

Many preclinical studies have suggested that administration of intranasal insulin may delay T1D development through mucosal tolerance, in which mucosal antigens have been shown to impact regulatory T cell development^[95]. To translate these findings to humans, individuals with high-risk HLA haplotypes and one or more islet autoantibodies were enrolled in the Intranasal Insulin Trial (INIT- I) ($n = 38$). This randomized, double-blinded, crossover pilot study suggested that intranasal insulin protects against the development of T1D by increasing antibody formation and decreasing T cell responsiveness^[96]. The INIT- II, a randomized, double-blinded, placebo controlled trial, is now enrolling individuals to determine if intranasal insulin can delay or prevent the progression to T1D (ClinicalTrials.gov Identifier: NCT 00336674).

However, a large study in Finland, the T1D Prediction and Prevention Trial enrolled and followed siblings of children with T1D or infants of mothers with T1D who had high-risk *HLA* genes for islet autoantibody development. Once two or more autoantibodies were detected ($n = 264$), they were randomized to intranasal insulin ($n = 137$) or placebo ($n = 127$). Interim analyses showed no benefit of intranasal insulin in delaying the onset of T1D^[97]. This indicates that intranasal insulin may not be effective at delaying diabetes onset at the administered dose and timing in the disease process. Potentially insulin antigen specific therapies may need to be administered earlier in the disease course to have an impact on delaying progression to T1D.

Several trials using non-antigen specific therapies including bacille calmette-guerin injections, Ketotifen (histamine antagonist), oral cyclosporine, and nicotina-mide (B6) have been completed. No study has prevented or delayed T1D development^[98-104].

Clinical trials with drugs aimed at modulating the immune response and preserving endogenous insulin secretion in patients with new-onset T1D are termed tertiary prevention trials^[51]. Only recently have these drugs expanded to prevention trials in islet autoantibody positive individuals (Figure 3). The CTLA4-Ig antibody (Abatacept) for Prevention of Abnormal Glucose Tolerance and Diabetes in Relatives At-Risk (ClinicalTrials.gov Identifier: NCT 01773707) and Anti-CD3 monoclonal antibody (Teplizumab) for Prevention Of Diabetes In Relatives At Risk For T1D mellitus (ClinicalTrials.gov Identifier: NCT01030861) are both TrialNet studies currently enrolling participants. Abatacept is a fusion antibody that binds to antigen presenting cells and blocks co-stimulation to T cells. Anti-CD3 monoclonal antibodies bind the CD3 molecule which is present on CD8 and CD4 T cells, thereby inhibiting T cell activation^[105]. Both of these drugs have shown some degree of success when used in new-onset trials^[106-111]. DIAPREV-IT is an antigen-based treatment currently enrolling individuals who are positive for GAD and one or more additional autoantibodies (ClinicalTrials.gov Identifier: NCT 01122446). A GAD/alum vaccine

is given at enrollment and 1 mo later. Although GAD vaccination is safe and easily administered, new-onset intervention trials have not shown long-term preservation of endogenous insulin secretion^[108,112,113].

NOVEL APPROACHES TO PREVENT T1D

Currently, insulin is the only medication approved by the United States Food and Drug Administration for the treatment of T1D. Despite T1D being a predictable chronic autoimmune disorder, there are not any therapies to preserve endogenous insulin production. As mentioned above, many large clinical intervention trials have not slowed the progression or prevented disease onset. We believe T1D will be preventable and that safe and specific therapies targeting the immune system are needed. One such approach is to target the trimolecular complex, which consists of a self-reactive CD4 T cell, insulin, and HLA molecule^[114]. It is well established that specific HLA alleles, namely HLA DQ8 which is present in approximately 60% of all T1D patients, confer significant disease risk. DQ8 is a molecular target for diabetes intervention by using small “drug-like” molecules to block antigen presentation, thereby inhibiting specific T cell activation. Preclinical studies have shown this to be a potential pathway for diabetes intervention^[115]. This concept has been advanced from bench to bedside as a clinical trial in which methyldopa (Aldomet), a clinically well-established antihypertensive drug, is being investigated to block DQ8 antigen presentation. The phase 1b dose escalation trial is using personalized medicine as methyldopa is being administered to recent onset adult T1D patients with the presence of the DQ8 gene (ClinicalTrials.gov Identifier: NCT01883804). Methyldopa is orally administered, safe as it has been used clinically for the last 50 years, and currently indicated for the treatment of pregnancy induced hypertension. Furthermore, all individuals have three class II molecules (DQ, DR, and DP), and by blocking a single class II molecule, there are two others to permit normal immune system function.

Other approaches have targeted components of the insulin trimolecular complex including antibodies that specifically bind to an insulin peptide in the HLA molecule. Preclinical studies in an animal model of spontaneous autoimmune diabetes indicate that this approach can delay diabetes onset^[116]. Efforts are currently being made to make a human antibody, which again is a very specific immune therapy for diabetes intervention. Finally, insulin antigen specific therapy has the potential to evolve with recent advances in the field of immunology. A peptide from the insulin B chain amino acids 9-23 (B:9-23) has been extensively studied in animal models and human T1D^[117,118]. It is now appreciated that insulin B:9-23 is a key autoantigen in the disease process of both mice and humans, sharing an identical amino acid sequence in both species^[119,120]. A mutated insulin B:9-23 peptide, but not the native peptide sequence, induced protective immune responses (regulatory T cells) and prevented

diabetes onset in preclinical animal models^[121]. With a deeper understanding of how the insulin peptide binds to HLA molecules and activates T cells, an insulin vaccine again holds promise for diabetes prevention.

In conclusion, T1D is now a predictable disease with the measurement of islet autoantibodies and prevention will naturally follow. To prevent T1D, general population screening for islet autoantibodies is needed along with a safe and specific therapy for disease intervention. The genes that confer diabetes risk are now molecular targets, and tailoring therapies to specific HLA genes is personalized medicine. The future holds promise for delaying the progression and ultimately preventing diabetes.

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Nociception at the diabetic foot, an uncharted territory

Ernst A Chantelau

Ernst A Chantelau, Diabetic Foot Clinic, Heinrich-Heine-University Düsseldorf, 40001 Düsseldorf, Germany

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Correspondence to: Ernst A Chantelau, Professor, Diabetic Foot Clinic, Heinrich-Heine-University Düsseldorf, Moorenstraße 5, 40001 Düsseldorf, Germany. chantelau@gmx.de

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Abstract

The diabetic foot is characterised by painless foot ulceration and/or arthropathy; it is a typical complication of painless diabetic neuropathy. Neuropathy depletes the foot skin of intraepidermal nerve fibre endings of the afferent A-delta and C-fibres, which are mostly nociceptors and excitable by noxious stimuli only. However, some of them are cold or warm receptors whose functions in diabetic neuropathy have frequently been reported. Hence, it is well established by quantitative sensory testing that thermal detection thresholds at the foot skin increase during the course of painless diabetic neuropathy. Pain perception (nociception), by contrast, has rarely been studied. Recent pilot studies of pinprick pain at plantar digital skinfolds showed that the perception threshold was always above the upper limit of measurement of 512 mN (equivalent to 51.2 g) at the diabetic foot. However,

deep pressure pain perception threshold at musculus abductor hallucis was beyond 1400 kPa (equivalent to 14 kg; limit of measurement) only in every fifth case. These discrepancies of pain perception between forefoot and hindfoot, and between skin and muscle, demand further study. Measuring nociception at the feet in diabetes opens promising clinical perspectives. A critical nociception threshold may be quantified (probably corresponding to a critical number of intraepidermal nerve fibre endings), beyond which the individual risk of a diabetic foot rises appreciably. Staging of diabetic neuropathy according to nociception thresholds at the feet is highly desirable as guidance to an individualised injury prevention strategy.

Key words: Foot ulcer; Neuroarthropathy; Insensitivity to pain; Pain perception; Diabetes mellitus; Amputation; Diabetic neuropathy

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Core tip: The diabetic foot is characterised by painless ulcers and/or arthropathy. Although painless diabetic neuropathy is known as the underlying condition, little is known quantitatively about the pain evoked by noxious stimuli (nociception) at the diabetic foot. Preliminary evidence shows that pinprick pain perception threshold at plantar digital skinfolds is supranormal, beyond the upper limits of measurement. It is suggested that measuring nociception at the foot in diabetes could specify the individual risk of painless ulcers and/or arthropathy and, thereby, provide the basis of an individualised graded injury prevention strategy.

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INTRODUCTION

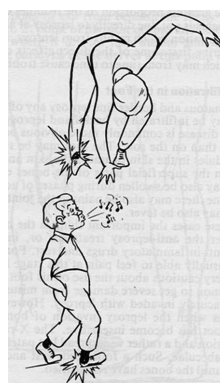
The diabetic foot (also called diabetic foot syndrome,

or diabetic podopathy) is characterised by painless foot ulceration and/or arthropathy in diabetes. Foot injuries and inflammations do not hurt, as illustrated on Figure 1.

Painless diabetic neuropathy (PLDN) is the underlying condition. Skin ulcers extending to the subcutaneous tissues are completely anaesthetic, while arthropathy may display faint deep dull aching upon load bearing. Nociception, that is the perception of pain originating from neural processes of encoding and processing noxious stimuli, is failing at the diabetic foot. Although insensitivity to pain at the diabetic foot is common knowledge, details are largely unknown^[1,2].

Research into painlessness in diabetes is scarce. Early studies date back to Pamela Margaret Le Quesne^[3] and her group almost 30 years ago. They tried to measure the pain perception (nociception) at the diabetic foot by pinching the skin with a custom made "pinchometer". The results were inconclusive at best^[4]. Other authors designed calibrated tools for assessing hypersensitivity of so-called symptomatic, *i.e.*, painful, diabetic neuropathy (SDN, see below). To this end, pinprick pain perception, axon-reflex reaction and temperature detection of the skin were studied. These modalities represent the functions of the afferent A-delta and C-fibres (so-called small fibres; see below) whose contributions to SDN, however, remain controversial^[5,6]. SDN will not be discussed here.

To date, measuring pain perception (*i.e.*, nociception) at the diabetic foot was deemed futile with the excuse that "The threshold of sensation that protects normal feet from injury is difficult to define... it is extremely difficult to define a 'significant loss of sensation', or at what level sensory loss becomes 'critical'."^[7] However, there is some dissent on this issue, as expressed by Dyck *et al*^[8]: "Sadly, the clinical assessment of decreased sensation by physicians is generally inadequate because it is not performed or is performed badly." Undeniably, many a clinical physician or expert was biased against measuring diminished nociception in diabetic neuropathy, and for several possible reasons: (1) quantitative sensory testing (QST, see below) was considered inferior to nerve conduction velocity studies in the detection of symptomatic (painful) diabetic neuropathy; (2) Experts of QST not always knew enough of the diabetic foot, this typical complication of symmetrical diabetic neuropathy, to understand what they read. For example those reviewers, who commented on a paper: "In a group of 20 patients (...) the authors compared sensation in ulcerated vs nonulcerated feet and were unable to find any difference. Had a difference been found, one might interpret the sensory dysfunction as a pathophysiological factor in ulcer development"^[9]; (3) QST findings were assumed to be poorly reproducible, an argument which was often mixed up with the natural inter-individual variability (on average 5-fold^[10]). Intra-individual coefficients of variation are acceptable, for example those of pain perception thresholds are well



Pain makes a healthy man fall when he begins to twist his foot. The man who feels no pain walks on without realising the damage he is doing.

Figure 1 Sketch reproduced from Brand P^[2].

below 25%^[10-14]; and (4) A really important obstacle against routine QST of nociceptive functions was the lack of normative data. However, since 2005, extensive reference material using a particular QST protocol was published^[12,14-17], see below. Although this protocol had primarily been devised for examining hyperalgesia in non-nociceptive pain syndromes like fibromyalgia, or neuropathic pains, and not for assessing hypoalgesia^[18], the author (Chantelau EA) believes that parts of it may well be applied for studying the failing nociception at the foot in diabetes. Diabetic hyperglycaemia in general does not interfere with QST^[19-21]; however, subclinical or clinical hypoglycaemia makes QST virtually impossible.

PLDN: THE PRINCIPAL RISK FACTOR FOR THE DIABETIC FOOT

Peripheral diabetic (poly) neuropathy is the most frequent sequel of diabetes mellitus. The nature of diabetic neuropathy has been clearly established by Martin^[22,23] in 1953 and 1954, and by Catterall *et al*^[24] in 1956. While today many people still believe that the condition is always symptomatic with spontaneous pains and dysaesthesias in the legs and feet, these authors had already noted more than half a century ago: "non-myelinated nerve-fibre degeneration occurs in a high proportion of diabetic patients, who, on clinical examination, show no evidence of 'clinical' diabetic neuropathy...the presence in diabetics of such nerve-fibre disturbance explains the frequent finding of persistently cold feet and their proneness to traumatic lesions"^[24]. Indeed, the basic feature of diabetic neuropathy is small fibre degeneration^[25] with reduced or absent nociception at the feet as the key sign (PLDN). By contrast, SDN develops secondary to PLDN, superimposes on PLDN, and affects only a minority of 15%-30% of patients^[25,26]. The symptoms of SDN like spontaneous neuropathic pains and tingling in the feet may be alleviated by drug treatment; they resolve gradually while nerve degeneration^[27] and PLDN progress. However, diminished nociception in PLDN does

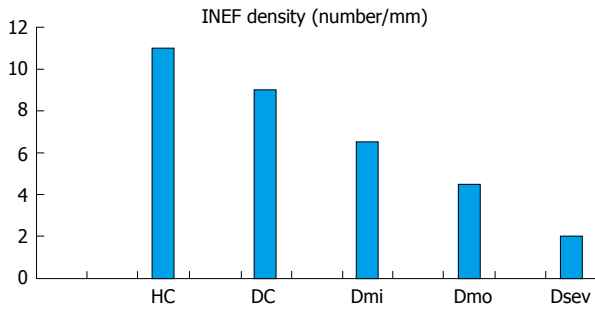


Figure 2 Intraepidermal nerve fibre ending density at the lower limb in relation to severity of diabetic neuropathy (adapted from Quattrini *et al*^[40,41]). HC: Healthy controls; DC: Diabetic controls; Dmi: Mild SDN; Dmo: Moderate SDN; Dsev: Severe SDN; SDN: Symptomatic diabetic neuropathy; INEF: Intraepidermal nerve fibre ending.

not resolve, nor does PLDN respond to drug treatment. Prevalence and incidence rates of PLDN are not known. However, the prevalence of the diabetic foot, indicative of severe, end-stage PLDN, in patients with SDN is about 10%, while the incidence is about 5%-7% per year^[28,29].

Most of the patients with PLDN never have complaints until they are facing a foot injury that -unexpectedly - does not hurt. To once more quote Catterall *et al*^[24]: "Cutaneous neuropathic changes commonly start as blisters about the tips of the toes or at the site of a corn or callosity in places constantly exposed to irritation by an ill-fitting shoe. Frequently the deceptive lack of normal sensation, and particular pain sensation, leads a patient to ignore the lesion and delay treatment until secondary infection with deep penetration of the tissues or severe inflammation is present." PLDN is associated to the duration of diabetes and to the cumulative effects of increased blood glucose (or deficient insulin function). It is symmetrical, age- and length-dependent (it starts in advanced age by affecting the endings of the longest axons in the body, in the skin of the toes^[30]). Thus, deficits in nociceptive function develop in the forefeet first, and subsequently ascend to the rearfeet, the ankles and the lower legs. The hands are affected only in most severe cases.

PLDN is a disease of the small primary afferent A-delta and C-fibres: they are thin and their conduction velocity is relatively slow. (A fraction of about 15% of small fibres extending to the skin are efferents; they belong to the sympathetic nervous system and are restricted to the dermis, innervating sweat glands, blood vessels, and the arrector pili muscles. Small fibre efferents will not be discussed here). Primary C-afferents (also named group IV fibres, axon diameter < 1.5 μm , conduction velocity 0.5-2 m/s) are unmyelinated nerve fibres. Primary A-delta afferents (also named group III fibres, axon diameter 1-6 μm , conduction velocity 5-30 m/s) consist of thinly myelinated axons whose intracutaneous endings, however, are unmyelinated^[31]. The endings of A-delta and C-afferents do not carry corpuscles but are "free"; they serve as receptors for noxious thermal,

mechanical and chemical stimuli (termed nociceptors), and as receptors for innocuous thermal stimuli. In the epidermis they are densely distributed, up to 1000 per 1 cm^2 , which is much more than in muscles, ligaments, joints and bones (periosteum). Most nociceptors are specific of one single modality, while some are polymodal. C-fibre afferents are estimated to account for 70% of all nociceptors in the skin (the remainder are A-delta nociceptors). Cutaneous A-delta nociceptor stimulation results in a short sharp, stinging "first" pain, whereas C-fibre nociceptor stimulation results in prolonged dull, burning "second" pain. In muscles, A-delta and C-fibre nociceptors evoke the same dull pain character^[32], see section 4.

In PLDN, the nociceptors degenerate progressively ("die back") by unknown molecular mechanisms. It is not clear, whether this insidious process of central-peripheral distal axonopathy^[33,34] begins already in a prediabetic stage^[25]. However, PLDN superimposes on the physiological age-related decline in sensory functions after the age of 50^[34-36], and is aggravated by other neuropathic conditions like vitamin B12 deficiency, alcohol toxicity, end-stage renal failure, or exposition to neurotoxic substances or conditions. Clinically, PLDN remains undetected until most nociceptors have died and painlessness of a foot trauma astounds the patient (and the doctor)^[24]. The vanishing of intraepidermal free nerve endings can be quantified by histomorphology^[31,37,38]; it correlates to increasing severity of diabetic neuropathy^[39-41] (Figure 2), and to rising thermal and pain perception thresholds^[34,38,40].

A-delta and C- fibre nociceptors and thermal receptors "die back" not only in the skin, but presumably also inside the adjacent subcutaneous tissues (equivalent to group III and IV fibre nociceptors in muscles, fascia, ligaments and joints) of the diabetic foot. However, this remains to be established by histopathology. A-delta (group III) fibres seem to be particularly susceptible to the neuropathic processes, as their functions deteriorate somewhat earlier than C- (group IV) fibre functions^[42].

Diabetes affects small fibres prior to large ones. When large myelinated A-beta afferents (axon diameter 6-12 μm , conduction velocity 30-70 m/s) become affected, the axon and the myelin sheet get damaged, and conduction velocity decreases. A-beta fibres mediate touch, deep pressure and vibration sensation by means of their corpuscular endings: Meissner's, Merkel's, and Pacinian corpuscles. A-beta fibres are not involved in nociception, but have a role in allodynia^[43] which, however, will not be discussed here.

Diabetic foot: End-stage complication of PLDN

The diabetic foot is characterised by painless ulceration and/or arthropathy. Since 60 years or so^[22,23,24,44] the condition is well known as an end-stage complication of diabetic neuropathy, of PLDN rather than SDN. The basic defect has frequently been termed "loss of protective sensation"^[44], which is, in fact, "loss of

nociception" (see below). Ulcers and arthropathy start from single injuries (mechanical, thermal or chemical skin wound, or skeletal trauma) which are subjected to persisting repetitive stress (load bearing by walking, because they do not hurt - Figure 1), whereby they enlarge enormously until they become apparent to the patient. The initial injury may also be caused by uninterrupted repetitive mechanical wear and tear (fatigue from overuse, remaining unperceived because of lack of nociception!) The ulcers become infected, and the infection spreads to the adjacent subcutaneous tissues. Bone and joint damage aggravates due to ongoing unprotected walking. When properly treated immediately (like any other acute foot injury in subjects with preserved nociception!), the initial injuries will heal uneventfully. Human experiments showed that wound healing is not impaired by PLDN^[45,46]. Perforating neuropathic foot ulcers heal if they are unloaded completely^[24], and if arterial blood flow is sufficient and infection is cured. The ulcers frequently break down again when repetitive stress from walking is resumed, "due to faulty and often excessive weight-bearing by the ulcerated area"^[23]. Hence, the overall amputation risk is increased: the diabetic foot is the most frequent cause of amputation in industrialised countries.

CURRENT CLINICAL PRACTICE OF DIAGNOSING DIABETIC NEUROPATHY

According to the common misconception that neuropathy in diabetes in general is SDN, current guidelines state that the condition be diagnosed and staged semi-quantitatively according to various symptom scores (e.g., Neuropathy Symptoms Score, Neuropathy Disability Score, Michigan Neuropathy Screening Instrument, Toronto Clinical Scoring System). However, testing sensory functions is also accepted: five simple nerve tests are considered diagnostic for symptomatic neuropathy, the 10-g monofilament plus 1 of the following 4: vibration using 128-Hz tuning fork, pinprick sensation, ankle reflexes, vibration perception threshold^[47]. All except vibration perception threshold are qualitative tests, all but pinprick sensation are unsuitable for diagnosing PLDN.

Vibration and touch represent A-beta fibre functions, not nociceptive functions. Nevertheless, vibration and touch sensation is used as surrogate markers of the risk of sustaining painless foot injuries (ulceration/arthropathy). To identify loss of protective sensation (LOPS) - without assessing nociception- "any of the five tests listed could be used (...). One or more abnormal tests would suggest LOPS, while at least two normal tests (and no abnormal test) would rule out LOPS"^[47].

Current guidelines claim that "estimating the severity of diabetic sensorimotor polyneuropathy has not received the attention it deserves. For a given patient with diabetes it is not sufficient to simply identify patients as having sensorimotor polyneuropathy - severity also

needs to be ascertained"^[48]. However, measuring loss of nociception (LON) as yet has no place in assessing neuropathy in diabetes^[47].

Current clinical practice of diagnosing a diabetic foot/ diabetic podopathy

Loss of touch or vibration sensation is not diagnostic of an active diabetic foot, while painlessness of a foot injury (soft tissue or skeletal lesion) or infection in a patient with diabetes mellitus is. Three different clinical pictures of active diabetic foot lesions may be discerned, according to the dominant clinical component: (1) septic; (2) ischaemic; and (3) arthropathic. After healing of such a lesion, the foot is an inactive diabetic foot (with scars, deformities, etc.) and remains a *locus minoris resistentiae* for the rest of the life. LON traditionally is not assessed systematically, but is taken as matter of fact when a patient with an injured foot does not limp. The patient history of traumatic events is often unproductive as far as normal symptomatology (pain!) is concerned. However, when concurrent symptoms of trauma onset other than pain are concerned, like swelling and erythema, patient history may be very well productive.

QST

The QST protocol, published by the German Research Network on Neuropathic Pain and supported by pharmaceutical companies, was designed for non-invasively diagnosing pain syndromes like spontaneous neuropathic pains, fibromyalgia or chronic lower back pain^[15,16,18]. It comprises 13 somatosensory sensory tests that measure the function of large (A-beta) and small (A-delta and C-) afferent nerve fibres, and the corresponding functions of the spinothalamic tract (cold detection threshold, warm detection threshold, thermal sensory limen, paradoxical heat sensation, cold pain threshold, heat pain threshold, mechanical detection threshold, mechanical pain threshold, mechanical pain sensitivity, dynamic mechanical allodynia, wind-up ratio, vibration detection threshold, and deep pressure pain threshold). Using this protocol in healthy subjects, a pool of normative reference data from face, hand and foot had been generated^[16,17].

QST of pain perception in diabetes

Of all sensory modalities that are impaired by diabetes mellitus, the nociceptive pain system is the most relevant. In the following sections, the focus will therefore be directed on pain perception thresholds: elevated thresholds indicate reduced perception of noxious stimuli, and *vice versa*. Pain tolerance or other sensory modalities (vibration, electrical current, chemicals, touch, thermal perception) will not be addressed. Some technical features of the threshold studies considered here are outlined below, concerning thermal (cutaneous heat and cold), and mechanical (cutaneous and deep pressure)



Figure 3 MSA Thermostest® (electronic device, expensive!).



Figure 5 MARSTOCKnervtest® PinPrick stimulator 512 mN (fibreglass, cheap!).

pain perception.

Cutaneous thermal pain perception: Thermal pain perception threshold is measured by applying warm or cold stimuli to the skin, generated by appropriate thermodes (Figure 3). The temperature of the thermode is gradually increased or decreased. The subject under study is asked to indicate whether a thermal sensation becomes painful. Heat pain represents C-fibre function, and cold pain represents A-delta fibre function. In healthy subjects, heat pain perception threshold may range from approximately 41 °C to approximately 47 °C (average approximately 44 °C), and cold pain perception threshold ranges from approximately 1 °C to approximately 30 °C (average approximately 12 °C).

Deep pressure pain perception: Deep pressure pain perception threshold (DPPPT) may be examined, for instance, by a hand held device (algometer, Figure 4) containing a mechanical or electronic force gauge, and a plunger with a flat blunt rubber tip. The diameter of the tip can be 11 mm (surface approximately 1 cm²), or larger, as appropriate. The plunger is pressed perpendicularly onto the skin and the underlying structures, with slowly increasing stimulus ramp (50 kPa/s, equivalent to 0.5 kg/s). The subject under study communicates when his pressure sensation turns to pain.



Figure 4 Algometer® (electronic device, expensive!).

The deep pressure pain sensation evoked by an algometer at muscle or joint is as yet not fully understood. For instance, the afferents involved (high and low threshold mechanosensitive units^[32]), the anatomical structures subjected to algometer pressure, and the role of spatial summation (pressure area 1 cm²)^[49,50], still have to be elucidated. The contribution of fascia, which is more pain-sensitive than muscle, and of periosteum, which is more pain-sensitive than bone marrow, remains to be determined. The pain quality at reaching DPPPT is dull and burning and probably more of a pressure discomfort than of a stinging or pricking^[49,50]. Inside deep tissues, stimulation of the few A-delta (group III) nociceptors simultaneously probably does not elicit a separate pricking pain sensation like a single punctuate stimulation at the skin does^[49,51]. DPPPT decreases at chronically ischaemic muscle^[52,53]. DPPPT at muscle (blunt stimulation) decreases with age, at variance to cutaneous pressure pain perception threshold (CPPPT, punctuate stimulation)^[42]. Using a pressure algometer with 1 cm² contact area to stimulate musculus abductor hallucis, DPPPT in healthy subjects may range from approximately 200 kPa to approximately 1000 kPa (average approximately 500 kPa, equivalent to 5 kg). Algometer stimulation of a joint evokes a DPPPT of the same range.

Cutaneous pressure pain perception: Cutaneous pressure pain perception threshold (CPPPT) is examined by the use of calibrated pinprick stimulators with a sharp edge, comparable to von Frey hairs. These punctuate stimulators are filaments of 50-350 µm diameter, with calibrated bending forces (or weight loads) over a range from 8 mN to 512 mN (equivalent to 0.8 g to 51.2 g) (Figure 5).

The investigator presses each single stimulator perpendicular to the skin (until bending of the filament) and the subject under study communicates, whether a painless touch is felt, or a painful (pricking or stinging) sensation, or nothing at all. The pain threshold is determined by the method of limits, or by the forced-choice-method. The stimulators excite single nociceptors representing A-delta (rather than C-fibre) functions; the

Table 1 Early studies of cutaneous pain perception thresholds (pressure, heat) at the diabetic foot, using various, non-standardised methods^[29,55,56,57]

Test	Diabetic subjects under study		Healthy controls	Ref.
	Diabetic foot	Neuropathy		
CPPPT	100 ¹	100	0	[29]
CPPPT	100 ¹	63 ¹	-	[55]
CPPPT	68	30	2	[56]
Heat	100 ¹	100	0	[29]
Heat	100 ¹	100	0	[57]

Percentages of supranormal results are shown. ¹Above upper limit of measurement. CPPPT: Cutaneous pressure pain perception threshold.

pain quality evoked is that of a stinging, pricking “first” pain. CPPPT increases with age (at variance to DPPPT, see above). At the non-callous plantar skin of healthy subjects, CPPPT may range from approximately 4 mN to approximately 450 mN (average approximately 100 mN, equivalent to 10 g).

Limitations of pain threshold measurements:

QST is a subjective psychophysical method, as it is based on the patient report. “A critical element in pain threshold determination is the particular sensory experience an individual considers painful. This factor will be influenced by, among other things, the subject’s pain experience history, and the instructions given by the experimenter. For instance, thresholds are likely to be different if a subject is instructed to indicate when he perceives ‘pain’ vs when he perceives ‘a sharp or burning sensation’ or ‘an uncomfortable situation’....”^[54]

In particular, pressure pain measurement is affected by the hardness of the skin at the site of measurement, and by the ramp rate of increasing the stimulus of hand-held algometers, amongst others.

QST of nociception at the diabetic foot

Early studies of pain perception thresholds:

Between 1988 and 2011, not a single study could be identified on cold pain perception threshold, or on DPPPT. However, there are at least 4 studies on CPPPT and/or heat pain perception threshold^[29,55-57], all of which found significantly elevated thresholds at the active (?) diabetic foot and the contralateral foot, as compared to neuropathic patients without diabetic foot (Table 1). In neuropathic patients with and without diabetic foot, pain perception thresholds were significantly higher than in non-neuropathic (healthy or diabetic) control subjects. Lang *et al*^[52] reported that perception thresholds for cold pain, heat pain and pinprick were elevated at legs with chronic severe foot ischaemia (rest pain with/without tissue loss); the 16 patients (8 of whom had diabetes mellitus) had polyneuropathy according to a vibration perception threshold < 4/8 (graduated tuning fork). DPPPT at ischaemic muscle, however, was lower than at healthy controls’ muscle (199 kPa vs 295 kPa^[52]). In another study of non-neuropathic patients with chronic leg ischaemia, CPPPT, and DPPPT at muscle, were lower

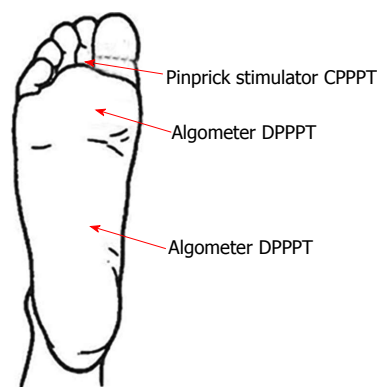


Figure 6 Pressure pain perception threshold measurements done in own studies^[58-61]. CPPPT: Cutaneous pressure pain perception threshold; DPPPT: Deep pressure pain perception threshold.

than in control subjects but cutaneous cold and heat pain perception thresholds were not^[53].

Recent pilot studies of pressure pain perception thresholds:

Between 2012 and 2014, we conducted four studies on the matter^[58-61] including explorative cross-sectional and follow-up studies. Our overall database comprised 123 subjects in whom pressure pain perception thresholds were measured: 43 control subjects, 23 of whom had an acute trauma of the foot skeleton (sprain, minor fracture), 59 diabetic patients with PLDN (46 of whom with a diabetic foot), and 21 diabetic patients without PLDN. Among the 46 patients with a diabetic foot, there were 33 with active painless foot ulcer, 13 with healed foot ulcer, 11 with healed Charcot foot (grade 1, inactive^[62]), and 13 with acute skeletal trauma (elective surgery). CPPPT and DPPPT were studied, as well as vibration perception threshold (not reported here). According to our study protocol, CPPPT was measured only at the forefoot (plantar digital skinfold), while DPPPT was measured at the hindfoot (m. abductor hallucis) and at the forefoot (metatarsophalangeal joint) (Figure 6).

By cross-sectional comparison, CPPPT at a plantar digital skinfold (glabrous skin) was above the safety limit of measurement of 512 mN (approximately 51.2 g) in 98% of cases with a past or present painless foot ulcer or healed arthropathy (Charcot foot). This is in line with the older reports cited above. Simultaneously, DPPPT at abductor hallucis muscle was above the safety limit of measurement (1400 kPa, equivalent to 14 kg) only in about 20% of cases. The DPPPT at metatarsophalangeal joint was above 1400 kPa in about 50% of cases (Table 2).

Serial pressure nociception studies to monitor pain thresholds after an acute foot trauma (ankle sprain, toe fracture) in otherwise healthy subjects revealed that CPPPT and DPPPT at the site of the trauma decreased slightly, indicating normal, inflammation-mediated posttraumatic hyperalgesia (Figure 7). This observation is consistent with previous reports (Martinez *et al*^[63]:

Table 2 Simultaneous measurements of pressure pain perception thresholds at the foot in subjects with and without painless diabetic neuropathy, with and without diabetic foot

Subject populations	Diabetic foot ¹	Healthy ²	PLDN ³	DM-controls ⁴
No. of subjects	46	43	13	21
% of subjects with CPPPT > 512 mN at toe skinfold	98%	4%	46%	5%
% of subjects with DPPPT > 1400 kPa at m. abductor hallucis	22%	0%	0%	0%
% of subjects with DPPPT > 1400 kPa at metatarsophalangeal joint	50%	2%	0%	5%

¹With previous or active ulcer/arthritis; ²Non-neuropathic, healthy; ³PLDN, no ulcer; ⁴Diabetes, no neuropathy, no ulcer. Percent subjects with thresholds above the safety limit of measurement are shown. Adapted from ref. [58-60]. CPPPT: Cutaneous pressure pain perception threshold (512 mN upper limit of measurement); DPPPT: Deep pressure pain perception threshold (1400 kPa upper limit of measurement).

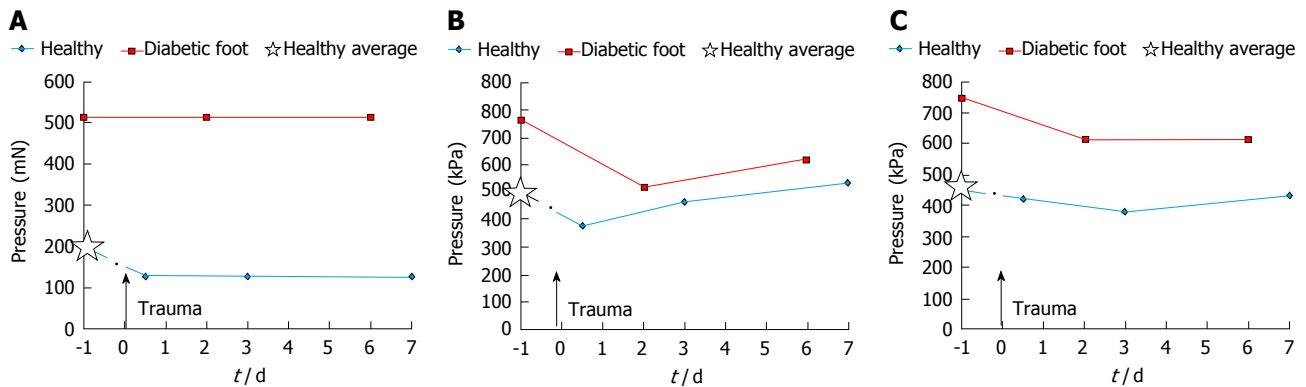


Figure 7 Lowered pain perception thresholds (hyperalgesia) after acute skeletal trauma of the foot in 13 healthy control subjects (sprain, toe fracture) and in 12 diabetic foot patients (elective surgery) at the injured site. Data are combined from ref. [59,61]. A: Cutaneous pressure pain perception threshold (mN) (pinprick) at plantar digital skinfold (medians); B: Deep pressure pain perception threshold (kPa) over metatarsophalangeal joint (medians); C: Deep pressure pain perception threshold (kPa) over musculus abductor hallucis (medians).

decrements in CPPPT on day 1-4 at an operated knee; Lang *et al*^[53]: reduction in CPPPT and DPPPT in a chronic ischaemic leg up to 3 mo after successful endovascular revascularisation, Dominguez *et al*^[64]: reduced DPPPT at the foot immediately after a sprain). This physiologic, inflammation-mediated posttraumatic hyperalgesia is the essential trigger of avoidance behaviour to preserve the injured limb from further noxious and innocuous impacts.

By contrast, at the diabetic foot CPPPT was extremely elevated (above the safety limit of measurement) prior to an acute foot trauma (elective surgery), and also immediately thereafter (Figure 7A); there was no posttraumatic decrement of CPPPT at the site of the trauma^[61]. This suggests absence of A-delta "first" pain quality and inflammation-mediated hyperalgesia. DPPPT at the diabetic foot, being elevated prior to the surgery, showed a small posttraumatic decrement from this baseline level (Figure 7B and C) which, however, was not accompanied by clinical signs of hyperalgesia, or avoidance reaction, respectively.

These data, however, have to be interpreted with caution since our study groups were small (only 12 and 13 subjects, respectively) and heterogeneous. Furthermore, it cannot be ruled out that our study subjects may have behaved like those young healthy women, who reacted to consecutive daily measurements of DPPPT over 4 d by increased pain sensitivity^[65] - a reaction that may be an exception rather than the rule^[13].

CPPPT within the normal reference range may decrease slightly upon re-testing^[14] but remains consistently above the safety limit of measurement in patients with diabetic foot^[58-61].

Miscellaneous studies of A-delta and C-fibre sensory functions at the diabetic foot:

At least 7 studies^[66-72] could be ascertained, all of which reporting that the axon-reflex reaction is not much smaller in neuropathic patients with a diabetic foot than in those without. Axon-reflex reaction in the feet of neuropathic patients was consistently smaller than in the feet of non-neuropathic control subjects.

At least 11 studies^[57,67,73-81] could be ascertained, all of which reporting that neuropathic patients with a diabetic foot have higher thermal detection thresholds than neuropathic patients without. Thresholds were higher in the feet of neuropathic patients than of control subjects, and were particularly high in patients with more severe, relapsing diabetic podopathy and/or Charcot foot^[76-79]. One study^[79] showed a lower cooling detection threshold in the active or inactive Charcot foot vs the contralateral foot, while the warming detection threshold was similar on both feet.

CONCLUSION

At the diabetic foot, cutaneous thermal and mechanical (pinprick) nociception is reduced dramatically. CPPPT

Table 3 Percentages of subjects with active diabetic podopathy (ulcer, arthropathy) according to loss of nociception

Foot morbidity	Degree of loss of nociception			Ref.
	None (CPPPT ¹ approximately 100 mN) 0%	Mild/moderate (CPPPT ¹ < 512 mN) 0%	Severe (CPPPT ¹ > 512 mN) 100%	
Past/present painless ulcer				[59]
Foot ulcer, incidence per year	Approximately 0%-1%	Approximately 6%-15%	Approximately 20%-100% ²	[28,59,77,86-101]
Charcot-foot, prevalence	Approximately 0%	Approximately 0.05%	Approximately 2%	[102]

¹Cutaneous pressure pain perception threshold, pinprick stimulator, upper limit of measurement 512 mN, measured at a plantar digital skinfold;

²Depending on compliance with prophylactic podiatry and/or special footwear. CPPPT: Cutaneous pressure pain perception threshold.

was elevated beyond the safety limit of measurement in most of the small number of published studies. Patients with diabetic foot ulcers unanimously display a CPPPT at a digital plantar skinfold which is beyond the safety limit of 512 mN. However, details of the causal role of increased CPPPT (indicating lack of A-delta nociception) for the manifestation of diabetic podopathy remain to be established, as well as the anatomical distribution of PLDN at the foot. Concerning DPPPT at the diabetic foot, the data is less clear, as differences to control subjects often were surprisingly small. Compared to baseline DPPPT, inflammation-mediated posttraumatic DPPPT was reduced at the diabetic foot, albeit at a high level and far from indicating hyperalgesia. Pending confirmation, this may suggest that DPPPT probably does not need to be extremely elevated for a diabetic foot to develop.

There are many more open questions, for instance: what number of residual intraepidermal free nerve fibre endings corresponds to the elevation of CPPPT at the diabetic foot? What elevation of CPPPT is necessary to appreciably increase a patient's risk of sustaining painless foot ulceration and/or arthropathy? How much pain threshold elevation, *i.e.*, how much "LON", is clinically meaningful? Is the nociception at healthy tissues relevant, or is it the pain perception (hyperalgesia, allodynia), that is typically caused by stimulation of inflamed tissue? Is superficial and deep tissue nociception similarly impaired by PLDN? How much are "silent" nociceptors^[82] affected? Is the stinging "first" pain more reduced than the burning "second" pain? Is there a defect in posttraumatic ongoing (non-stimulated) pain? Is absolute or relative reduction of individual pain perception essential for the diabetic foot to develop? Is pain perception or pain tolerance more relevant? Do abnormalities of the central nervous system contribute to the painlessness of the diabetic foot? To what extent is sensitization, either peripheral or central, affected at the diabetic foot? Concerning the discrepancies between CPPPT (at the forefoot) and DPPPT (at muscle of hindfoot) in our own studies^[58-61] - does the distal-to-proximal gradient of PLDN provide an explanation?

Measuring nociception at the diabetics' feet-what is it good for?

LON is the principal pathogenic component of diabetic podopathy, as it is the principal functional component

of PLDN. Loss of pressure perception does not increase the risk of diabetic foot ulceration, whereas a history of foot ulceration (equivalent to cutaneous LON) does^[83]. Measuring nociception provides the opportunity for a clinically meaningful staging of the severity of PLDN. A critical LON needs to be elaborated (probably corresponding to a critical number of intraepidermal nerve fibre endings), "to be able to inform the patient adequately about the risk of ulcer formation and to prescribe preventative measures... otherwise, sensation loss and resultant ulcers may lead to amputation", as Assal *et al*^[84] advocated 25 years ago. Perhaps every patient may have his/her own individual critical LON to be taken into account. Small reductions in intraepidermal nerve fibre density may be mirrored by respective increases in CPPPT, as recent work by Selim *et al*^[85] shows. Hence, measuring CPPPT prospectively may be suitable for monitoring progression of PLDN.

Shifting the emphasis from amputation prevention by wound healing to amputation prevention by injury prevention:

Once an active diabetic foot/podopathy has become manifest, a multitude of cumbersome, laborious, tedious, expensive and costly therapeutic interventions is necessary to save the foot and prevent an amputation. Injury prevention in high-risk PLDN - to prevent the diabetic foot- is certainly much more efficient. Preventing the first or recurrent ulcers^[86] should be no question. Having had a first ulcer strongly predisposes to subsequent ones, most likely due to comorbidities from structural abnormalities that resulted from the (healed) first ulcer (scars, deformities, contractures, tissue loss). The same holds true for arthropathy. Hence, preventing the first ulcer/arthropathy must have absolute priority. To this end, intensive prophylactic measures are necessary in high-risk patients who should be identifiable by simple means. As a proposal, the following tentative risk-stratification is construed from various published data (assuming a "normal" average walking activity of 5000 strides per day^[87]); (Table 3).

Subjects with critical LON at their feet require signals other than foot pain to control foot usage and avoid over-usage. These signals can only come from technical devices, like step counters or load monitors incorporated into footwear, that send acoustic or optical alarms when a critical load (or an equivalent number of steps) has accumulated. Then, the feet have to be rested - for an

appropriate period of time as physiologically required to regenerate the stressed tissues - before their usage (walking) may be resumed. Moreover, feet with critical LON need special footwear to avoid rubbing of the skin while walking in order to prevent skin abrasion and blister formation.

Pain is a homeostatic emotion that drives behaviour, just as hunger and thirst are. Patients who cannot have it, either from acquired or inherited insensitivity to pain^[103,104], either in the feet or elsewhere in the body, need our special protection, and much more specific than we, their caregivers, may have been willing to concede in the past. Research into nociception at the foot in diabetes must continue and expand. Chances to improve injury prevention will be missed, if this uncharted territory remains unexplored.

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Gut microbiota and Ma-Pi 2 macrobiotic diet in the treatment of type 2 diabetes

Francesco Fallucca, Lucia Fontana, Sara Fallucca, Mario Pianesi

Francesco Fallucca, "In Unam Sapientiam" Foundation, University La Sapienza, 00161 Rome, Italy

Francesco Fallucca, Mario Pianesi, International Study Center for Environment, Agriculture, Food, Health and Economics, 00161 Rome, Italy

Lucia Fontana, Unit of Dietology and Diabetology, Sandro Pertini Hospital, 00157 Rome, Italy

Sara Fallucca, Department of Endocrinology and Diabetes, University Campus Bio-Medico, 00128 Rome, Italy

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Correspondence to: Francesco Fallucca, Professor, "In Unam Sapientiam" Foundation, University La Sapienza, Largo Ettore Marchiafava 1, 00161 Rome, Italy. francesco.fallucca@gmail.com

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a rise in the numbers of overweight and obese individuals. A number of diets have been shown to be effective for the management of T2DM: the Mediterranean diet, the vegetarian diet and the low-calorie diet. Results of studies clearly indicate, however, that the efficacy of these diets is not solely related to the biochemical structure of the individual nutrients they contain. This review discusses this point with reference to the potential role of the intestinal microbiota in diabetes. The macrobiotic Ma-Pi 2 diet is rich in carbohydrates, whole grains and vegetables, with no animal fat or protein or added sugar. In short- and medium-term trials conducted in patients with T2DM, the Ma-Pi 2 diet has been found to significantly improve indicators of metabolic control, including fasting blood glucose, glycosylated hemoglobin, the serum lipid profile, body mass index, body weight and blood pressure. The diet may also alter the gut microbiota composition, which could additionally affect glycemic control. As a result, the Ma-Pi 2 diet could be considered a valid additional short- to medium-term treatment for T2DM.

Key words: Ma-Pi 2 macrobiotic diet; Type 2 diabetes; Low-grade inflammation; Gut microbiota; Metabolic control

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Core tip: Imbalances in the intestinal microbiota (dysbiosis) have been linked to diseases, including diabetes. In short- and medium-term trials conducted in patients with type 2 diabetes mellitus (T2DM), the Ma-Pi 2 diet, which is rich in carbohydrates, whole grains and vegetables, with no animal fat or protein or added sugar, has been found to significantly improve indicators of metabolic control, including fasting blood glucose, glycosylated hemoglobin, the serum lipid profile, body mass index, body weight and blood pressure. The diet may also alter the gut microbiota composition. Hence, the Ma-Pi 2 diet could be considered a valid additional short- to medium-term treatment for T2DM.

Abstract

In the past 10 years the prevalence of type 2 diabetes mellitus (T2DM) has increased hugely worldwide, driven by

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INTRODUCTION

In the past 10 years, the prevalence of type 2 diabetes mellitus (T2DM) in Italy has increased greatly; currently, approximately 3.3 million people are diagnosed with T2DM (5.5% of the total population) and another million people are thought to be affected but as yet undiagnosed. These numbers are expected to increase in the near future, and it has been estimated that 9.0% of the population will have T2DM by 2030^[1]. Similar increases are anticipated in other European countries and, globally, the number of people with diabetes is predicted to reach 592 million by the year 2035 - an increase of 55% from 2013 data^[2].

This increase in the prevalence of T2DM, which accounts for approximately 90% of all diabetes cases, is clearly being driven by an increase in the numbers of individuals who are overweight or obese^[3]. Indeed, the risk of diabetes appears to literally soar as the weight piles on; a rise in body mass index (BMI) from 21 kg/m² (healthy) to 35 kg/m² (obese) can increase the likelihood of developing the disease by a factor of 80^[4]. In line with the increase in prevalence of diabetes, the number of obese adults, together with the rates of obesity-related diseases (e.g., coronary heart disease and stroke, hypertension and arthritis) and associated healthcare costs, are all expected to increase dramatically worldwide over the next 20 years^[4,5].

T2DM and obesity, and their associated complications and costs, are therefore among the most pressing healthcare problems that society is currently facing. Despite improvements in our understanding of T2DM and the development of new diagnostic and treatment methods, we are still failing to control this epidemic. There is a crucial need to find effective, simple, pragmatic, sustainable and cheap interventions (e.g., those aimed at changing lifestyle, such as exercise and diet).

Recent reviews highlight the benefits of physical exercise in T2DM^[6]. In Italy, the Italian Diabetes and Exercise Study^[7-10] has contributed to this evidence and has helped provide definitions and standards used by the American College of Sports and Medicine and the American Diabetes Association (ADA). Yet, despite the accepted benefits of physical exercise for patients with T2DM, the implementation of exercise recommendations has proved difficult. Reasons for this include a lack of patient compliance, insufficient knowledge/awareness of benefits among general practitioners, diabetologists or exercise professionals, and a lack of dedicated facilities^[11]. In addition, in order to be successful, exercise interventions in individuals

with T2DM tend to require intensive counseling and supervision from medical or exercise professionals.

As a result, recent attention has focused less on exercise and more on diet; dietary therapy (as a life-style intervention), with or without additional drug treatment, represents an efficacious and safe alternative for T2DM management^[12]. Various cohort studies have shown that selected healthy eating patterns, mainly characterized by a higher intake of fruit and vegetables, are associated with a lower risk of diabetes^[13]. In this review, we look at some of the various diets that have been shown to be effective for the management of T2DM, in particular the Ma-Pi 2 macrobiotic diet, and explore the potential role of the intestinal microbiota in T2DM.

MEDITERRANEAN DIET

A Mediterranean-type diet is one involving: a high consumption of cereals, grains, fruits, vegetables, legumes, nuts; olive oil as the principal source of fat; low-to-moderate consumption of fish and poultry; relatively low consumption of red meat; and moderate consumption of wine, normally with meals^[14].

Based on this definition, numerous epidemiological studies have indicated the favorable effects of this diet^[15-17]. Such diets have been reported in two prospective studies^[18,19] and one intervention study^[20] to be associated with a reduced risk of diabetes. However, the role of the Mediterranean diet in weight control remains controversial^[21], which suggests that the protective effect of this diet against diabetes are not based on weight control but through several of its dietary characteristics. Currently it is unclear which component of the Mediterranean diet contributes most to its favorable effects.

LOW CALORIE DIET

Weight loss following a reduction in energy intake (caloric restriction) and/or an increase in energy expenditure (through exercise) improves insulin sensitivity, hyperglycemia and other cardio-metabolic risk factors^[22]. Caloric restriction (CR) is defined as a reduction in caloric intake by around 20%-40%, with adequate intakes of protein and micronutrients to avoid malnutrition^[23,24]. Caloric intake can be reduced by eliminating the consumption of energy-dense foods (e.g., refined carbohydrates, potatoes, white bread, white rice, sweets and sweetened drinks) and by increasing the intake of a nutrient-dense foods (a wide variety of vegetables, fruits, nuts, low-fat dairy products, egg whites, wheat and soy proteins, fish and meat). The energy intake of such a diet is around 1100-2000 kcal/d, with approximately 26% of calories obtained from protein, 28% from fat and 46% from complex carbohydrates^[23]. CR may be administered as a short- (1 mo-1 year) or long-term diet. CR in healthy individuals as well as those who are obese or have T2DM has been reported to lower blood glucose

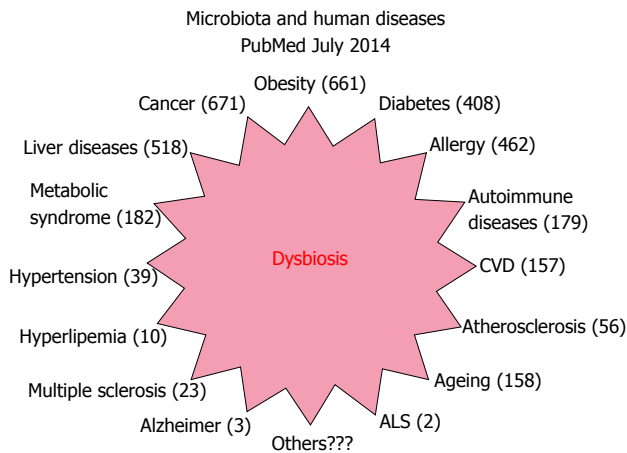


Figure 1 Articles indexed in PubMed concerning the microbiota and human diseases. Results of a research in PubMed looking for articles concerning gut microbiota that associated dysbiosis and human diseases. Number of articles for each kind of disease is indicated in brackets. CVD: Cardiovascular diseases; ALS: Amyotrophic lateral sclerosis.

levels, fasting insulin values and BMI^[25]. Weight loss induced by caloric restriction mediates a reduction in inflammatory markers and improves insulin resistance and sensitivity^[26]. With adequate nutrition, CR can improve cardio-metabolic health, prevent T2DM and may be a powerful tool against obesity and insulin resistance.

VEGETARIAN DIET

Vegetarian diets typically include large amount of fruits, vegetables and legumes, nuts and soya-protein and usually aim to eliminate all animal products. Studies have shown that, generally, vegetarians residing in Western countries have a lower BMI and a higher ratio of polyunsaturated to saturated fat intake than individuals consuming a non-vegetarian diet (people eating meat and/or fish)^[27,28]. Vegetarians have also been shown to have lower concentrations of low-density lipoprotein (LDL)-cholesterol^[29-31], lower blood pressure^[32,33] and a lower risk of diabetes^[34] than non-vegetarians. Factors associated with the higher fiber content in vegetarian diets promote increased insulin sensitivity^[35]. Vegetarian diets are also associated with other health benefits. According to the largest study ever conducted in the United Kingdom from the University of Oxford, the risk of hospitalization or death from heart disease was 32% lower in vegetarians than in people who ate meat and fish, with most of the difference in risk probably caused by effects on cholesterol and blood pressure^[36]. In addition to a lower risk of cardiovascular disease^[37,38], vegetarian diets have been reported to reduce the incidence of cancer in a low-risk population^[39,40].

MICROBIOTA

The results of some studies indicate that the efficacy of a diet is not solely related to the biochemical structure

of the individual nutrients it contains and the current standard definition of macronutrients fails to capture important information^[41]. This has become more obvious as knowledge of the gut microbiota^[42-46] has been gained and the "food as hormone" hypothesis^[47] has emerged.

The human gut microbiota weighs about 1500 g and the number of intestinal microbial cells it contains is tenfold greater than the total number of human body cells^[48]. The gut microbiome, which represents the collective genomes of all gut microbiota, is 150 times greater than the human gene complement^[46]. The gut microflora plays several metabolic active roles: it produces vitamins, synthesizes amino-acids, transforms bile acid, and is able to ferment non-digestible substrates and endogenous mucus, stimulating bacterial growth and producing short-chain fatty-acids (SCFA)^[49], which work in the gut and at distance after absorption^[49]. Moreover, the gut microbiota also causes pathogen displacement, by competing for attachment sites and nutrients, and by secreting antimicrobials^[50]. The microbiota plays a fundamental role in the development of the immune system^[51], as SCFA have a strong immunomodulatory activity, leading to the production and release of cytokines, chemokines and phagocytes^[52].

The gut microbiota is also involved in the development of cells and tissues^[53], and is responsible for the integrity of the gut barrier^[53] through its involvement in glucagon-like peptide (GLP)-2 production by enteroendocrine L-cells, and the circulation of endotoxins^[54]. In addition, the microbiota triggers the production of peptide YY, which regulates gut motility and the hungry sensation^[55].

Dysbiosis is a bacterial imbalance in the gut resulting in a modification of the normal local distribution of microbial communities that correlates with biochemical and clinical modifications in the host: hyperglycemia and T2DM have been observed in the presence of a low percentage of bacterial *Firmicutes* and *Clostridia* species^[56]. Diet and increase in bodyweight are linked to gut microbial imbalance, in both the presence and absence of obesity^[57].

Recent studies have shown a link between dysbiosis and several diseases, including diabetes (Figure 1).

Obesity and T2DM are associated with chronic low-grade inflammation and endotoxemia. Lipopolysaccharide (LPS) consumption and a diet rich in fat increase adiposity, impaired glucose tolerance, and insulin resistance those raises are reversible by the consumption of a pre-probiotic rich diet^[44,58,59], or a meal based on fiber and fruit, as recommended by the American Heart Association^[60]. Bacterial LPS derived from Gram-negative bacteria residing in the gastrointestinal microflora could act as a trigger for the development of diabetes and obesity; in a series of experiments in mice, it was observed that high-fat feeding favors an increase in the Gram-negative to Gram-positive colonizing bacteria ratio, which has been associated with an increased intestinal permeability that precedes the development of metabolic endotoxemia, inflammation, and associated disorders (obesity and diabetes)^[44]. Different nutrients have different enhancing-

effects on endotoxemia (LPS production by microflora or for higher gut permeability) and the consequent inflammatory response^[61]. Other factors may also influence our microbiota, such as age^[62-64], chemical products and antibiotics^[65], and prebiotics and probiotics^[58,66-68].

The internationally endorsed definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host"^[69], whereas, definition for prebiotics is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits upon host well-being and health"^[66]. Therefore, in order to be classified as a prebiotic, the ingredient should resist gastric acidity, hydrolysis and should be fermented by the intestinal microflora. The consumption of prebiotic foods enhances a reduction of triglyceride and postprandial glucose levels, gut permeability and inflammatory effect^[66,70]. It has been observed that administering prebiotics to obese mice induces a microbiota modulation, lowering both diet-induced LPS endotoxemia, and systemic inflammation and liver inflammation^[44]. The main positive effects of pro- and prebiotic administration are increases in GLP-1 and peptide YY secretion^[71-73], a reduction in the complications of pregnancy^[74], and improvements in metabolic diseases (obesity, T2DM, cardiovascular disease)^[75].

New findings in microbiota knowledge are opening new fields of study about human health and disease. However, the research regarding the interaction between bacteria and human health is at the beginning and many aspects are still not properly understood; well-defined clinical studies are needed in order to develop the potential of this new therapeutic area.

Until now, scientists have focused primarily on identifying human genetic markers, but research has shown that other factors, such as the intestinal flora, play a role in the development of T2DM^[45,46]. The results of metagenome-wide association studies in T2DM patients and non-diabetic controls^[45,46] clearly indicate that T2DM is a significant factor in the observed variation in gut microbial samples, and suggest that the gut microbiota in T2DM patients features dysbiosis. These studies^[45,46] indicate that a gut-microbiota-based index could be more effective and accurate in assessing and predicting the risk of T2DM than a human genome variation-based one.

Knowledge of the microbiota and the general concept of "food as hormone", as proposed by Ryan *et al.*^[47], suggest that diet has an enormous impact on our health. In fact, circulating substrates derived from food have both direct and indirect actions and, ultimately, food may be considered a cocktail of hormones that exert their effects on target tissues, activating cell-surface or nuclear receptors. Food components also interact with the gut flora to induce indirect signals. Therefore, if we consider this new role for food, we can make dietary recommendations to promote health or treat specific diseases, taking into account that until now macronutrients have been classified according to their energy-yielding biochemical properties and not by their

ability to work in a manner similar to that of hormones. Identifying these food- and food metabolite-receptor interactions will provide new opportunities for studying the relationship between the food we eat and diseases, including obesity.

MA-PI 2 MACROBIOTIC DIET

Macrobiotic diets are originally derived from an ancient Eastern philosophy of life based on two ancient Asian theories (Yin/Yang and the Five Transformations), formulated for Western culture by the Japanese philosopher Georges Ohsawa^[76] and further updated by Mario Pianesi who created the 5 Ma-Pi diets^[77]. The Ma-Pi 2 diet was specifically designed for patients with metabolic disorders, and consists of 50%-55% whole-grain (rice, millet and barley), 35%-40% vegetables (carrots, savoy cabbage, chicory, red radish, onions, parsley, cabbage) and 8%-10% legumes (adzuki beans, chickpeas, lentils and black beans), plus gomashio (roasted ground sesame seeds with unrefined sea salt), and fermented products [miso, wandadou jiangyou (soy sauce) and yanzimei (pickled ume plums)], which could have probiotic effect; seaweeds [Kunbu (*Laminaria japonica*, Aresch), Qundaicai (*Undaria pinnatifida*, Harv.), Haitai (*Porphyria tenera*, Kjell.) and Hiziki (*Sargassum fusiforme*, Harv.)] and Beicha tea (caffeine-free green tea). The daily average energy intake is in the range of 1700-2200 kcal (12% from proteins, 18% from fats and 70% from carbohydrates, mainly complex). The Ma-Pi 2 diet contains, on average, 18% saturated, 46% monounsaturated and 36% polyunsaturated fat, with no trans-fatty acids and an n-6:n-3 polyunsaturated fatty acid ratio of 5:1. It provides nutrients and phytochemicals with antioxidant, hypoglycemic and hypolipidemic effects, such as vitamin C, β carotene, magnesium (average 700 mg/d), manganese (average 16 mg/d), zinc (average 15 mg/d), chromium, phytosterols (average 326 mg/d), dietary fiber (average 50-60 g/d), inulin (average 9 g/d), polyphenols, tocotrienols, folates (more than 500 μ g/d), quercetin, and prebiotic and probiotic products. The Ma-Pi 2 diet excludes all animal products, (including egg and dairy), and has no added sugars^[77].

All of the ingredients used to prepare the Ma-Pi 2 diets are grown, stored and processed without the use of synthetic chemicals. The crops are from old seed varieties, which were produced using natural methods. All of the products used are labeled, providing detailed information on the origin and characteristics of the product and its supply chain (the Pianesian Transparent Label)^[78]. The nutritional content of one Ma-Pi 2 diet used in a clinical study in T2DM patients is presented in Table 1^[79].

Since 2001, various clinical studies have been carried out to assess the effect of the Ma-Pi 2 diet in T2DM patients.

Single-arm studies of the Ma-Pi 2 diet in patients with T2DM conducted in several countries and continents (e.g., America, Asia, Africa and Europe) have consistently

Table 1 Example of the average daily energy and nutrient intake for the Ma-Pi 2 diet during in one dietary intervention study^[79]

Nutrient	Ma-Pi 2 diet
Energy (kcal)	2174
Protein (g)	66
Tryptophan ¹	13
Threonine ¹	35
Isoleucine ¹	41
Leucine ¹	73
Lysine ¹	42
Met + cystine ¹	34
Phen + tyrosine ¹	78
Valine ¹	50
Total fat (g)	38
Cholesterol (mg)	0
Carbohydrates (g)	392
Fiber (g)	54
Vitamin C (mg)	164
Folic acid (g)	751
Vitamin B1 (mg)	3.52
Vitamin B2 (mg)	1.3
Vitamin B6 (mg)	5.55
Niacin (mg)	25
Vitamin B12 (g)	0.45
Vitamin E (mg)	10
Vitamin A (g)	3266
Potassium (mg)	3646
Manganese (mg)	16
Iron (mg)	24
Calcium (mg)	982
Phosphorus (mg)	1632
Zinc (mg)	15.4
Magnesium (mg)	754
Sodium (mg)	1724

¹mg of amino acid per gram of protein.

shown that consumption of the diet for 3 or 6 mo resulted in statistically significant improvements from baseline in indicators of metabolic control, including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), the serum lipid profile [including reductions in total cholesterol, LDL cholesterol and triglyceride values, and the LDL:high-density lipoprotein (HDL) cholesterol ratio], BMI, and insulin resistance, as well as body weight and blood pressure ($P < 0.001$) (Table 2)^[79,80-84]. These results were apparent even in patients with poor glycemic control (HbA1c $> 8.5\%$)^[85]. In patients being treated with insulin, use of this agent fell during the intervention^[79,85].

Similar results were obtained later in short-term (21-d) intervention studies^[86,87], thus demonstrating the ability of the diet to achieve rapid metabolic control.

The first randomized, controlled trial comparing the Ma-Pi 2 diet (in 25 participants) with Italian dietary recommendation for T2DM (in 26 participants)-the MADIAB trial-was carried out in 2013^[88]. After 21 d on the prescribed diet, which was administered under supervised conditions, the average daily energy intake was 1803 kcal (12% protein, 15% fat, and 73% complex carbohydrates, with 29 g/1000 kcal fiber) in the Ma-Pi 2 group and 1798 kcal (18% protein, 32% fat,

Table 2 Metabolic effects of the Ma-Pi 2 dietary intervention after variable periods of time

Metabolic parameters	Duration of Ma-Pi 2 intervention	Effect
Body mass index	3 wk-6 mo	↓
Body weight (kg)	3 wk-6 mo	↓
Body fat mass (%)	3 wk-6 mo	↓
Body fat free mass (%)	3 wk-6 mo	↔
Fasting glucose (mg/dL)	3 wk-6 mo	↓
Fasting insulin (mIU/mL)	3 wk	↓
HbA1c	3 wk-6 mo	↓
HOMA-R index	3 wk-6 mo	↓
Triglycerides (mg/dL)	3 wk-6 mo	↓
Total cholesterol (mg/dL)	3 wk-6 mo	↓
HDL-cholesterol (mg/dL)	3 wk-6 mo	↔
LDL-cholesterol (mg/dL)	3 wk-6 mo	↓
Systolic blood pressure (mmHg)	3 wk-6 mo	↓
Diastolic blood pressure (mmHg)	3 wk	↓
Urinary pH	3 wk	↑
Serum anion gap (mEq/L)	3 wk	↓
Serum bicarbonate (mEq/L)	3 wk	↑
Tumor necrosis factor alpha (ng/mL)	3 wk	↓
Interleukin 6 (pg/mL)	3 wk	↔
Insulin-like growth factor 1 (ng/mL)		↓

Taken from the results of studies investigating the Ma-Pi 2 diet in men and women with type 2 diabetes over variable periods. ↑: Increase; ↓: Decrease; ↔: No effect; HbA1c: Glycosylated hemoglobin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

and 49% complex carbohydrates, with 20.5 g/1000 kcal fiber) in the control group ($P = 0.860$).

The multivariate analysis (adjusted for age, gender, BMI at baseline, and physical activity) showed that significantly higher percentage reductions in FBG, post-prandial blood glucose (PPBG), HbA1c, total cholesterol, LDL-cholesterol, LDL:HDL ratio, BMI, weight, waist and hip circumference, and insulin resistance were seen in the Ma-Pi 2 group than in the control group. Although the triglyceride levels were reduced in all subjects, the reduction detected in the controls was significantly higher than that in intervention subjects. Furthermore, all subjects in the Ma-Pi 2 group achieved FBG and PPBG target levels (< 110 mg/dL for FBG and < 140 mg/dL for PPBG) at the end of the 21-d dietary treatment^[88].

Further results from this trial, presented at the 2014 ADA 74th Scientific Sessions, have also demonstrated that the Ma-Pi 2 diet is a safe strategy by which to reduce markers of insulin resistance and inflammation. In addition, a significant reduction in insulin-like growth factor-1 was observed in the Ma-Pi 2 subjects vs the controls ($P < 0.001$)^[89].

The MADIAB trial is also currently assessing the hypothesis that the Ma-Pi 2 diet may affect the composition of the gut microbiota to a greater extent than the control diet. The results of this analysis will be published later this year.

These results strengthen the idea that the Ma-Pi 2 diet could play an important role in the treatment of T2DM,

as whole cereals, non-animal fats and protein, and the high fiber content (> 50 g/d) and probiotic presence could improve gut microbiota composition, favoring a reduction in inflammation and insulin resistance^[90].

Eliminating all animal products from the diet may increase the risk of certain nutritional deficiencies in some micronutrients such as vitamin D, calcium, iron, zinc and long-chain n-3 (omega-3) fatty acids^[40]. However, the Ma-Pi 2 diet content of those micronutrients seems to be adequate^[77,79]. Special concern should be given to possible deficiencies of vitamin B-12^[77], even though clinical evidences for deficiency in this vitamin are described only after several years of insufficient consumption^[91]. The MA-PI 2 diet was especially conceived for the treatment of T2DM and studies in adults with T2DM consistently showed that a 3 or 6-mo consumption of the Ma-Pi 2 diet was nutritionally safe, at least for the evaluated time^[79-83,85]. These evidences suggest that Ma-Pi 2 diet can be used as a short- and medium-term treatment, aimed to achieve a good metabolic control and that further research is needed to demonstrate the safety of this diet in the long-term.

However, currently available studies on the Ma-Pi 2 diet in patients with T2DM have a number of limitations, such as the use of relatively small sample sizes. This was due to the particular design of the studies, where subjects were required to dwell in isolated environment 24 h per day, in order to ensure a good compliance to the diets^[80-85,87-89]. However the MADIAB randomized trial was adequately powered and corroborative analyses were performed to ensure consistency and robustness of the clinical trial results^[88]. Other limitations in available studies on the Ma-Pi 2 diets include their short- or medium-term durations and the fact that, in some studies, participants (in both the intervention and control groups) were studied in a supervised environment^[84,85,87-89]. Subject compliance to dietary recommendations for T2DM are required to obtain clinically significant changes in outcomes, and improvements obtained with dietary interventions in clinical trials are not easy to reproduce in daily life. Future studies should aim to address all of these issues.

CONCLUSION

A variety of diets have been shown to be effective for the management of T2DM, including the Mediterranean-style, vegetarian and low-calorie diets. However, the Ma-Pi 2 diet, in both uncontrolled and controlled short- and medium-term trials conducted in patients with T2DM in several countries and continents, has been found to achieve a speed of metabolic control that has not been reported in studies of other diets.

This finding could be attributable to the composition of the Ma-Pi 2 diet, which is high in whole grains, vegetables and legumes, and fermented products, with no animal products and no added sugars. These characteristics allow to improve glycemic control, reduce insulin requirements, improve insulin sensitivity, lower blood cholesterol and

triglycerides, reduce body weight control, and lower systemic blood pressure. The dietary habits may also modify the gastrointestinal microflora composition, which could influence glucose control.

Hence, the Ma-Pi 2 diet could be considered a valid additional short- to medium-term treatment for T2DM, particularly when glycemic control needs to be rapidly improved. Further research is needed to demonstrate the efficacy of this diet in the long-term management of T2DM.

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Some of the experimental and clinical aspects of the effects of the maternal diabetes on developing hippocampus

Javad Hami, Fatemeh Shojae, Saeed Vafaee-Nezhad, Nasim Lotfi, Hamed Kheradmand, Hossein Haghir

Javad Hami, Fatemeh Shojae, Saeed Vafaee-Nezhad, Nasim Lotfi, Department of Anatomy, School of Medicine, Birjand University of Medical Sciences, Birjand 97178, Iran
 Nasim Lotfi, Hossein Haghir, Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad 91857, Iran

Hamed Kheradmand, Hazrat Rasoul Hospital, Tehran University of Medical Sciences, Tehran 19168, Iran

Hossein Haghir, Medical Genetic Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad 91857, Iran

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Correspondence to: Hossein Haghir, MD, PhD, Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Azadi Square, Mashhad 91857, Iran. haghirh@mums.ac.ir

Telephone: +98-513-8002486

Fax: +98-513-8002486

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of hippocampus structure and function. Although, the exact mechanism by which maternal diabetes affects the developing hippocampus remains to be defined. Multiple biological alterations, including hyperglycemia, hyperinsulinemia, oxidative stress, hypoxia, and iron deficiency occur in pregnancies with diabetes and affect the development of central nervous system (CNS) of the fetus. The conclusion from several studies is that disturbance in glucose and insulin homeostasis in mothers and infants are major teratogenic factor in the development of CNS. Insulin and Insulin-like growth factor-1 (IGF-1) are two key regulators of CNS function and development. Insulin and IGF-1 receptors (IR and IGF1R, respectively) are distributed in a highly specific pattern with the high density in some brain regions such as hippocampus. Recent researches have clearly established that maternal diabetes disrupts the regulation of both IR and IGF1R in the hippocampus of rat newborn. Dissecting out the mechanisms responsible for maternal diabetes-related changes in the development of hippocampus is helping to prevent from impaired cognitive and memory functions in offspring.

Key words: Maternal diabetes; Cognition complications; Teratogenic factor; Hippocampus

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Core tip: Diabetes mellitus is the most seriously metabolic condition in pregnancy that affects the hippocampal development and function of the offspring. Multiple biological alterations, including hyperglycemia and hyperinsulinemia are occurring in maternal diabetes and impair the neurodevelopment of the fetus. Insulin-like growth factor-1 (IGF-1) and insulin are important regulators of development of central nervous system. It has clearly showed that maternal diabetes disturb the regulation of both insulin receptors and IGF-1 receptors in the hippocampus of rat newborn. This article is a brief review of the literatures that suggests a probable

Abstract

Diabetes mellitus during pregnancy is associated with an increased risk of multiple congenital anomalies in progeny. There are sufficient evidence suggesting that the children of diabetic women exhibit intellectual and behavioral abnormalities accompanied by modification

mechanism of how diabetes during pregnancy affects the hippocampus development in the offspring.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic common metabolic illness characterized by hyperglycaemia associated with insulin deficiency or insulin resistance. There are two major types of DM: type 1 DM (T1DM) results from chronic and progressive destruction of the beta-cells of the islets of Langerhans in the pancreas. T2DM is primarily characterized by both insulin resistance and relative insulin deficiency^[1,2].

DM is one of the most common and most important metabolic condition affecting up to 7% of pregnancies. Several population studies show that the diabetes during pregnancy affects the health of both mothers and their infants^[3-6].

There are two main types of DM occurs in pregnancy period: pregestational DM (PGDM) and gestational DM (GDM)^[7]. GDM accounts for approximately 90% of all cases of diabetes in pregnancy. Of the remaining cases, 60% have preexisting T2DM before pregnancy, while 40% have a diagnosis of pre-conceptual T1DM^[8].

In a study in the United Kingdom, it was found that PGDM occurs in approximately 1 out of 250 pregnancies, with two-thirds of them having T1DM and the remaining one-third having T2DM^[9]. At present, type 2 diabetes is the most prevalent form of diabetes affecting women of reproductive age in the developed countries, with a mean prevalence of 27.6% in United Kingdom^[10]. Last data from Australia also showed that T2DM affected about 55% of pregnancies^[11]. Overall, GDM complicated from 2% to 12% of all pregnancies. So, however, the incidence of T1DM appears to be relatively constant and similar to the rate in young adults throughout the world; the incidence of T2DM is specially rising in developed world^[9,12]. Furthermore, the overall numbers of children born to mothers with diabetes will significantly rise in the next decades.

COMMON CONGENITAL MALFORMATIONS IN DIABETIC MOTHER INFANTS

An increasing number of evidence clearly showed that infants born to diabetic mothers are in increased risk of fetal and neonatal anomalies which results in increased infant mortality and morbidity rates. Although, the

wide spectrum of the effects of diabetes in pregnancy on the fetus development are determined by the time of onset and the severity of DM^[6,13,14].

Despite considerable progress in the clinical management of pregnant women who have diabetes, the prevalence of major congenital anomalies is approximately 3 times more in infants of diabetic women in comparison to the offspring of normal mothers^[12,15,16].

According to previous works, diabetes in pregnancy has been accompanied by increased risk of a wide range of fetal complications and malformations, including respiratory distress syndrome, macrosomia, organomegaly, obstetric, metabolic and hematological complications^[13,14,16,17].

The overall rate of major congenital anomalies in infants born to diabetic mothers is about 6% to 13%, which is approximately 2-3 times higher than that of the normal condition. However, the prevalence of major structural malformations in the children born to mothers with diabetes are to 10 times greater in comparison to the general population. The frequencies of perinatal mortality are also five-fold higher in women with diabetes compared with normal women^[12,14,16,18].

The prevalence of Minor congenital anomalies in infants born to 802 mothers with GDM, 117 mothers with PGDM, and 380 normal mothers were assessed by Hod *et al*^[19] (1992). They found a range between 19.4% and 20.5% in prevalence of minor congenital anomalies in their infant in all groups studied without any marked difference between groups. In their study, there was no correlation between the type and prevalence of minor congenital anomalies with the severity and the time of onset of the diabetes in mothers. Neither there was any relationship between the type or prevalence of minor congenital anomalies with the type or appearance of major congenital anomalies^[19].

Overall, the defects in central nervous system, heart, kidney, and skeletal system are among the commonest congenital malformations in the offspring of diabetic pregnancies^[3,14,16,18].

PATHOGENESIS OF COMMON COMPLICATIONS IN DIABETIC INFANTS

As yet, the exact mechanisms by which maternal diabetes alters the development of growing fetuses are not completely understood, and no distinct teratogenic mechanism has been identified to clearly explain a reason for this range of congenital anomalies observed in the infants born to diabetic mothers^[20,21].

Many of the developmental effects of diabetic pregnancies on the fetus can be attributed to maternal glucose (metabolic) control. In normal pregnancies, the blood glucose level in women remains within a tight range. Consequently, the blood glucose concentration in the fetus is fairly constant as the glucose in the mother's blood crosses the placenta freely. Nevertheless, in pregnancies complicated by DM, there are great

variations in the maternal blood glucose level^[12,14,22]. Pedersen *et al*^[23] (1954) emphasized the correlation between high blood glucose concentration in pregnant women with hyperglycemia during fetal period^[23], which stimulated the pancreas of the fetuses, leads to beta-cell hyperplasia and hypertrophy with increased insulin secretion and content^[23,24].

Several lines of studies also emphasized the direct correlation between high blood glucose concentration in mothers as revealed by increased level of glycosylated hemoglobin (HbA1c) with increased frequency of congenital malformations in offspring^[22,25]. Towner *et al*^[26] (1995) found a striking correlation between pregestational elevated HbA1c in early pregnancy with the increased risk of fetal congenital malformations^[26]. There is enough evidence accumulates to support this hypothesis that the tight glycaemic control in early and before pregnancy may reduce the frequency of anomalies in the infants born to diabetic mothers^[22]. Therefore, the prevalence of congenital abnormalities in children born to mothers with diabetes has decreased in the last three decades probably as a result of the overall progress in monitoring of blood glucose concentrations in diabetic pregnant women^[27-29].

In addition, fetal hyperinsulinemia also has been shown in the offspring of diabetic mothers. On separation of the newborn from the mother, the glucose former no longer is supported by placental glucose transfer, may develop neonatal hypoglycemia^[30,31].

In earlier works, chronic fetal hyperinsulinemia in the third trimester of pregnancy has been produced in monkeys. The researchers showed that *in utero* hyperinsulinemia results in an organomegaly and fetal macrosomia, except for kidney and brain^[32,33]. Increased plasma erythropoietin levels and body fat content was also evident in infants of diabetic mothers^[34].

Insulin is a hormone that primarily functions as an anabolic hormone of fetal development and growth resulting in macrosomia and visceromegaly^[14]. On the basis of *in vitro* and animal studies on the pancreas, the simplified hyperinsulinemia - hyperglycemia hypothesis has been expanded in earlier studies^[35,36].

Ketones in the mother's blood readily cross the placenta, but they cannot be cause fetal hyperinsulinemia; so, they might not influence fetal growth and development^[14].

In experimental diabetic rodents, fetal hyperglycemia, neonatal hypoglycemia, and disturbances in prostaglandin and arachidonic acid metabolism have been reported to result in congenital anomalies in their offspring^[14,37]. In diabetic pregnancies in rats, it is clearly established that the elevated intracellular free oxygen radicals concentrations have been accompanied by the increased risk of embryopathies^[38]. Nevertheless, hyperglycemia-induced fetal malformations in rodents have been demonstrated to associate with the number of genomic DNA mutations^[39]. Therefore, there are animal

studies showing that the free radical scavengers and antioxidants may decrease the risk of teratogenicity of maternal diabetes^[40,41].

DIABETIC PREGNANCY AND NEURODEVELOPMENTAL SEQUELAE IN HUMAN

Both PGDM and GDM can affect fetal neurodevelopment^[42-45]. Although the untoward effects of maternal diabetes on pregnancy outcomes with respect to congenital malformations have generally been appreciated, the effect of maternal diabetes on the development of central nervous system (CNS) in the fetuses and its behavioral sequelae remains to be completely defined. A limited number of long-term follow-up studies of neurodevelopmental outcomes in diabetic pregnancies have been reported^[46-50]. Nevertheless, it is demonstrated that children born to diabetic mothers are more likely to have neurodevelopmental abnormalities including impairments in learning ability, activity level, attention span, and motor functioning. Interestingly, some of these deficiencies are well-known as risk factors in children who develop schizophrenia later, but the degree of risk is variable and may be related to the severity of metabolic derangement in the mothers with diabetes^[42,46,50,51].

Examination of cognitive functioning and the behavior of the children born to diabetic mothers offers the opportunity to functionally assess the CNS development^[52]. Hence the assessment of the behavior and cognition in the offspring of diabetic mothers may clarify the maternal diabetes effects on the development of CNS.

The results of earlier studies clearly demonstrated that the diabetes during pregnancies may results in intellectual and behavioral functioning disturbances in the infants^[45,46,49,53]. These data suggest a teratogenic effect of diabetes in pregnancy on the function of CNS in fetuses and provides the earliest indicator of postnatal CNS deficiencies reflected in intellectual and behavioral problems observed in the children of mothers with diabetes^[54].

Earlier reports on the neurologic development in infants of diabetic mothers revealed serious CNS deficits, even in the absence of structural malformations. These alterations were significantly less severe when maternal diabetes was medically controlled and treated, but some alterations in cognitive function may persist throughout childhood^[6,42,45,48,51].

To elucidate the effects of diabetes during pregnancy period on cognitive functioning in the children, Yamashita *et al*^[45] (1996) studied 33 pregnant women (24 with T2DM, 6 with T1DM, and 3 with GDM). Their long term follow-up research showed that maternal diabetes significantly affects the development of intellectual functioning in infants. Although, they report no differences in IQ score among offspring of three groups^[45]. In another

study, Churchill *et al.*^[55] (1969), found that diabetic mothers had offspring with significantly lower mean IQs than control infants. In a cohort study of fifty infants at 1, 3, and 5 years of age born to diabetic mothers, Stehbens *et al.*^[56] (1977) reported an adverse effect of diabetes in pregnancy on CNS structure and function in their children. They also found that three infants born to diabetic mothers had major neurologic anomalies and six of them had IQs less than 80.

Rizzo *et al.*^[57] (1991) reported a strikingly correlation between diabetes in mothers during pregnancy and lower IQ in their children. In another study, the same researcher found significant correlations between second- and third-trimester regulation of glycemia and results on the Brazelton Neonatal Behavioral Assessment Scale; they reported an association between increases in maternal glucose levels with poorer infant responses^[58].

Other investigators have not found any differences in cognitive scores^[59-61]. Children born to diabetic mothers may sustain minor neurological damage which does not necessarily affect their scores in IQ tests^[49]. For example, Persson *et al.*^[60] reported more encouraging results in 73 infants born either to mothers with T1DM or to mothers with GDM; all subjects available for follow-up at 5 years of age had normal results on neurologic examinations, as well as normal IQs, and there was no relation between maternal diabetes and IQ^[60].

In a retrospective study at considerable variance with most other reported studies, Yssing *et al.*^[62] (1975) reported a 36% incidence of cerebral dysfunction or related conditions in 740 infants of diabetic pregnancies; 18% had a major cerebral disability. In a study by Haworth *et al.*^[63] (1976) on infants of diabetic pregnancies, noted an about 30% increase in the incidence of neurological and intellectual development impairments^[63]. No differences in behavioral adjustment and academic achievement between children born to T1DM mothers and children of normal mothers were reported in Hadden *et al.*^[64] study.

In addition, poorer habituation performance were also found in fetuses of diabetic pregnancies when compared to fetuses born to normal mothers^[65]. Since habituation reflects the fetal CNS performance, the observed habituation abilities differences between the diabetic and normal groups suggests differences in their CNS function or maturation^[65,66]. Recent investigations also have been demonstrated that there is a significant correlation between diabetes during pregnancy and increased risk of some psychologic disturbances including schizophrenia in their children^[67,68].

Together, these studies suggest a wide-range of teratogenic effects of maternal diabetes on the development and function of fetal CNS^[67]. However, no single molecular mechanism can fully explain the effects of maternal diabetes on fetal neurodevelopment because the CNS development is complex and regulated by a number of signaling molecules and transcription factors.

DIABETES IN PREGNANCY AND NEURODEVELOPMENTAL ABERRATION IN ANIMALS

Neurodevelopmental assessment of the offspring born to diabetic dams have been revealed a wide spectrum of behavioral, neurochemical, cellular, and molecular impairments^[69-72]. Experimental models subjected to streptozotocin - induced type 1 diabetic pregnancies developed significant deficits in cognitive behaviors^[68]. Kinney *et al.*^[73] (2003) found that the only female offspring born to diabetic dams showed deficits in long-term memory and learning. These results have suggested that the *in utero* diabetic condition has gender-specific effects on CNS development^[73].

In a study by Plagemann *et al.*^[74] (1998), alterations in catecholamines levels in the hypothalamic nuclei of newborns born to diabetic animals were evaluated. They reported an increased hypothalamic dopamine (DA) and norepinephrine (NE) concentrations in the offspring born to diabetic rats at birth. Twenty one-day-old pups born to diabetic mothers, NE levels were strikingly increased in the ventromedial hypothalamic nucleus and the lateral hypothalamic area (LHA), while DA levels were significantly elevated in the paraventricular hypothalamic nucleus and the LHA. The authors concluded that there are strikingly differences in hypothalamic catecholaminergic systems during early development in the rat newborns born to diabetic animals^[74].

It has been shown that PGDM is associated with an increases risk of neural tube defects (NTDs), also known as diabetic embryopathy. The prevalence of NTDs in the offspring of diabetic mothers is 3 to 10-fold higher than that of in general population^[75-77]. In earlier studies, the mitochondrial morphological changes has been shown in developing neural tubes subjected to a diabetic environment during the same time period when the NTDs are induced in diabetic pregnancies^[72,78]. Altered activity of cytochrome-c oxidase also has been manifested in the rat hippocampus that have iron deficiency in their brains due to fetal hyperglycemia^[79]. It has also been hypothesized that dysfunction in energy metabolism of developing brain leads to oxidative stress elevation and abnormal regulation of intracellular calcium^[80-82].

NEUROPATHOPHYSIOLOGY OF DIABETIC PREGNANCY

Several well-known biological alterations occur in mothers with diabetes that affect fetal neurodevelopment. These biological changes lead to alterations in neurotransmitter systems, synaptic membranes, neuronal integrity, and growth factors expression that have also been implicated in the development of neurodevelopmental and neurocognitive sequelae in offspring born to diabetic mothers^[14,42,69,72,74].

The hyperglycemia is the most distinct mechanism by which these predispositions might be mediated. The defining characteristics of diabetes during pregnancy, these are clearly showed to have effects on developing CNS and to induce fetal hyperinsulinemia, chronic tissue hypoxia, decreased in fetal iron levels and increased oxidative stress^[20,83-85].

Hyperinsulinemia, hypoxia, polycythemia and iron deficiency

It is revealed that fetal polycythemia induced by DM in pregnancy is likely triggered by the chronic *in utero* hyperglycemia and hyperinsulinemia and develops in response to higher concentration of blood glucose in mothers^[20,42]. A study by Mimouni *et al.*^[86] (1986) showed an incidence of 29.4% fetal polycythemia in infants born to diabetic mothers in comparison to 5.9% in normal conditions. One of the reasonable explanations is that *in utero* hyperglycemia and hyperinsulinemia produces a hypermetabolic state in the developing fetuses, which results in relative tissue hypoxia, leading to polycythemia *via* excess production of erythropoietin. Elevated fetal plasma erythropoietin concentrations in diabetic pregnancies suggests an increased prevalence of chronic fetal hypoxia during development. Although there are no distinct mechanisms for fetal hypoxia in diabetic pregnancies^[34].

Hypoxia affects the fetal CNS development including alterations in myelination, changes in cortical connectivity, excitotoxicity, and neuronal or glial cell death^[87-89].

In the hypoxia state, excess erythropoietin and hemoglobin are produced^[90]. So, the developing fetuses need for iron exceeds its supply that leads to Iron mobilization from vital tissues including the fetal brain^[91,92]. It is showed that human infants born to diabetic women possess brain iron content 40% less than that in normal pregnancies^[93].

Several authors have reviewed the role of iron on CNS development and function^[94-96]. Iron is one of the key component of the many enzymes involve in essential reduction or oxidation reactions, neuronal replication, synthesis and catabolism of neurotransmitters and myelin production^[97-101]. The uptake of Iron into the brain is maximal during the rapid brain development and growth, which coincides with the peak of myelinogenesis^[99-101]. In experimental animals, iron deficiency demonstrated to affect the development of neurotransmitter systems in the brain results in behavioral alteration^[98,102]. Moreover, iron deficiency in fetuses is also known to manifest as increased negative emotionality and higher levels of irritability in infants and is a predictive factor in the behavioral and developmental problems at 5 age old children^[103]. There are studies suggesting that iron deficiency during brain development leads to alterations in the hippocampal structure and function^[104].

Moreover, in animal models, hyperinsulinemia during *in utero* period reduced the fetal amino acid

concentrations, including levels of the nonprotein amino acid taurine, which is showed to be involved in the development of the brain^[105].

Diabetes during pregnancy as a proinflammatory milieu

Diabetes during pregnancy is associated with the disbalance of pro-inflammatory pathways supported by increased circulating concentrations of inflammatory molecules^[106,107]. It is demonstrated that inflammatory cytokines affect the neuronal development and metabolism of neurotransmitters^[108]. In experimental models, cytokines reduced the survival of dopaminergic and serotonergic cells^[109]. Earlier investigations demonstrated that cytokines, including tumor necrosis factor alpha and interleukin-6, are increased in infants exposed to diabetes *in utero* and have been implicated in neuronal damage^[110,111]. It is also clearly showed that imbalances in the regulation of cytokines have been increasingly associated by a number of prevalent and severe neurodevelopmental disorders^[112,113].

Currently, a limited number of human studies demonstrating the effects of inflammatory prenatal environment on neurobehavioural development in children^[114]. Evidence from a few studies suggests that immune alterations that occur in prenatal period may persist into postnatal life^[115,116]. Therefore, increased inflammatory cytokines in diabetic pregnancies may be relevant to neurodevelopment alterations exhibited by the infants born to diabetic women.

Diabetes in pregnancy and oxidative stress

Enhanced oxidative stress plays important roles in embryo development and implicated in the pathogenesis of some neurodevelopmental disorders observed in infants of diabetic mothers^[83-85]. Free radicals can inactivate the biological functions of proteins and lipids and potentially leading to cell death^[117,118]. It is showed that GDM is associated with an increased concentration of oxidative stress, due to both defect in the antioxidant defenses and/or overproduction of free radicals^[83-85]. Although, there are several studies demonstrating the presence of elevated oxidative stress in PGDM and GDM states. Some works also suggested that this milieu can be shared with the developing fetus^[83-85,119].

In experimental animals, it is emphasized that oxygen radicals play crucial roles in the progression and timing of the development and differentiation of neurons, and synaptic plasticity; so, imbalance in these signals can lead to changes in development of CNS^[120,121]. On the other hands, the developing CNS is particularly susceptible to increase in oxidative stress, owing to its poor antioxidant defenses and/or high oxygen consumption^[122].

Oxidative stress might contribute to the pathogenesis of some of neurodevelopmental disorders *via* an attenuation with gamma-aminobutyric acid receptor function in the brain, decreased synaptic efficiency in hippocampal cells, and inhibition of dopamine β -hydroxylase^[123-125]. On the other hand, there are evidence

indicating that maternal diabetes-induced NTDs are associated with some metabolic difficulties such as increase in superoxide dismutase activity and decreased in arachidonic acid level^[126]. There are evidence from human models indicating a potential role of elevated oxidative stress in neurodevelopmental diseases^[127-131]. Moreover, the incidence of congenital anomalies in infants of diabetic mothers has been reported to be significantly reduced by antioxidants supplements therapy including Ascorbic Acid and vitamin E. These data suggest that elevated oxidative stress is associated with the pathophysiology of fetal dysmorphogenesis^[132,133].

Role of insulin-like growth factor-1 and insulin in maternal diabetes - induced neuropathies

Insulin- like growth factor-1 (IGF-1) and insulin belong to the insulin superfamily and exert profound effects in the CNS development and function. Evidence of these peptides actions on CNS comes from a wide variety of *in vitro* and *in vivo* experimental data, the latter predominantly derived from rodent studies. In several studies, the authors demonstrated that the insulin and IGF-1 have a wide spectrum of biological actions in the developing CNS including neuronal/glia cell proliferation, differentiation, survival, synaptogenesis, longevity, and neuroregeneration. Most of these biological functions are mediated *via* two transmembrane receptors: the insulin receptor (IR) and the IGF-1 receptor (IGF1R)^[69,70,134-142].

The IR and IGF1R, which are homologous in structure, are composed of two α -subunits and two β -subunits linked by disulphide bonds. The extracellular α - subunit binds to their cognate ligand. The β -subunit comprises an extracellular domain and a cytoplasmic domain that contains intrinsic protein tyrosine kinase activity. The binding of either IGF-1 or insulin to α -subunit of their cognate receptors stimulates phosphorylation of the β subunit on serine and tyrosine residues. The autophosphorylation of the β subunit then results in the phosphorylation of cellular substrates and signal transfer cascade for IGF-1 and insulin^[141,143,144].

Using ligand binding autoradiography and *in situ* hybridization, several line of human and animal investigations have found that IR and IGF1R are irregularly distributed in developing and mature brain with the highest densities in the hippocampus, cerebral cortex, olfactory bulb, cerebellum, and hypothalamus. Interestingly, some of brain regions show a marked difference in the density of InsR and IGF1R between embryonic vs mature brain, implying a key role for these ligands in brain development^[145-147].

There are evidence from several researches implicating defects in IR and IGF1R signaling result in congenital developmental defects seen in offspring of diabetic mothers^[145,148-150]. Ramsay *et al*^[151] (1994) in their study was found that expression of IGF-1 in the brain were declined in swine newborns born to diabetic dams when compared to that of control animals.

The hippocampal formation subserves important physiological and behavioral functions including spatial

learning and memory and is a part of brain that particularly vulnerable to changes in blood glucose level^[152-154]. In an investigation by Tehranipour *et al*^[155] (2008) the effects of maternal T1DM on density of hippocampal pyramidal cells immediately after birth were examined. Those study results were clearly indicated that maternal diabetes can decreased the numerical density of pyramidal cells in the hippocampus of rat newborns, especially in CA3^[155].

Interestingly, the studies By Hami *et al*^[156] (2012) showed that there are prominent gender-and laterality-differences in expression and distribution pattern of InsR and IGF1R in the developing rat hippocampus. The authors concluded that these differences may be a probable mechanism for the control of sex and laterality differences in development and function of the rat hippocampus^[156].

In another study by Hami *et al*^[69] (2013), the effects of diabetes in pregnancy on gene expression and protein concentration of IGF1R and IR in the developing rat hippocampus at postnatal days 0, 7, and 14 were evaluated. In that study, the authors found a markedly upregulation of both IR and IGF1R expression in the hippocampus of diabetic group newborns at first postnatal day. At the same time point, they showed only slight changes in their hippocampal protein transcripts. In 7-d old rats, there was a significant decreased in IGF-1R gene expression and protein levels in the newborns born to diabetic dams. Moreover, they found a down regulation in hippocampal IGF1R transcripts in 14-d old diabetic group offspring. Two weeks after birth, the IR gene expression was significantly declined in the hippocampus of diabetic newborns. The authors claimed that maternal diabetes strongly altered the regulation of both IR and IGF1R during development of rat hippocampus^[67].

CONCLUSION

The Incidence of congenital anomalies in infants born to diabetic women is more common in comparison to children of normal population. There are multiple lines of evidence that suggest the disturbances in intellectual and behavioral functioning observed in the children of diabetic women are accompanied by modification of hippocampus structure and function. The etiology and pathogenesis of these impairments induced by diabetes during pregnancy have spurred considerable efforts for clinically and basically researches. The final goal at these investigations was to find the teratogenic factors, which may enable preventive or protective measures to be taken in pregnancies with diabetes. Nevertheless, the exact mechanism by which diabetes during pregnancy affects the CNS development remains to be defined. Until now, several biological changes are defined to occur in diabetic mothers and to affect development of CNS (*i.e.*, disturbance in glucose and insulin homeostasis, oxidative stress, hypoxia, and iron deficiency). Moreover, the new researches on genetic

predisposition involves in teratogenicity of diabetes in pregnancy starts to define new genes and their products involved in the etiology of CNS malfunctions and malformations observed in offspring born to diabetic mothers. Hyperglycaemia and hyperinsulinemia in the mothers and their fetuses are thought to be major factors in teratogenicity of maternal diabetes on the CNS development. There are sufficient evidences for this hypothesis as concise control of glycemia in mothers reduces the incidence of anomalies exhibited with the offspring born to diabetic mothers. Recent evidence clearly indicated that maternal diabetes markedly influences the regulation of both IR and IGF1R - as two important regulators of development and function of CNS - in the developing rat hippocampus. Dissecting out the mechanisms responsible for maternal diabetes-related changes in the development of hippocampus is helping to prevent from impaired cognitive and memory functions in offspring.

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Endothelial and platelet markers in diabetes mellitus type 2

Peter Kubisz, Lucia Stančiaková, Ján Staško, Peter Galajda, Marián Mokáň

Peter Kubisz, Lucia Stančiaková, Ján Staško, National Centre of Haemostasis and Thrombosis, Clinic of Haematology and Transfusiology, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, 03659 Martin, Slovak Republic

Peter Galajda, Marián Mokáň, Department of Internal Medicine I, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, 03659 Martin, Slovak Republic

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Correspondence to: Peter Kubisz, MD, DSc, Professor, National Centre of Haemostasis and Thrombosis, Clinic of Haematology and Transfusiology, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, Kollarova Str. N. 2, 03659 Martin, Slovak Republic. kubisz@jfm.uniba.sk
 Telephone: +421-43-4203232
 Fax: +421-43-4132061

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which carries a risk of vascular impairment. DM type 2 (DM2) can be characterized by the dysfunction of haemostasis manifesting by stimulated coagulation process, disorder of platelet function and decreased fibrinolytic activity. These all are the reasons why DM2 is the most common acquired thrombophilia. Endothelial dysfunction along with platelet hyperactivity are unquestionably involved in the hyperactivation of platelets and clotting factors in DM. As a natural consequence of continuous investigation, many markers of endothelial dysfunction and diabetic thrombocytopenia have been identified and considered for implementation in clinical practice. Endothelial function can be assessed by the evaluation of endothelial markers, circulating molecules synthesised in various amounts by the endothelium. These markers precede the signs of evident microangiopathy. Platelets have an ethiopathogenic relation to the microangiopathy in DM. Their increased activity was confirmed in both types of DM. Predictors of endothelial and platelet disorder could improve the screening of individuals at increased risk, thus leading to the early diagnosis, appropriate treatment, as well as to the effective prevention of the complications of DM2. In the article we deal with the mechanisms involved in the pathogenesis of endothelial and platelet functional abnormalities, endothelial and platelet markers of DM2 considered for implementation in clinical practice and possibilities of their detection.

Key words: Diabetic thrombocytopenia; Endothelial markers; Platelet markers; Endothelial dysfunction

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Core tip: Number of diabetics increases, what leads to worldwide increasing diabetes-associated vascular events. Moreover, diabetes mellitus type 2 is the most common acquired thrombophilia. Therefore, to prevent life-threatening vascular complications in subjects with diabetes, mechanisms and markers of endothelial and platelet dysfunction have been investigated. In order to contribute to better management of discussed patients and to increase knowledge about their origin, in this article we tried to summarize the pathogenesis of

Abstract

Diabetes mellitus (DM) is an extremely common disorder

endothelial and platelet dysfunction and to characterize possible predictors of abnormalities of endothelium and platelets, as well as methods of their detection.

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INTRODUCTION

Diabetes mellitus (DM) is an extremely common disorder which carries a risk of vascular impairment. In 1970s the attention has shifted towards haemostatic mechanisms with particular emphasis on the relationship between platelets and the vessel wall^[1].

The term "endothelial dysfunction" is usually defined as a disorder of the endothelial capacity to maintain vascular homeostasis^[2]. Endothelial dysfunction along with platelet hyperactivity are unquestionably involved in the hyperactivation of platelets and clotting factors in DM^[3]. It is an early sign of vascular damage in DM type 2 (DM2) but late in DM1. Endothelial dysfunction occurs even in normoalbuminuric (NAU) patients with DM2^[4]. Endothelial function can be assessed by the evaluation of endothelial "markers", circulating molecules synthesised in various amounts by the endothelium^[5]. In fact, markers precede the signs of evident microangiopathy^[4].

Microalbuminuria conventionally represents albumin excretion rate 30-300 mg in a 24 h urine collection. It is associated with endothelial dysfunction and serves as a predictor of DM at levels out of the characterized reference range^[4,6].

DM2 can be characterized by the dysfunction of haemostasis manifesting by stimulated coagulation process, disorder of platelet function and decreased fibrinolytic activity. These all are the reasons why DM2 is the most common acquired thrombophilia^[7].

Markers of activated haemostasis, for instance prothrombin activation fragment 1 + 2 (F1 + 2) and thrombin-anti-thrombin complexes, plasma levels of fibrinogen, factor VII, factor VIII, factor XI, factor XII, kallikrein, and von Willebrand factor (vWF) are elevated in DM^[3]. Impairment of fibrinolytic system because of imbalance between regulators of plasminogen represents the typical sign of thrombophilia present in diabetes^[7]. Increased amount of platelet aggregates in circulation, increased aggregation of platelets after addition of platelet agonists, increased platelet contractility, and the presence of elevated plasma levels of their contents, for example beta-thromboglobulin (β -TG), platelet factor 4 (PF4), and thromboxane B2 (TXB2), show platelet hyperactivity in DM^[3].

Eighty percent of individuals with DM are dying because of thrombosis^[3]. The most effective tool to preserve dysfunction of the endothelium and vascular

impairment in DM is the management of hyperglycaemia^[4].

This review will characterize mechanisms responsible for the development of endothelial and platelet functional abnormalities, endothelial and platelet markers of DM2 considered for implementation in clinical practice and possibilities of their detection.

MECHANISMS OF ENDOTHELIAL DYSFUNCTION IN DM2

The pathogenesis of endothelial dysfunction in both DM1 and DM2 is multifactorial^[4].

Altered cell signaling

The alteration of the insulin-mediated activation of nitric oxid synthase derived from endothelium is important factor of endothelial damage in the setting of DM and insulin resistance (IR). On the other hand, the activation of insulin-signalling cascade is associated with the expression of endothelin-1 (ET-1), a vasoconstrictor and mitogenic substance, and proinflammatory molecules as intercellular adhesion molecule 1 (ICAM-1)^[2].

Increased oxidative stress

Reactive oxygen species and circulating markers of oxidative stress are elevated in DM, IR and obesity^[2].

Pro-inflammatory activation of the endothelium

Systemic inflammation present in DM can impair the function of the endothelium and lead to atherosclerosis. Individuals with diabetes or obesity have elevated circulating levels of markers of inflammation, e.g., C-reactive protein, tumor necrosis factor α , interleukin 6 (IL-6), and ICAM-1. Moreover, elevated levels of pro-inflammatory substances predict vascular complications in diabetics^[2].

Activation of protein kinase C

Activation of protein kinase C beta can be the cause of the association between inflammation, endothelial damage, and IR in DM^[2].

Mitochondrial dysfunction

Recent works relationship between endothelial dysfunction and impaired mitochondrial biogenesis in subjects with DM^[2].

ENDOTHELIAL MARKERS OF DM2

vWF

vWF represents a marker of the activation of the endothelium^[8]. It is synthesised, stored and secreted both by megakaryocytes/platelets and by endothelial cells^[9]. vWF takes part in the processes of platelet adhesion, platelet aggregation and acts as a plasma carrier for factor VIII, thus enabling its stability in the circulation. Its role in platelet adhesion is particularly

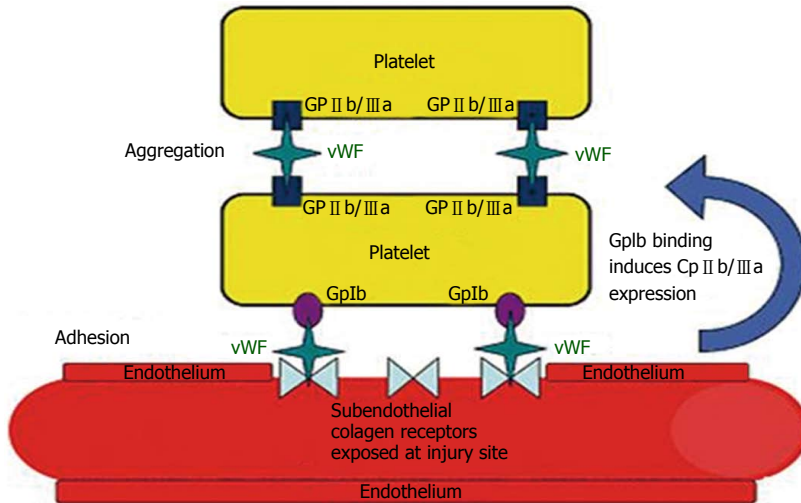


Figure 1 The role of von Willebrand factor in the platelet adhesion to the damaged vessel wall and the subsequent platelet aggregation. Adapted from: Trombose^[11]. vWF: Von Willebrand factor; GP: Glycoprotein.

important under conditions of high shear stress^[10] (Figure 1).

According to the results of the Framingham Offspring Study there was strong evidence that vWF is an independent risk factor for DM (relative risk = 1.37, 95%CI: 1.07-1.75; $P = 0.01$)^[12]. vWF level is already increased in NAU individuals, while in a few studies vWF level was increased firstly in microalbuminuric (MAU) individuals^[8]. For instance, Galajda *et al.*^[13] have found significant increase of vWF in DM2 subjects without vascular impairment comparing to healthy individuals (1.33 ± 0.39 IU/mL vs 1.01 ± 0.27 IU/mL, $P < 0.01$)^[13].

On the contrary, in the study of Kubisz *et al.*^[8] circulating vWF levels did not significantly increase in diabetic subgroups when comparing with the controls. Since then, many studies have confirmed no or no independent link between vWF and DM^[14,15]. This can be explained by the fact that the association between vWF and DM relates to the concentrations of the proinflammatory cytokine IL-6. Thus, vWF as a product of the acute phase response is associated with the risk of DM complications, but the relation between vWF and DM is probably indirect^[14,16].

Thrombomodulin

Plasma (soluble) thrombomodulin (TM) is a marker of endothelial damage^[8]. TM represents major substance of the protein C anticoagulant system^[17].

It was suggested that the elevation of plasma TM concentration in patients with DM2 could be the consequence of widespread vascular damage in diabetic patients with incipient nephropathy^[18]. Actually, vWF and TM levels are increased in group of subjects with DM2 and endothelial dysfunction^[19].

Widespread endothelial dysfunction is present in diabetic nephropathy. Hence, plasma vWF and TM represent valuable markers of endothelial dysfunction potentially useful in early confirmation of diabetic

microvascular complications^[20]. Surprisingly, our recent study found soluble TM more sensitive marker than vWF in individuals diagnosed DM2 with both, the normo- and microalbuminuria (TM $P < 0.0001$ in NAU, $P < 0.005$ in MAU)^[8]. Galajda *et al.*^[13] reported on the contrary to the previous studies, when comparing the DM2 patients without vasculopathy and control group, the concentrations of TM as calcium-independent marker of endothelial injury to be similar. This is the reason to assume that only elevated concentrations of intracellular calcium (Cai)-depending endothelial and platelet indicators can predict the dysfunction of the endothelium^[13].

Thrombin-activatable fibrinolysis inhibitor

Thrombin-activatable fibrinolysis inhibitor (TAFI) removes C-terminal lysine part from fibrin and inhibits plasminogen activation. Taking all the facts into the consideration, TAFI represents a substantial link between coagulation and fibrinolytic processes^[7]. Positive correlation between TAFI and F1 + 2 in MAU group ($r = 0.427$, $P = 0.001$) as well as TAFI and fibrinogen in the NAU group confirmed activation of TAFI in the course of stimulated coagulation^[6].

TAFI antigen levels and activity are significantly ($P < 0.05$) increased in diabetics when comparing with healthy subjects. Inverse and significant correlation of TAFI antigen and D-dimers was found in diabetic subjects supporting the function of TAFI in diabetes-induced inhibition of fibrinolytic activity^[6,7,21]. Elevated levels of TAFI may also be important in endothelial injury in MAU individuals^[6,8].

A significantly elevated plasma level of TAFI was present in diabetics with microalbuminuria when compared with diabetics with normoalbuminuria ($P \leq 0.001$)^[8]. Progression of DM2 therefore contributes to marked TAFI mediated downregulation of fibrinolytic activity. It is also suggested that fibrinolysis inhibition can be mediated by TAFI in early stages of diabetes

Table 1 The most important endothelial markers of diabetes mellitus type 2 with the method used for their detection

Endothelial markers	Method of detection	Normal values
PAI-1 antigen	ELISA	5-40 ng/mL
TAFI antigen	ELISA	5.8-10.0 µg/mL
t-TFPI antigen	EIA	70 (40-110) ng/mL
f-TFPI (men)	EIA	16 ± 4 ng/mL
f-TFPI (women)	EIA	16 ± 3 ng/mL
TM antigen	EIA	10-50 ng/mL
tPA antigen	ELISA	1-20 ng/mL
vWF antigen	EIA	Less than 1.4 IU/mL (140%)

EIA: Enzyme immunoassay; ELISA: Enzyme-linked immunosorbent assay; PAI-1: Plasminogen activator inhibitor 1; TAFI: Thrombin-activatable fibrinolysis inhibitor; f-TFPI: Free tissue factor pathway inhibitor; t-TFPI: Total tissue factor pathway inhibitor; TM: Thrombomodulin; tPA: Tissue plasminogen activator; vWF: Von Willebrand factor.

defined by microalbuminuria in DM2 subjects without macrovascular complications. It seems that discussed process is independent from the level of plasminogen activator inhibitor-1 (PAI-1)^[6,8].

Elevated circulating levels of TAFI were present not only in individuals diagnosed DM2, but also in patients with obesity, IR and arterial hypertension^[8]. On the contrary, increased total cholesterol level probably downregulates TAFI^[6].

PAI-1

PAI-1 represents one of the most significant and quick natural inhibitors of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator^[7].

PAI-1 is produced in vascular and metabolic tissues^[7]. Therefore, up-regulation of PAI-1 occurs predominantly in obese patients and presence of endothelial dysfunction^[6].

PAI-1 is released to plasma predominantly from the endothelial cells^[7]. This is the cause of the elevation of PAI-1 concentrations in individuals with DM2 and hypertension with endothelial dysfunction^[6,22]. The inflammatory cytokines, as well as metabolic components are activators of PAI-1 production in the endothelial tissue^[7,23]. Insulin has inhibiting influence on the PAI-1 synthesis in endothelial cells. Significant decrease of PAI-1 level in DM2 individuals with endothelial impairment on long-term insulin therapy was confirmed^[7,24]. However, the mechanism of insulins action depend upon the metabolism in hepatic cells and endothelium^[7].

PAI-1 overproduction belongs to the most typical signs of thrombophilia in DM2 leading to the dysfunction of fibrinolytic system^[7]. Plasma levels of PAI-1 are significantly increased in both cohorts (MAU and NAU) compared with the controls ($P < 0.0001$ in both subgroups)^[8].

When comparing with other parameters, PAI-1 is the most complex one associated with IR. Level of PAI-1 highly correlates with parameters of the IR syndrome in healthy individuals, patients with IR, DM2, or subjects with coronary artery disease. Positive correlation was found between PAI-1 concentrations

and body mass index ($r = 0.43$, $P < 0.05$) in the MAU patients and between PAI-1 and triglycerides (TAG) ($r = 0.67$, $P = 0.01$) in NAU subjects, proving that obesity, dyslipidaemia and decreased fibrinolytic activity are related to each other^[6,7]. Subjects with DM2 and hyperinsulinaemia non-diabetics had significantly increased PAI-1 levels which correlated with the C-peptide ($r = 0.519$, $P < 0.001$), TAG ($r = 0.685$, $P < 0.001$), body mass index ($r = 0.607$, $P < 0.001$) and with levels vWF and soluble TM^[25].

Moreover, increased PAI-1 levels are an independent risk factor for DM2. It was proposed that a decrease of PAI-1 level may be linked with a decrease in transformation to DM2. On the other hand, the finding of elevation of PAI-1 preceding DM2 thus being, in the preexistence of IR, independent of glycaemia gives unquestionable evidence that abnormality of fibrinolytic system characterized with increased PAI-1 and tPA antigen occurs nearly at the beginning of the development of metabolic disorders and is considered a risk factor for future DM and metabolic complications^[7].

Tissue factor pathway inhibitor

Tissue factor pathway inhibitor (TFPI) acts as an inhibitor of tissue factor (TF)-initiated coagulation. TF binds to the activated factor X, subsequently TFPI-Xa complex binds to the TF/factor VIIa (FVIIa) complex and regulates its action^[26].

Enhanced TFPI activity was confirmed in individuals with DM and mainly in those with microalbuminuria. Increases in TFPI reflect endothelial dysfunction or impaired binding of TFPI to endothelial cells by glucosaminoglycans because TFPI is predominantly synthesized in endothelial cells^[26]. It may be the first consequence of increased glycosylation, and thus the deterioration of the antithrombotic potential of endothelial cells. Mentioned increase in TFPI activity does not compensate the procoagulant state associated with increased thrombinogenesis (excess of F1 + 2), and inversely correlates with the internal thrombin potential^[27,28]. Plasma levels of TF, TFPI and FVIIa are significantly increased in DM2^[26].

Total TFPI (t-TFPI) has a poor anticoagulant effect. On the contrary, free form of TFPI (f-TFPI) has an increased anticoagulant capacity. Moreover, the study of Morange *et al.*^[29] showed that f-TFPI has a strong correlation with endothelial markers and t-TFPI has increased relation to parameters indicating cardiovascular risk. Normal values of f-TFPI varying according to gender, as well as informative normal values of t-TFPI can be seen in Table 1.

tPA

tPA is a serine protease which converts plasminogen to plasmin, the form active in the process of fibrinolysis, thus being the most valuable initiator of fibrinolytic process. tPA is synthesised particularly in the endothelial cells, smooth muscle cells, monocytes and megakaryocytes^[6,7]. Quick tPA release to the circulation occurs

following the Cai - dependent stimulus. Minority of tPA is present in plasma in the form of complex binding PAI-1^[7].

tPA is present mainly on the endothelial surface and may be released to circulation following its injury^[7]. tPA levels are elevated in subjects with DM and metabolic syndrome. Increased tPA concentrations were proposed as a risk factor of DM in healthy subjects, because total tPA antigen levels were elevated in DM, but the level of free tPA and the tPA activity not. Therefore, the elevated total tPA is a facade masking the dysfunctional fibrinolysis in DM2^[6]. Moreover, an elevated tPA antigen concentration is a substantial feature of the IR syndrome and also relates to the inflammation. tPA antigen levels have strong correlations with IR, and can serve as helpful markers of diabetes^[14]. The increased tPA levels correlate with vWF and soluble TM in individuals with various locations of atherosclerotic lesions. This supports the position of tPA as an indicator of endothelial injury^[7].

The tPA levels were described to be increased in DM2 in an early phase of disease while tPA levels in DM1 are increased later in presence of vascular complications. Dependence between tPA levels and PAI-1 and IR is higher in the phase of complications than dependence between tPA and presence of complications in DM2. There is a correlation between tPA and PAI-1 found in patients with DM2 and the principle cause of the tPA increase is formation of the tPA/PAI-1 complexes^[7].

As it was mentioned earlier, insulin can inhibit the endothelial production of PAI-1 with the following decrease of tPA/PAI-1 complexes levels but it does not influence the tPA release from endothelium and tPA plasma levels. This can be explanation of the absent correlation between tPA and PAI-1 found in DM2 patients treated with insulin^[7,25].

E-selectin

E-selectin (CD62E), expressed by endothelium, is rapidly enhanced by proinflammatory molecules^[30].

At first the adhesion molecules were considered to be endothelial markers because of their general elevation in various vasculopathies^[25]. It was confirmed that individuals with increased E-selectin had a significantly higher probability of the development of DM2^[4]. Moreover, in the study of Thorand *et al.*^[15] soluble E-selectin and ICAM-1 significantly preceded DM2. In particular, soluble E-selectin remained an independent marker of diabetes after exclusion of inflammation, levels of insulin and hemoglobin A1c in both men and women^[15,30].

ICAM-1 and vascular cell adhesion molecule 1

ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) are synthesised in the endothelium and leukocytes. Increased concentrations of these cell adhesion molecules (CAMs) are early markers of endothelial dysfunction, cardiovascular disease and DM2^[30].

Increased concentrations of E-selectin, ICAM-1,

and VCAM-1 raise the relative risk of DM by 1.5- to 7.5-fold^[30]. Taking everything into account, elevated concentrations of CAMs representing an increased probability of the development of diabetes highlight the link between dysfunction of the endothelium and IR^[15].

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a growth factor derived from endothelial cells, stimulating their proliferation, differentiation and survival, mediating endothelium-dependent vasodilatation, inducing increased permeability in the microcirculation and participating in other supporting functions. VEGF enhances the glomerular permeability to macromolecules and leads to profound proteinuria including albuminuria. Described action has probably a role in the development of endothelial dysfunction in diabetes^[8]. The beneficial effects of VEGF-A (principal member of VEGF family) inhibition in the early phases of diabetic glomerulopathy was also confirmed^[31].

Highly increased plasma VEGF levels were measured in individuals with retinopathy and nephropathy when compared with the control group. Widespread endothelial dysfunction occurs at the beginning of the development of diabetic nephropathy and precedes biochemical finding of the disorder of renal functions since the significantly increased VEGF level was present already in NAU diabetics without manifestations of microvascular impairment. Because VEGF levels were significantly elevated in the NAU cohort and had a tendency to be significant in the MAU patients, VEGF can be concluded as an indicator of diabetic endotheliopathy. VEGF ought to be evaluated only along with further markers representing the dysfunction of the endothelium, particularly soluble TM^[8]. It is assumed that VEGF may be a more sensitive indicator of renal changes than microalbuminuria^[8,32]. On the other hand, since there were no marked differences in VEGF concentrations in individuals with DM with physiological renal functions and at the beginning of the development of diabetic nephropathy, VEGF was not confirmed as a reliable marker of the progression in DM2 patients^[8].

ET-1

ET-1 is considered to be the most potent vasoconstrictor form of endothelin and is produced by the endothelial cells. During prolonged periods of stress caused by hyperglycaemia and IR in the course of DM2, there is a shift in the balance with more vasoconstrictors being produced compared to vasodilating agents. This leads to endothelial dysfunction and affects the permeability of the glomerular filtration barrier leading to microalbuminuria^[33].

Even experimental studies suggested that ET-1 drives development of glomerulosclerosis and podocyte loss^[34]. ET-1 is really significantly associated with early development of the endothelial dysfunction in the

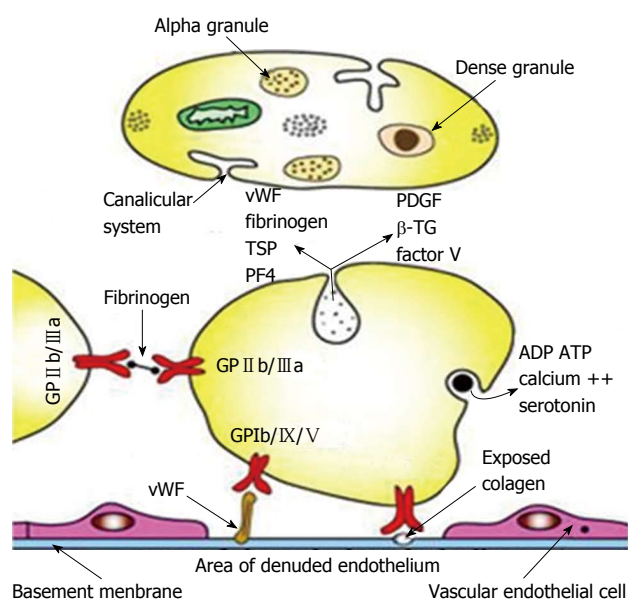


Figure 2 The structure of the platelet and its most important functions, including the secretion of stored products, as well as the binding of specific surface glycoproteins to the endothelium (bottom), and other platelet (left side). Adapted from: Trombore^[11]. ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; β -TG: Beta-thromboglobulin; GP: Glycoprotein; PDGF: Platelet-derived growth factor; PF4: Platelet factor 4; TSP: Thrombospondin; vWF: Von Willebrand factor.

glomerulus^[33].

MECHANISMS OF DIABETIC THROMBOCYTOPATHY

Primary dysfunction of megakaryocyte-platelet system

One of the manifestations of DM is the production of subpopulation of large and hyperreactive platelets in the bone marrow. Thrombocytopoiesis is increased^[35,36].

IR in platelets

In obesity and DM2 increased aggregability of platelets, as well as decreased sensitivity to antiplatelet effect of insulin was confirmed^[36].

Influence of endothelial dysfunction

Substances produced by the endothelium, represented by nitric oxide, prostacyclin and adenosine normally inhibit platelets. In diabetic patients due to the hyperglycaemia and oxidative stress the capacity of endothelium to produce prostacyclin and nitric oxide is decreased. In the cases of microangiopathy consumption of platelets in the sites of damaged endothelium causes paradoxically the decrease in the subpopulation of large activated platelets^[36].

Changes of the activity of membrane pumps and increase of Cai in platelets^[36]

Platelet markers of DM2: Platelets have an ethiopathogenic relation to the microangiopathy in DM. Their increased activity was confirmed in both types of

DM^[36,37]. The structure of the platelet with components involved in its physiological function and markers of platelet activity are illustrated in Figure 2.

Mean platelet volume

Mean platelet volume (MPV) is the parameter informing about the platelet size and activation. Bigger platelets indicate more activity^[38]. Patients with DM have significantly higher MPV due to the elevated alpha-granule content in cytoplasm as the sign of dysfunction of megakaryocyte-platelet system^[36,38]. Thus, platelets can be a useful tool to evaluate complications in DM^[38]. Further studies concluded that MPV is strongly and independently associated with the presence and severity of diabetes and higher MPV is the manifestation of early diabetic thrombocytopathy in both types of DM without vascular complications^[39,40].

Soluble P-selectin

P-selectin (CD62P) is a highly glycosylated membrane glycoprotein constitutively expressed in endothelial cells and megakaryocytes and present in the membrane of Weibel-Palade bodies of endothelium and in α granules of platelets^[36,41]. It is a marker of platelets' activation. Its increased levels in patients with arterial hypertension, lower extremity peripheral artery disease and coronary artery disease correlate with levels of β -TG, and they do not have a relation to vWF and TM^[42-44]. Increased levels of P-selectin occurred already in early stages of DM1 and DM2 without vascular complications. Elevated levels of P-selectin were also found in subjects diagnosed IR and impaired glucose tolerance^[45-47].

Elevated synthesis of P-selectin and ICAM-1 in diabetic human retina and choroid was confirmed. This is the reason why circulating levels of soluble adhesion molecules have been proposed as biomarkers playing a significant role in the pathogenesis of macrovascular and microvascular dysfunction and neuropathy of DM^[48]. Galajda *et al.*^[45] found in patients with DM2 significantly elevated P-selectin that paradoxically did not correlate with levels of PF4, but they correlated with vWF. This can propose also its function in the endothelial dysfunction in patients with DM2^[45].

P-selectin was significantly increased in patients with DM suffering myocardial infarction than in control group or those without myocardial infarction. It indicates contribution of elevated levels of P-selectin to the atherosclerosis leading to coronary heart disease in diabetics^[49]. Thus, detection of P-selectin level could be useful marker of possible cardiovascular event in individuals with DM^[50].

PF4

PF4 is a basic protein found exclusively in a granules of platelets. After release it is able to modulate haemostasis. PF4 has both procoagulant and anticoagulant properties. It has also angiostatic effect - it inhibits proliferation and migration of endothelial cells^[36].

Table 2 The most important platelet markers of diabetes mellitus type 2 with the method used for their detection

Platelet markers	Method of detection	Normal values
βTG	EIA/RIA	Less than 40 ng/mL
GPV	EIA	80 (40-160) ng/mL
MPV	Electric impedance or laser diffraction	6.5 (4.5-8.5) fl
PF4	EIA/RIA	Less than 10 ng/mL
TSP-1	EIA	40 (10-90) ng/mL, respectively 20-300 ng/mL

βTG: Beta-thromboglobulin; EIA: Enzyme immunoassay; GPV: Glycoprotein V; MPV: Mean platelet volume; PF4: Platelet factor 4; RIA: Radioimmunoassay; TSP-1: Thrombospondin 1.

In patients with both types of DM the increased levels of PF4 and β-TG as the manifestation of degranulation of activated platelets without influence on their lifespan were confirmed. In DM2 these levels are increased already in the early stage of the disease without the presence of vascular complications^[36].

In the study of Galajda *et al.*^[13] the correlation between vWF concentrations and calcium dependent substances as C-peptide ($r = 0.680$, $P < 0.001$) and PF4 ($r = 0.613$, $P < 0.01$) and no correlation with calcium-independent markers of endothelial damage, as TM ($r = 0.287$, $P = 0.196$) or TFPI ($r = 0.296$, $P = 0.181$) in diabetics was confirmed^[13]. In advanced cases of DM associated with retinopathy and nephropathy increased levels of PF4, β-TG, P-selectin, Cai in platelets, as well as their increased aggregability were confirmed^[36]. Moreover, patients with diabetes type 2 treated with sulphonylurea preparates and individuals treated 2-3 mo with insulin did not differ by endothelial and platelet parameters (vWF, TM and PF4)^[7].

β-TG

β-TG is a protein with 50% structural homology with PF4 acting as a leukocyte chemoattractant. Measurement of β-TG is considered to be a golden standard in the detection of activation of platelets in patients with normal renal function. Its levels are increased in renal insufficiency and correlate with levels of PF4^[39].

Glycoprotein V (CD42d)

Glycoprotein V (GPV) is exclusively expressed in platelets and megakaryocytes, where it is non-covalently bound to the complex GPIb/GPIX as the part of the receptor for vWF and thrombin^[51].

According to the fact that platelets are the only source of GPV it can be considered as a marker of specific activation of platelets by thrombin^[51].

TXB2

Metabolism of arachidonic acid with incorporation of its metabolites to membrane phospholipids of platelets in DM is upregulated. Production of TXA2 in platelets is increased. Plasma levels of its stable catabolite TXB2 produced in liver, kidneys, platelets and lungs are also

increased and correlate with raised levels of β-TG and PF4^[52].

Increased synthesis of TXA2 indicates that "resting" platelets of diabetics in general tend to be activated. TXA2 is a vasoconstrictor and prothrombotic factor with the risk of atherothrombogenesis present in DM^[53].

Moreover, synthesis of TXB2 has inverse correlation with the minimal level of arachidonic acid inevitable to 50% platelet aggregation. As the consequence, platelet aggregates in the circulation of patients with DM2 than in the healthy subjects. Platelets of diabetics therefore exhibit the signs of hyperactivity. This fact may also be very important for the arise of vascular disease in DM^[52].

Thrombospondin-1

This molecule represents a multifunctional glycoprotein synthesised in a wide range of cell types, to be more exact in platelets (increased expression), vascular smooth muscle cells and various kinds of renal cells^[53]. Unlike PF4 and β-TG, after release of thrombospondin (TSP) from activated platelets it binds to platelet surface, so levels of TSP-1, PF4 and β-TG usually do not correlate with each other. Last but not at least, its levels are influenced by hepatopathy. These are the reasons why analysis of TSP is not considered to be the golden standard in detection of platelet activity. Therefore, TSP is evaluated as the sign of activation of the whole vascular system^[36].

TSP-1 with antiangiogenic and proatherogenic properties is involved in diabetic vasculopathy. The endothelial dysfunction in DM was confirmed in many studies, and TSP-1 surely may contribute to this impaired function because it shows antiproliferative and apoptotic effect on the endothelium. It was confirmed that TSP-1 level has a relation with renal damage and vascular impairment^[53].

DETECTION OF MARKERS OF DM2

Informative normal values of the most important endothelial and platelet markers with the method used for their detection are documented in Tables 1^[36,54-56] and 2^[36].

Size of the platelet up to 7 μm and its volume more than 10 fl contribute to increased MPV^[36].

Levels of soluble P-selectin are measured using enzyme immunoassay method and their normal values vary between laboratories and are dependent on the commercial kit used for the detection^[36].

Values of β-TG 50 ng/mL and more are considered to be pathological^[36].

Plasma levels of VEGF, F1 + 2 and TXB2 can also be detected using enzyme-linked immunosorbent assay^[6,8,57].

CONCLUSION

Number of patients with DM2 is increasing incredibly and is expected to rise even in the future. Researchers

all over the world have made strong efforts to increase knowledge and improve understanding of mechanisms of abnormalities of endothelium and platelets unquestionably involved in the pathogenesis of this challenging health problem. As a natural consequence of continuous investigation, many markers of diabetic thrombocytopathy and endothelial dysfunction have been identified and are considered for implementation in clinical practice. These substances may improve the screening of high-risk individuals and lead to the early diagnosis, appropriate treatment, as well as to the effective prevention of the complications of DM2. However, these conclusions have to be confirmed by further research.

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Diabetic neuropathic pain: Physiopathology and treatment

Anne K Schreiber, Carina FM Nones, Renata C Reis, Juliana G Chichorro, Joice M Cunha

Anne K Schreiber, Carina FM Nones, Renata C Reis, Juliana G Chichorro, Joice M Cunha, Department of Pharmacology, Biological Sciences Sector, Federal University of Parana, Curitiba 81540-970, Brazil

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Correspondence to: Dr. Joice M Cunha, Professor, Department of Pharmacology, Biological Sciences Sector, Federal University of Parana, Rua XV de Novembro, 1299 - Centro, Curitiba 81540-970, Brazil. joice.cunha@ufpr.br

Telephone: +55-41-33611720

Fax: +55-41-32662042

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Abstract

Diabetic neuropathy is a common complication of both type 1 and type 2 diabetes, which affects over 90% of the diabetic patients. Although pain is one of the main symptoms of diabetic neuropathy, its pathophysiological mechanisms are not yet fully known. It is widely accepted that the toxic effects of hyperglycemia play an important role in the development of this complication, but several other hypotheses have been postulated. The management of diabetic neuropathic pain consists basically in excluding other causes of painful peripheral

neuropathy, improving glycemic control as a prophylactic therapy and using medications to alleviate pain. First line drugs for pain relief include anticonvulsants, such as pregabalin and gabapentin and antidepressants, especially those that act to inhibit the reuptake of serotonin and noradrenaline. In addition, there is experimental and clinical evidence that opioids can be helpful in pain control, mainly if associated with first line drugs. Other agents, including for topical application, such as capsaicin cream and lidocaine patches, have also been proposed to be useful as adjuvants in the control of diabetic neuropathic pain, but the clinical evidence is insufficient to support their use. In conclusion, a better understanding of the mechanisms underlying diabetic neuropathic pain will contribute to the search of new therapies, but also to the improvement of the guidelines to optimize pain control with the drugs currently available.

Key words: Diabetes; Neuropathic pain; Hyperglycemia; Anticonvulsants; Antidepressants

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Core tip: Diabetic neuropathic pain is a common complication of diabetes and the most common form of neuropathic pain. In this review, we will discuss the various factors that may contribute to the pathogenesis of diabetic neuropathic pain, including metabolic, vascular, autoimmune and oxidative stress-related mechanisms. In addition, we will review the possibilities of pain treatment, taken into consideration the first line drugs clinically used, the antidepressants and anticonvulsants, but also other options such as opioids, tapentadol and drugs for topical use, such as lidocaine and capsaicin cream.

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INTRODUCTION

According to the International Diabetes Federation, 382 million people worldwide are currently affected by diabetes^[1], one of the leading causes of neuropathy^[2]. The distal symmetrical polyneuropathy (DSPN) is the commonest clinical form of diabetic neuropathy, affecting more than 90% of the patients^[3]. Generally, DSPN affects the toes and distal foot, but slowly progresses proximally to involve the feet and legs in a stocking distribution. It is also characterized by a progressive loss of nerve fibers affecting both the autonomic and somatic divisions, thereby diabetic retinopathy and nephropathy can occur^[3]. Foot ulceration and painful neuropathy are the main clinical consequences of DSPN, linked with higher morbidity and mortality^[4]. Frequently, patients look for medical help only when pain appears^[5], a symptom that affects 10% to 26% of this population^[6,7].

Diabetic neuropathic pain (DNP) is characterized by tingling, burning, sharp, shooting, and lancinating or even as electric shock sensations^[3,8]. It is usually considered moderate to severe and often worse at night, causing sleeping disturbs. The pain can be constant and accompanied of cutaneous allodynia, which can substantially affect the quality of life of patients, impacting the ability to perform daily activities and having a negative influence on mood. The pain may also be a reason of withdrawal of recreational and social activities and may be associated with depression^[3,9,10].

The pathogenesis of DNP is not fully understood. Several theories have been proposed to explain the pain related to the diabetic neuropathy, such as changes in the blood vessels that supply the peripheral nerves; metabolic and autoimmune disorders accompanied by glial cell activation, changes in sodium and calcium channels expression and more recently, central pain mechanisms, such as increased thalamic vascularity and imbalance of the facilitatory/inhibitory descending pathways^[3]. Additionally, several risk factors are associated with DNP including worsening glucose tolerance, older age, longer diabetes duration, drinking alcohol and cigarette smoking^[10].

Currently, only three agents are approved in the United States for the treatment of DNP: duloxetine, a selective serotonin and norepinephrine reuptake inhibitor, pregabalin, an anticonvulsant, and the dual-effect drug tapentadol, an opioid receptor agonist and norepinephrine reuptake inhibitor^[11]. However, as pain relief is unsatisfactory for most patients, several pharmacological interventions have been used based on pre-clinical and/or clinical evidence, as well as an inference of mechanism of action.

PHYSIOPATHOLOGY OF NEUROPATHIC PAIN IN DIABETES

Although there is a great advance in understanding

the pathophysiological mechanisms leading to the development of diabetic complications, there is not yet a plausible hypothesis to explain why some patients develop the painful form of disease while others do not. In general, researchers seek to elucidate neuropathy underlying mechanisms as a bigger event, and include pain and other sensorial manifestations as direct consequences of neuropathy. However, interestingly, pain intensity normally is not associated with neuropathy severity, and can occur even in the absence of nerve injuries^[12,13]. In this review, it will be addressed the pathophysiological mechanisms currently believed to promote the DNP.

In this sense, the mechanisms that lead to DNP are not fully understood, although there is a consensus that toxic effects of hyperglycemia represent an important factor for the development of this complication^[14,15]. Nonetheless, other factors besides hyperglycemia should not be discarded^[16], and will be discussed as follows.

Polyol pathway hyperactivity

Metabolic disorders are the primary cause of diabetic neuropathy. Hyperglycemia, induced through decreased of insulin secretion or insulin resistance, is responsible for the enhanced of the polyol pathway activity. The rate-limiting first enzyme of this pathway aldose reductase catalyzes the formation of sorbitol from glucose, with the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP⁺. Sorbitol is further oxidized to fructose by sorbitol dehydrogenase, which is coupled with the reduction of nicotinamide adenine dinucleotide (NAD⁺) to NADH. It is described that during hyperglycemic states, the affinity of aldose reductase for glucose is higher, generating intracellular osmotic stress due to accumulation of sorbitol, since sorbitol does not cross cell membranes. Interesting, the nerve damage following the diabetic state seems not to be due to this osmotic stress since it has been reported insignificant sorbitol concentrations in the nerves of diabetic patients^[17-19]. However, the current accepted hypothesis states that polyol pathway hyperactivity is pathogenic primarily by increasing the turnover of cofactors such as NADPH and NAD⁺, which leads to a decrease in the reduction and regeneration of glutathione, as well as to an increase of advanced glycation end products (AGEs) production and activation of diacylglycerol and protein kinase C (PKC) isoforms. Depletion of glutathione could be the primary cause of oxidative stress and be related to the accumulation of toxic species^[19]. In fact, aldose reductase inhibitors are effective in preventing the development of diabetic neuropathy in animal models, but they have demonstrated disappointing results and dose-limiting toxicity in human trials^[20].

Oxidative and nitrosative stress

As already mentioned, the polyol pathway activation could be the primary cause of oxidative stress associated

with diabetes. However, oxidative stress could be also initiated by autooxidation of glucose and their metabolites, increased intracellular formation of AGEs, increased expression of the receptor for AGEs and its activating ligands, altered mitochondrial function, activation of PKC isoforms and overactivity of the hexosamine pathway^[21-23]. Moreover, there is mounting evidence that oxidative stress is caused by enhanced free radical formation due to glucose metabolism itself and/or deficits in antioxidant defense and it may play a major role among the putative pathogenic mechanisms of diabetic neuropathy. It seems that, in addition to oxidative stress, reactive nitrogen species, especially the peroxynitrite also play an important role in the development of diabetes and its complications^[24-26]. Although it has been clearly demonstrated significant changes in oxidative status in animal models of diabetes^[27], tissue concentrations of known carbonyl compounds are nearly negligible and plasma ET-1, nitric oxide, catalase and glutathione levels did not differ in neuropathic diabetic patients when compared to non-neuropathic diabetic ones^[28]. In line with this observation, clinical results have been contradictory for antioxidants as alpha lipoic acid, ranging from little benefit^[29,30] to interesting advantages^[31,32].

Microvascular changes

DNP is frequently associated with microvascular impairment^[33,34]. In clinical and preclinical studies, it was found that peripheral perfusion is reduced, not only in the nervous tissue^[35,36], but also in the skin^[37], being an important physiological evidence of microvasculature alteration. As a result, nerve ischemia occurs, caused by raise in wall thickness and hyalinization of the basal lamina of vessels that nurse peripheral nerves^[38,39], together with luminal reduction^[38]. These alterations are caused by plasma protein scape of capillary membrane to endoneurium, promoting swelling and augmented interstitial pressure in the nerves, accompanied by higher capillary pressure, deposition of fibrin and thrombus development^[40]. Hyperglycemia *per se* can evoke nerve hypoxia, especially in sensory nerves, altering their electrical stability^[41]. Apparently controversial data from clinical studies described that diabetic patients suffering from the DNP presented higher levels of intravascular oxygen and augmented blood flow in the lower limbs than painless patients. Nevertheless, authors still consider a hypoxic state inside the endoneurium^[42]. Alternatively, a potential sympathetic dysfunction can be the cause of higher blood flow^[43].

As a result of nerve ischemia, both diabetic patients and animals have shown a progressive nerve loss in proximal and distal segments^[44,45], resulting in reduction of intraepidermal nerve fiber density^[12]. Consequently, axonal degeneration and regeneration also occurs, but more frequently in patients that do not experience pain. Besides axonal retraction and regeneration, another structural modification related to hyperglycemia is myelin sheath alteration. The observed demyelination

can be related to Schwann cells altered capacity to support normal myelin sheath^[46].

It is also important to point out that endothelial function in patients with DNP is also altered. The vaso dilatation induced by acetylcholine (*i.e.*, the endothelium-dependent response) in dermal vessels of diabetic patients was reduced in comparison with healthy volunteers. In addition, vasoconstriction mediate by the sympathetic system (*i.e.*, endothelium-independent response) was also defective, what can also be implicated in the pathophysiology of diabetic neuropathy and then, in the DNP^[47].

It is believed that one potential cause of the microvascular changes described above may be the oxidative stress, since the treatment with antioxidant agents can maintain regular perfusion, restoring sensory transmission in type 1 diabetes model^[48].

Channels sprouting

Damaged nerve endings are believed to contribute to pain in DNP^[49,50]. The most accepted hypothesis states that disturbed action potentials can be produced by damaged nerve endings, being interpreted by central nervous system (CNS) as pain or dysesthesias^[51]. Changes in ion channel expression in peripheral fibers are direct consequences of nerve injury, leading to hyperexcitability^[52], that is far linked with neuropathic pain^[53].

In this regard, up-regulation of voltage-gated sodium channels (Nav) has been widely demonstrated in neuropathic pain models^[54,55]. These channels are involved in generation and transmission of action potential, and can be classified into sensitive (TTX-S) or resistant (TTX-R) to tetrodotoxin^[56]. There are several reports that the TTX-S channels Nav1.3, which primary function relays during the embryonic development^[57], and Nav1.7, that is constitutively expressed in peripheral sensory neurons^[58], are both up-regulated in the dorsal root ganglion (DRG) of diabetic animals^[59-61]. Nav1.3 expression was also found increased in small and large diameter DRG neurons of diabetic rats presenting allodynia^[62]. Considering TTX-R sodium channels, Nav1.8 and Nav1.9 are normally expressed in peripheral nociceptive neurons^[63], playing an important role in the generation of electrical activity in DRG^[64]. Intriguingly, DRGs of allodynic diabetic rats showed a reduction of Nav1.8 expression in the following days after diabetes induction^[62], and this reduction lasted 6 mo post diabetes induction^[61], indicating that other sodium channels may play an important role in DNP. The same reduction was detected for Nav1.6, another TTX-S channel, normally present at Ranvier nodes^[60,62].

In addition, an increase of Nav1.6, Nav1.7 and Nav1.8 phosphorylation is another feature observed in the diabetic state, which leads to augmentation in their activity. Thus, both abnormal expression and function of TTX-R and TTX-S sodium channels is linked to abnormal activity of nociceptive fibers^[60]. In this way, Sun *et al.*^[65] showed that TTX-S and TTX-R sodium

currents are increased in small neurons in the DRG of diabetic animals, being this related not only with sensory disturbances, but also with the rise of efficiency of conductance in polymodal C fibers, which in turn, facilitates nociceptive transmission.

In a recent *in vitro* study, it has been described that hyperglycemia evokes higher TTX-R Na^+ currents in a time and concentration-dependent manner, demonstrating a straight relationship between glucose levels and biophysical changes^[66]. In DNP patients, it was reported an increase in nodal Na^+ currents when compared to painless diabetic patients, what can also contribute to hyperexcitability in peripheral nerves^[67].

A new concept proposed by Hoeijmakers *et al.*^[68], links the beginning of pancreatic beta cells failure and DNP with genetic disruptions on Nav1.7 channels. Since both pancreatic beta cells and peripheral neurons express Nav1.7 channels, a susceptible genetic background could facilitate generation of Nav1.7 mutations, leading to gain-of-function that evokes beta cell lesions, and thereafter, diabetes and hyperexcitability in neurons^[69]. According to these authors, this theory could explain why some patients have neuropathy before diabetes onset^[68].

Another interesting finding related to sodium channels modulation is the increased levels of methylglyoxal in type 2 diabetes DNP patients, when compared with those painless^[70,71], and in complication-free type 1 diabetic patients^[72]. This glycolytic metabolite can activate nerve endings through transient receptor potential cation channel subfamily A member 1 activation in the DRG^[73], and also change the Nav1.7 and Nav1.8 function through posttranslational changes^[70]. In line with this clinical observation, in preclinical models methylglyoxal was found to reduce nerve conduction velocity, to elevate calcitonin gene-related peptide release from sensory nerves and to induce thermal and mechanical sensibility^[70]. In addition, in diabetic states methylglyoxal is also involved in the formation of AGEs^[74].

Calcium channels can also be misregulated in a diabetic condition, leading to an enhanced calcium influx in sensory neurons^[75], what can deflagrate both substance P and glutamate release^[76]. It was verified in two different animal models of insulin dependent diabetes that high voltage activated Ca^{2+} current amplitudes were increased in small diameter neurons^[75,77] and the activity of T-type channels (Cav3.2) is also augmented in small diameter fibers^[78], what could be normalized by molecular knockdown of this calcium channel^[79]. However, there was no translation of these results to patients in clinical trials^[80]. A possible future target for pharmacological intervention over calcium channels has been proposed by Orestes and colleagues (2013), which observed that glycosilation of Cav3.2 augments the current density, accelerates kinetics, and also increases the number of channels on neuron membrane, which can be directly involved in DNP. Interestingly, the deglycosylation treatment with neuraminidase inhibits native calcium currents in nociceptors and completely

and selectively reverses hyperalgesia in a pre-clinical model of type 2 diabetes^[80].

Resting membrane potential can also be altered by K^+ voltage dependent channels (Kv), also participating in the electrical properties of neurons^[81]. Regarding the currents generated by activation of these Kv channels in primary afferents there are two main types: rapidly inactivating A-type currents (I_A), and slowly inactivating currents (I_K)^[82,83]. It was verified that the total density of Kv currents as well as the mRNA of I_A subunits of Kv 1.4, 3.4, 4.2 and 4.3 were reduced in large and medium size DRG neurons of diabetic rats^[84]. So, this down regulation can increase neuronal excitability and peptide release^[83], which might also participate in hyperexcitability of peripheral nerves of diabetic subjects.

Microglial activation

It is becoming increasingly recognized that glial cells play an important role in the pathogenesis of many diseases of the nervous system, including chronic pain states^[85]. Glia comprises both macroglia (including astrocytes, radial cells and oligodendrocytes) and microglia cells, which are mainly responsible for maintain homeostasis, form myelin, and provide support and protection for neurons from both central and peripheral nervous system^[85]. Normally, microglial cells comprise less than 20% of spinal glial cells but in response to dorsal root ganglia and spinal cord after nerve injury there is a robust proliferation at spinal level^[86]. Activation of microglia occurs right after peripheral nerve injury, lasting for less than 3 mo, and is responsible for a production of several inflammatory mediators as cytokines, chemokines, and cytotoxic substances such as nitric oxide and free radicals, prompting to a pro inflammatory milieu^[83]. Diabetes has impact on all glial cells of the spinal cord since persistent microglial activation was observed in streptozotocin-induced diabetic rats lasting from 4 wk^[87,88] to 6 or 8 mo^[61,89]. This microglial activation has been associated with sensorial changes and up-regulation of Nav1.3 sodium channels in the DRG^[61], possible through p38 mitogen activated protein kinase dependent mechanism^[90,91]. Conversely, diabetes is associated with a reduction in glial fibrillary acidic protein (*i.e.*, glial fibrillary acidic protein) immunoreactive astrocytes in the spinal cord, which may affect the functional support and role of astrocytic cells in the nervous tissue, such as the clearance of neurotransmitters within the synaptic cleft^[92]. Considering the potential of microglial activation in driving spinal sensitization, in the near future, drugs that target these cells may become an important therapeutic alternative in chronic pain control.

Central sensitization

As already demonstrated in different neuropathic pain states, DNP may be a consequence of both peripheral and CNS changes^[93,94]. It was well described that during DNP, primary afferents are sensitized, inducing dorsal horn hyperactivity and neuroplastic changes in

central sensory neurons^[93]. The common occurrence of allodynia in DNP patients supports the idea that CNS pain processing is altered^[95].

Among the factors that can lead to the hyperactivity of spinal neurons in diabetic neuropathy is the increased glutamate release from primary afferents in the spinal cord^[96,97]. Moreover, spinal N-Methyl-D-aspartate (NMDA) receptor expression is augmented in this condition^[98], generating increased and more frequent excitatory post synaptic currents in the lamina II^[97]. Additionally, it has been described that cAMP response element-binding protein signaling, which directly regulates NMDA receptors activity^[99] is enhanced in DNP^[98,100]. Thus, it is plausible that augmented NMDA expression and glutamate release might contribute to spinal cord hyperactivity. On the other hand, GABA_B receptors seem to be downregulated in the spinal cord in diabetic neuropathy^[98]. Activation of GABA_B receptors results in inhibition of NMDA receptor activity through a direct inhibition of voltage-sensitive Ca²⁺ channels^[101] and opening of inwardly rectifying K⁺ channels^[102]. Furthermore, GABA_B receptor activation also causes downregulation of NMDA receptors at the spinal level in diabetic rats^[98]. Considering the importance of central sensitization in the hypersensitivity associated with DNP, strategies that aim to control spinal neurons hyperexcitability are very useful in pain control in this condition, as will be discussed below.

Brain plasticity

Functional changes in pain processing areas in the CNS, besides the spinal cord, have been ultimately linked with DNP^[103], in a tight relation to increased peripheral input^[93,104]. Among these areas, marked changes in the thalamus, cortex and rostroventromedial medulla (RVM) have been reported in DNP patients and or experimental models.

The ventral posterolateral nucleus (VPL) of the thalamus is the main receiving area of nociceptive stimuli that is processed in the spinal cord^[105]. Projection neurons reach the thalamus through the spinothalamic tract (STT), which represents a major ascending nociceptive pathway. It has been demonstrated that in diabetic rats, these neurons present increased spontaneous activity, enlargement of the receptive field and augmented responses to mechanical noxious and innocuous stimuli. The hyperexcitability of STT neurons probably accounts to hypersensitivity to external stimuli and spontaneous pain^[93], increased in primary afferents activity^[93,104] and to plastic changes in spinal neurons^[93].

In addition, in studies that assessed brain imaging in diabetic rats it was reported increased activity not only at VPL, but also in different thalamic nuclei that control sensory-motor aspects^[106]. In diabetic patients, a recent study showed increased activation of diverse brain areas, including medial thalamus after application of noxious thermal stimuli in feet^[107]. Moreover, it has been described that DNP patients has a marked reduction in the levels of N-acetyl-aspartate (NAA) levels in the

thalamus compared to painless diabetic individuals^[108]. It is important to point out that patients with brain disorders in which neuronal loss or dysfunction are involved have consistently decreases in brain NAA concentrations^[109]. Other clinical finding related to thalamus alterations in diabetic patients is that subjects with painful type 1 diabetic neuropathy presented increased thalamus blood flow, when compared with those without pain, which was considered to reflect higher neuronal activity^[110]. Taking account the thalamus relevance in the nociceptive pathway, it is plausible to suggest that the alterations reported in this area might contribute to the development and/or maintenance of DNP^[108,110].

Likewise, in a model of type 1 diabetes, increased glutamate transmission was reported in the anterior cingulate cortex a brain area involved in the processing of the affective-motivational dimension of pain^[111]. The consequence of higher stimulation of this area by glutamate is suggested to be a sustained negative perception of affective component of pain^[111].

Changes in the endogenous pain control system have also been described in pre-clinical and clinical studies of DNP. The RVM is a structure that receives direct influences of periaqueductal gray matter, which is, in turn, affected by other structures, such as amygdala and hypothalamus^[112]. Three different populations of cells have been describe within the RVM: activation of ON cells act in a pronociceptive way, while activation of OFF cells has the opposite effect^[113] and neural cells which activation is still contradictory and remains to be better clarified^[114,115]. In diabetic animals, there is evidence of a reduction on the OFF cells and increase on the ON cells population. In addition, basal activity is augmented in ON cells, and reduced in OFF cells, in a resultant misbalance between pain facilitatory and inhibitory descending modulation in diabetic animals^[103]. After noxious mechanical stimulation in the periphery, there was no difference between diabetic and control ON cells activity. Thus, the mechanical hyperalgesia detected in diabetic rats could be associated with OFF cells impairment and consequently reduction on descending inhibitory tone^[103].

Some studies have also addressed the levels of the main neurotransmitters of the endogenous pain control system in different areas of the CNS in diabetic rats, but they have shown discrepant results. While some researchers found reduced release of norepinephrine in the spinal cord in diabetic rats^[116], others have described opposite findings^[117]. There is also evidence of diminished norepinephrine levels in supra spinal areas, such as brainstem and thalamus, but higher concentration in the cortex of diabetic animals^[118]. Additionally, impaired spinal opioid-induced release of serotonin (5HT) has been demonstrated in diabetic rats, and this finding may be related to opioid hyporesponsiveness in experimental DNP^[119]. Increased norepinephrine and 5HT levels in the spinal cord, as well as, augmented expression of norepinephrine and 5HT in RVM neurons

was also demonstrated in diabetic rats^[117]. Considering the facilitatory role of serotonergic and noradrenergic descending modulation during chronic pain, these changes may probably account for enhanced pain during diabetic neuropathy^[117]. There is also clinical evidence for misbalance between excitatory and inhibitory neurotransmitters in the CNS of diabetic patients with positive symptoms of neuropathy. In this regard, it has been found reduced levels of GABA and higher levels of glutamate in the posterior insula of diabetic patients, as well as a higher glutamate/GABA ratio within the thalamus^[120]. These changes may contribute to pain development in DNP, but further studies are necessary to determine their clinical significance.

TREATMENT OF DNP

DNP continues to represent a therapeutic challenge as its pathophysiology is not yet fully understood and pain relief is still unsatisfactory. The pharmacological treatments, with exception to those targeted to the glycemic control, are symptomatic, not focused on the pathophysiological mechanisms, limited by side effects^[3,121] and by the development of tolerance^[121].

A wide variety of drugs, used alone or in combination, has been shown to significantly reduce neuropathic pain compared with placebo in randomized controlled trials, but pain relief remains inadequate for most patients^[122]. Generally, in clinical trials, treatment is considered successful if patients would obtain 50% of reduction in the pain level^[123-125] associated with some additional beneficial effects on sleep, fatigue, depression and quality of life^[125]. Thus, the management of this condition basically consists of excluding other causes of painful peripheral neuropathy, improving glycemic control as a prophylactic therapy and using medications to alleviate pain^[126].

Despite of multimodal and multidisciplinary approaches to the treatment, the primary pathway is pharmacologically based^[127]. Three different agents have regulatory approval in the United States for the treatment of DNP: pregabalin, duloxetine and tapentadol^[11,128]. However, as pain relief is still suboptimal and challenging for clinicians^[95], drugs from various pharmacological classes have been used and some of them are included in this review.

Anticonvulsants

Pregabalin was the first anticonvulsant to receive approval from the Food and Drug Administration (FDA) for the treatment of postherpetic neuralgia, DNP^[129,130] and neuropathic pain after spinal cord injury^[131]. Pregabalin is a GABA analogue that selectively binds to pre-synaptic voltage-gated calcium channels containing the $\alpha 2\delta$ subunit in the brain and spinal cord, causing inhibition of the release of excitatory neurotransmitters^[128,132]. Moreover, $\alpha 2\delta 1$ subunits are responsible for increasing the functional expression of these channels, as a consequence of increased trafficking. Thus, the analgesic

action of pregabalin is also proposed to be the result of impaired trafficking of $\alpha 2\delta 1$ subunit with a consequent diminished expression of functional calcium channels^[133].

Several clinical trials evaluating pregabalin in DNP showed efficacy in the management of this condition^[3,134,135] with a number needed to treat (NNT) of 6.3^[125]. In addition to its analgesic effects, pregabalin presents anxiolytic activity^[132,135] and it has a beneficial effect on sleep and quality of life^[132], contributing, therefore, to improve the general condition of the patients. The side effects include dizziness, somnolence, peripheral edema, headache and weight gain^[3].

Some guidelines have also recommended gabapentin to treat DNP^[136]. Besides pregabalin, gabapentin is the only other anticonvulsant drug that demonstrated efficacy in the treatment of this condition^[128] with an NNT of 5.8^[137]. Gabapentin and pregabalin have a similar mechanism of action and the first is licensed for neuropathic pain in the United Kingdom, but not in the United States^[128]. Some clinical trials have suggested that gabapentin and pregabalin present better analgesic efficacy than tricyclic antidepressants or opioids^[138] and other important aspects of these drugs include their tolerability and lack of serious toxicity^[139].

Antidepressants

Antidepressants represent the first line drugs in DNP management. Duloxetine, a serotonin and norepinephrine reuptake inhibitor, is rated level A for efficacy and is approved in the United States for the treatment of this condition. Additionally, some clinical trials have pointed out the effectiveness of duloxetine in other chronic pain conditions, such as fibromyalgia and chronic musculoskeletal pain^[140,141].

Results from a meta-analysis that included randomized, double blind, placebo controlled studies in patients with DNP showed the superiority of duloxetine over placebo in reduction of pain severity and in Patient Global Impression of Improvement/Change, as well as efficacy similar to gabapentin and pregabalin^[142]. Moreover, in a 2-wk open-label randomized trial in diabetic patients poorly responsive to gabapentin, duloxetine was able to reduce the pain score to levels similar to those achieved with pregabalin^[143,144]. Furthermore, analgesic efficacy of duloxetine in the treatment of DNP is maintained over a 6-mo period^[145], reinforcing its importance as a treatment option for this condition. The NNT for duloxetine varies from 1.3 to 5.1 in DNP patients^[146,147], which experience more frequently nausea, somnolence and dizziness as side effects^[146].

Venlafaxine is also a selective serotonin and norepinephrine reuptake inhibitor, that predominantly inhibits serotonin reuptake at low doses and norepinephrine at higher doses^[148]. Venlafaxine was also shown to be effective in reducing pain intensity in diabetic neuropathic patients^[149], with an NNT between 2.2 and 5.1 and a number needed to harm (NNH) of 9.6, to minor adverse effects, and of 16.2, for major adverse effects^[150].

Tricyclic antidepressants can also be an alternative to

treat DNP^[151]. Amitriptyline was shown to be as effective as gabapentin in a direct meta-analysis study^[152] and as duloxetine in a randomized, double-blind, crossover trial^[153]. Likewise, nortriptyline was reported as being as effective as gabapentin in attenuating neuropathic pain in a double-blind crossover trial enrolling diabetic patients^[154].

Tricyclic antidepressants are estimated to have an NNT of 1.3^[151,155], and an NNH from 4.2 to 10.7^[151] in DNP. The most common side effects related to the use of these drugs are dry mouth, postural hypotension, arrhythmias, cognitive impairment, constipation and urinary retention, which are more frequently observed after amitriptyline than nortriptyline treatment^[155].

Opioids

Opioids are recommended to be used as second or third line treatment for DNP^[3,151]. One multicenter, randomized, placebo-controlled study reported the tramadol effectiveness to significantly improve scores on physical and social functioning ratings in patients with DNP, but beneath some side effects such as nausea, constipation, headache and somnolence^[156]. Morphine was also shown to be effective in reducing mean daily pain scores related to diabetic neuropathy and postherpetic neuralgia^[128,157]. Moreover, results of clinical trials indicated that diabetic neuropathic patients experienced a significant reduction in pain intensity and an improvement on quality of life during oxycodone treatment, compared to placebo-exposed group^[158,159]. Besides, oxycodone improved gabapentin but not the pregabalin effectiveness in promoting DNP relief^[160,161].

The clinical evidence for the effectiveness of opioids in the control of DNP is corroborated by some pre-clinical data, which have reported the effectiveness of morphine^[162,163] and buprenorphine^[164] in reducing thermal or mechanical hypersensitivity in DNP animal models.

There is also evidence that the anti-hyperalgesic effect of opioids is improved by the association with some drugs, such as the antidepressants amitriptyline, moclobemide and reboxetine^[165]. In line with this idea, new molecules that integrate additional mechanisms to the opioid receptor agonism have been shown efficacy in reducing nociceptive behavior in animal models of DNP, such as the cebranopadol, a nociceptin/orphanin FQ peptide and opioid receptor agonist^[166], and the mu-opioid receptor agonist and norepinephrine reuptake inhibitor, tapentadol^[162,167]. The latter was approved by FDA for DNP treatment since 2012^[111]. Tapentadol has been shown to be effective in the management of different types of chronic pain, including osteoarthritis knee pain, low back pain and DNP, with a tolerable safety profile^[168,169]. Specifically concerning DNP, a randomized-withdrawal, placebo-controlled trial reported reduction of at least 30% in pain intensity in about 50% of the patients that received tapentadol^[170]. Similar data were obtained in a recent clinical trial in diabetic

neuropathic patients with moderate to severe pain, which experienced nausea (21.1%) and vomiting (12.7%) as side effects^[171].

Others agents

The drugs discussed below are currently associated to the pharmacological treatments already described according to the patients' symptoms and needs in order to achieve a better relief of pain in DNP conditions. However, further studies are necessary, specially controlled clinical trials, to determine the more efficacious, safe and successful combinations to be applied in the management of DNP^[128].

Capsaicin topical cream: Topical agents may be associated with fewer and less clinically significant adverse events than systemic agents^[172]. In addition, the possibilities of drug interactions are markedly reduced with the use of local treatments, which represent good options for patients with multiple medical problems^[128]. The capsaicin cream has been shown to be effective in the treatment of neuropathic conditions^[150] and is approved for topical relief of neuropathic pain^[128]. Capsaicin is the pungent component of hot chilli peppers^[11] and an agonist of the transient receptor potential vanilloid 1. This receptor is a ligand-gated, nonselective cation channel, predominantly expressed on unmyelinated C nerve fibers^[173], which, after repeated exposure to topical capsaicin, are depleted of their content of substance P and other neurotransmitters^[173,174]. The C fibers depletion and desensitization reduce painful stimuli transmission from peripheral nerves to the central nervous system^[173]. Some clinical trials have demonstrated the effectiveness of low-concentration (from 0.025% to 0.075%) capsaicin cream in DNP^[11,174,175]. Higher concentrations are not indicated because of desensitization of nociceptive sensory nerve endings, which may increase the risk of skin injuries in DNP patients^[173,174]. Some adverse effects include itching, stinging, erythema, transient burning sensation and initial pain at the application site, that diminishes with repeated use^[132,175], leading many patients to withdraw from the treatment^[128,173].

Lidocaine patch: Lidocaine patches act as peripheral analgesics with minimal systemic absorption and are used in combination with other analgesic drugs^[132,172]. Lidocaine blocks sodium channels and counteracts the hyperexcitability of peripheral nociceptors that contributes to neuropathic pain^[132,176]. The blockade reduces ectopic discharges and raises the peripheral sensory neuron discharge threshold^[176]. The few DNP clinical trials that compared topical lidocaine with other relevant interventions suggested that the effects in pain reduction are comparable to other drugs, such as capsaicin, gabapentin, amitriptyline^[172] and pregabalin^[172,174]. Adverse events include local irritation^[172], contact dermatitis and itching^[132].

Alpha lipoic acid: The benefit provided by alpha

lipoic acid (ALA) in the treatment of DNP possibly is due to its direct effects on the neuropathy, by reducing the oxidative stress, which has been defined as an important factor in the physiopathology of the diabetic neuropathy^[122]. Its antioxidant and anti-inflammatory actions may contribute to an all-round improvement of diabetic neuropathy symptoms^[135]. In some clinical trials that evaluated ALA effect in diabetic patients, pain was not a primary end point. However, they have shown a moderate benefit in terms of pain reduction^[132]. In a randomized double-blinded trial, ALA-treated patients reported a greater reduction in neuropathic pain when compared to placebo-treated subjects^[122]. Compared to several drugs currently in use for DNP treatment, ALA has fewer side effects^[30], being nausea and vomiting the most common^[132].

Isosorbide dinitrate spray: Isosorbide dinitrate is a nitric oxide-dependent vasodilator with effects on both arteries and veins^[177]. The improvement of pain and burning sensation could be associated with the increased generation of nitric oxide, improving microvascular blood flow^[178]. In a clinical trial with diabetic patients, the isosorbide dinitrate spray reduced overall neuropathic pain and burning sensation in about 50% of the patients, which also reported improvement in their quality of life, with improvements in sleep, mobility and mood^[178,179].

Final considerations about DNP treatment:

Besides the fact of many diabetic complications can be reduced with improved blood glucose control and other lifestyle interventions^[132,150], such as quit smoking and reducing alcohol consumption^[150], the efficacy of these measures, as well as the pharmacological treatments on DNP are not predictable. The medications rated as level A based on their efficacy are able to reduce pain and improve some aspects of patients' quality of life, but are not able to fully eliminate pain or prevent/revert the neuropathy. Even their combination does not result in satisfactory pain control, being the best improvement in pain, restricted to 50% of relief for the majority of the patients. Considering the available pharmacological options, DNP treatment has to be based mainly on patients' symptoms, pain level and tolerance of side effects.

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New-onset diabetes mellitus after kidney transplantation: Current status and future directions

Sneha Palepu, G V Ramesh Prasad

Sneha Palepu, G V Ramesh Prasad, Division of Nephrology, University of Toronto, St. Michael's Hospital, Toronto ON M5C 2T2, Canada

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Correspondence to: G V Ramesh Prasad, MBBS, MSc, MA, FRCPC, FACP, FASN, Associate Professor of Medicine, Division of Nephrology, University of Toronto, St. Michael's Hospital, 61 Queen Street East, 9th Floor, Toronto ON M5C 2T2, Canada. prasadr@smh.ca

Telephone: +1-416-8673722

Fax: +1-416-8673709

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Abstract

A diagnosis of new-onset diabetes after transplantation (NODAT) carries with it a threat to the renal allograft, as well as the same short- and long-term implications of type 2 diabetes seen in the general population. NODAT usually occurs early after transplantation, and is usually diagnosed according to general population guidelines. Non-modifiable risk factors for NODAT include advancing age, African American, Hispanic, or South Asian ethnicity, genetic background, a positive

family history for diabetes mellitus, polycystic kidney disease, and previously diagnosed glucose intolerance. Modifiable risk factors for NODAT include obesity and the metabolic syndrome, hepatitis C virus and cytomegalovirus infection, corticosteroids, calcineurin inhibitor drugs (especially tacrolimus), and sirolimus. NODAT affects graft and patient survival, and increases the incidence of post-transplant cardiovascular disease. The incidence and impact of NODAT can be minimized through pre- and post-transplant screening to identify patients at higher risk, including by oral glucose tolerance tests, as well as multi-disciplinary care, lifestyle modification, and the use of modified immunosuppressive regimens coupled with glucose-lowering therapies including oral hypoglycemic agents and insulin. Since NODAT is a major cause of post-transplant morbidity and mortality, measures to reduce its incidence and impact have the potential to greatly improve overall transplant success.

Key words: Cyclosporine; Graft; Kidney; New-onset diabetes; Tacrolimus; Transplantation

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Core tip: New-onset diabetes after kidney transplantation (NODAT) is detrimental to patient and graft survival. Early diagnosis through the identification of modifiable and non-modifiable risk factors for NODAT and appropriate screening, accompanied by good glycemic control that involves a multidisciplinary care approach will help result in good short- and long-term kidney transplant outcomes.

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INTRODUCTION

Kidney transplantation is widely considered to be the therapy of choice for patients with end-stage renal disease (ESRD) because of its ability to provide the maximum amount of replacement of renal function and a consequent improvement in patient morbidity and mortality. Among the various complications experienced by kidney transplant recipients (KTR), new-onset diabetes after transplantation (NODAT) is a well-known condition by which an organ transplant recipient such as a KTR develops diabetes mellitus (DM) at some point after the transplant procedure. The purpose of this review is to discuss the epidemiology and management of this common and important post-transplant disorder. A special emphasis will be placed on studies performed during the past ten years, with the intent of providing management recommendations for both diabetes and transplant professionals.

A diagnosis of NODAT carries with it a threat to the renal allograft, as well as the same short- and long-term implications of type 2 DM, but these occur at an accelerated pace^[1]. DM can both cause and expedite the course of cardiovascular disease (CVD) as well as the failure of multiple organ systems^[2]. According to the World Health Organization (WHO), the number of patients worldwide with DM has doubled from 170 million to 340 million over the past ten years. In addition, the WHO predicts that DM will be the seventh leading cause of death by 2030. This increase in incidence of DM has extended across many population groups, including perhaps transplant recipients.

Over the past decade, concomitantly with DM, there has also been a corresponding increase in the number of patients seeking kidney transplants. According to the 2010 United States Renal Data System Annual Data Report, the annual number of kidney transplants in that country increased from 13425 to 17350 over the decade between 1998 and 2008^[3]. Thus, there is also a larger KTR population at increased risk for NODAT. Although type 2 DM is an important cause of ESRD and consequently an important contributor to clinical consequences in incident KTR cohorts, we focus here on NODAT, as an entity distinct from pre-existing DM in a patient receiving a kidney transplant.

INCIDENCE AND PREVALENCE OF NODAT

The prevalence of NODAT after kidney transplantation has increased^[4], and varies between 2% and 53% based on several estimates^[5]. One reason for the wide variation in reported prevalence may be due to the challenges faced in making the diagnosis. Until about a decade ago, there was no consensus definition for NODAT. Previously, it was defined by fasting or random blood glucose levels of varying thresholds, or perhaps quite naively, by the need for insulin or oral

hypoglycemic agents in the post-transplant period^[6]. This issue was addressed by the development of the 2003 Consensus Guidelines, developed by the American Diabetes Association (ADA) and the WHO^[7]. According to these guidelines, NODAT is present if the patient has symptoms of DM, casual plasma glucose ≥ 11.1 mmol/L, or 8-h fasting plasma glucose ≥ 7.0 mmol/L. In 2010, the definition for DM was revised by the ADA to include the 2-h oral glucose tolerance test (OGTT), with a 2-h value of ≥ 11.1 mmol/L being indicative of DM. The general principle behind standardized NODAT incidence reporting is that the diagnostic criteria should reflect what is used in the general population. Hopefully, these developments will allow for more consistent reporting of NODAT in the future, leading in turn to more precise estimates of incidence rates.

It is well established that NODAT usually occurs early after kidney transplantation. One long-term study^[8] conducted between 1976 and 2004 in 1580 Egyptian KTR demonstrated a diagnosis of NODAT in 18.2% patients overall, in whom 52.4% were diagnosed by 6 mo and 11.5% between 6 and 12 mo. A French study of 527 KTR indicated a median time-to-onset of 1.6 mo, with an incidence of 7% over 2 years^[9]. Incidence estimates in the United States are 9.1% at 3 mo, 16% at 12 mo, and 24% at 36 mo^[10]. The incidence may be increasing even in children^[11]. Most studies reporting NODAT prevalence do not include OGTT data, as a result of which significant underestimation of incidence may occur. Variation in studies with regards to length of follow-up, intensity of routine screening, and degree of use of standardized definitions will all have an impact on incidence estimates. Furthermore, glycemic control improves in some patients with increasing time post-transplant, as a result of immunosuppressive medication dose reduction and other interventions. This in turn affects prevalence estimates and interpretation of incidence rates. Addition of HbA1c testing may also modify reported incidence rates. However, HbA1c testing has not been routinely employed at most transplant centres.

RISK FACTORS FOR NODAT

The risk factors specific to NODAT have been well-delineated. These can be broadly classified into non-modifiable and modifiable risk factors. These are summarized in Table 1.

NON-MODIFIABLE RISK FACTORS FOR NODAT

Non-modifiable risk factors include advancing age, black race, genetic background, a positive family history for DM, and previously diagnosed glucose intolerance^[12]. Polycystic kidney disease is also believed to be a risk factor. All of these risk factors can usually be identified

Table 1 Risk factors for new-onset diabetes after transplantation

Non-modifiable	Modifiable
Advanced age	Obesity
African American, Hispanic, or South Asian descent	Sedentary lifestyle
Genetic, <i>e.g.</i> , HLA B27	Metabolic syndrome
Adult polycystic kidney disease	Viral infections, <i>e.g.</i> , HCV, cytomegalovirus
Previous glucose intolerance, <i>e.g.</i> , during pregnancy, steroid therapy for renal or non-renal disease	Corticosteroids
Male donor	Calcineurin-inhibitors (tacrolimus > cyclosporine)
Deceased donor	Sirolimus
	Acute rejection

HCV: Hepatitis C virus; HLA: Human leukocyte antigen.

before transplantation.

Since the ESRD population continues to age, it is possible that the incidence of NODAT will increase as a result of more elderly patients being transplanted for their ESRD. Age is considered to be the strongest risk factor for NODAT^[13]. It was long ago shown that patients above the age of 45 may be at higher risk for NODAT^[14]. A later study estimated that risk at about 2.9-fold^[15]. The incidence of NODAT may increase by as much as 50% for every 10-year increase in age^[16]. Age over 60 has been associated with a risk of 2.6-fold^[10]. However, age *per se* is not a precluding factor to kidney transplantation^[17] and the risk for NODAT does not typically dissuade transplant centres from performing transplants in older patients.

Besides African-American or Hispanic descent^[12], KTR of South Asian origin may also be at higher risk for NODAT^[18]. The relative risk for NODAT has been estimated at 1.68 for blacks and 1.35 for Hispanics compared to Caucasians^[10]. Using the 2003 consensus criteria, blacks are considered to be at a two-fold risk for NODAT^[19]. The increased risk for NODAT, at least in blacks, is believed to be at least partly due to variation in the pharmacokinetics of various immunosuppressive agents^[1]. Variability in dosing requirements based on ethnicity has been demonstrated recently for tacrolimus, with increased amounts of this diabetogenic agent needed in East Asians^[20]. It is unclear at this time, however, if the diabetogenic effects of immunosuppressive medication are influenced by ethnicity, although this has been speculated^[7].

Genetic predisposition to NODAT includes traditional associations with the alleles human leukocyte antigen A28, A30, B27, and Bw42. A number of genetic associations with NODAT have been identified in the last decade. For example, there has been a reported association with the R325W polymorphism in the SLC30A8 zinc transporter gene of pancreatic islets, with the R/r inheritance being associated with higher risk^[21]. Another study from the same group suggested the association of *TCF7L2* gene variants with NODAT^[22]. This

association with *TCF7L2* has also been demonstrated by another group^[23]. Other associations of specific gene variants with NODAT include *KCNQ1*^[24], *NFATc4*^[25], adiponectin 276G/T^[26], and mitochondrial haplotype H^[27]. While all of these associations are interesting, it remains to be seen if such findings can be replicated in different populations, and whether they can be demonstrated to be independent risk factors for NODAT in prospectively conducted studies. Furthermore, the expense and inconvenience of such assessments greatly limits the ability to study these and other gene candidates further.

In the past decade, NODAT has also been associated with autosomal dominant polycystic kidney disease (ADPKD)^[28,29], as well as autosomal recessive polycystic kidney disease^[30]. ADPKD in particular is a common disease and cause of ESRD, particularly in Caucasians, and so it remains to be definitively proven as a risk factor for NODAT since both ADPKD and NODAT are common in KTR. A plausible mechanism for the association also needs to be developed.

Finally, previously diagnosed glucose intolerance is a risk factor for NODAT^[13]. Glucose intolerance may have been diagnosed at the time of pregnancy, or when corticosteroids were used as part of the therapy for the primary renal disease or a related or unrelated systemic disease. In such cases, even though DM may have resolved with cessation of steroid therapy, the risk for DM may persist lifelong. To our knowledge, these risks have not been evaluated prospectively. Also, donor factors for NODAT considered "non-modifiable" include male gender and deceased, as opposed to living donor kidneys. However, these risk factors are unlikely to ever be evaluated prospectively, and so will not be discussed further.

MODIFIABLE RISK FACTORS FOR NODAT

There are many more risk factors for NODAT that may be considered modifiable, at least on a theoretical basis. The most significant risk factors are as follows.

Obesity has detrimental effects to transplantation for a variety of reasons. Obesity has been estimated to increase the risk for NODAT, with a relative risk of 1.73^[13]. The risk for NODAT increases linearly for every 1 kg above 45 kg^[13]. While obesity in the context of transplantation is traditionally understood as body mass index > 30 kg/m², body fat percentage may also be a useful marker^[31]. It remains undetermined, however, whether it is pre-transplant weight that increases the risk for NODAT, or whether it is the weight gain that occurs soon after transplantation that is the cause. At least one study indicates that is the former^[32]. Nonetheless, longer-term studies may be needed to demonstrate the association of weight gain with NODAT^[13]. Adiponectin is a hormone that is reduced with increasing adiposity. Increased adipose tissue is also associated with inflammation. NODAT has been associated with reduced

adiponectin and increased C-reactive protein levels^[33], indicating that these are also possible mechanisms for the association seen.

The metabolic syndrome is often associated with obesity. When a definition for metabolic syndrome such as the National Cholesterol Education Program Adult Treatment Panel III is used, which does not contain diabetes as a mandatory component, the presence of metabolic syndrome has been associated with an increased risk for NODAT. In the Patient Outcomes in Renal Transplantation study^[34], metabolic syndrome was independently associated with NODAT (HR = 3.46, 95%CI: 2.40-4.98, $P < 0.0001$).

Hepatitis C virus (HCV) infection has been associated with DM in the general population, particularly over the age of 40^[35]. Just like DM, HCV also causes ESRD by causing glomerular disease, and HCV can be acquired through blood contamination in hemodialysis units. As a result, it is not uncommon for HCV-infected patients to be considered for kidney transplantation when there is no evidence for hepatic dysfunction and viral titres are sufficiently low or undetectable. The one-year incidence of NODAT was found to be 25.6% in those who were HCV-positive, compared to 14.4% in those who were negative^[10]. Another study found an adjusted Odds ratio for NODAT approaching 4.0 in those with HCV^[36]. The risk for NODAT with HCV may be exacerbated by the use of tacrolimus^[37]. Cytomegalovirus (CMV) infection has also been considered as a risk factor for NODAT, with the mechanism being impaired insulin release^[38]. Unlike HCV however, CMV is much more easily treated in the post-transplant setting and so receives considerably less attention as a risk factor.

Corticosteroids remain a mainstay of post-transplant immunosuppression and are a part of most medication regimens. The risk for NODAT with steroids relates both to the dose used and the duration of therapy^[13]. Steroids induce gluconeogenesis and glycolysis, increasing both fasting and post-prandial glucose levels. Glycogenesis is decreased. Insulin resistance, to which KTR may be predisposed, is an important effect of steroid therapy. They also impair pancreatic beta-cell function^[39]. While a maintenance dose of 5 mg/d of prednisone is typically used for KTR, higher doses (such as 1 mg/kg per day) are used in the early post-transplant phase. Higher doses are also used as bolus therapy when acute rejection of the transplant occurs. There is a dose dependent relationship between steroid dose and glucose levels^[40].

Tacrolimus and cyclosporine are widely used calcineurin-inhibitor (CNI) drugs in KTR. Most patients receive one or the other drug as part of their immunosuppressive drug regimen. Between these two, tacrolimus is considered to be more diabetogenic by about 50%^[10,41]. Unlike in the case of steroids, this effect is not believed to be dose-dependent^[16,42], although this is controversial^[43]. Tacrolimus is being increasingly preferred as the CNI of choice at most transplant centres

due to its demonstrated superior efficacy and safety^[44], despite the risk for diabetes. However, it is possible to use much lower doses presently^[44]. Deficiency of calcineurin leads to decreased insulin production. CNI inhibit the uptake of glucose molecules by cells due to a reduction in the number of glucose transporter type 4 (GLUT-4) receptor molecules on the cell membrane surface of adipocytes^[45]. GLUT-4 is an insulin-regulated protein present primarily in adipose tissue and striated muscle, enabling the translocation of glucose into the cell cytoplasm^[46]. Thus, reduction in GLUT-4 leads to hyperglycemia. Tacrolimus also reduces glucokinase activity in pancreatic islets, thereby suppressing glucose-induced insulin release^[47]. Although both impaired insulin release and increased insulin resistance are both believed to be mechanisms for CNI-induced NODAT, the former may be more important. Islet cell damage in the form of cytoplasmic swelling, vacuolization, and altered insulin staining can be demonstrated as a result of CNI therapy^[48]. CNI also cause reduced insulin gene expression^[49].

Sirolimus is another immunosuppressive agent used in transplantation that has been contextualized to NODAT. The association of sirolimus with NODAT has been believed to be due to its combination with CNI, and so conversion from a CNI to sirolimus as the main immunosuppression has been perceived as beneficial^[50]. However, some studies seem to indicate that sirolimus is a risk factor for NODAT. These include both large database studies^[51] and smaller single-centre reviews^[52]. The effect may be mediated by hypertriglyceridemia^[53]. Sirolimus may also inhibit pancreatic beta cell proliferation. At best, sirolimus is neutral with respect to NODAT risk^[13].

The potential pathogenic mechanisms for drug-induced NODAT are summarized in Table 2.

CLINICAL SIGNIFICANCE OF NODAT

RTR who develop NODAT are most likely to be at the same risk for developing the short- and long-term complications of diabetes as people with type 2 diabetes. However, there have been few prospective, long-term, or interventional studies with adequate statistical power to support this statement. It is clear, however, from smaller studies that NODAT has an adverse effect on important transplant- and patient-related outcomes.

An older study has demonstrated that after 12 years of post-transplant follow-up, graft survival in patients with NODAT was only 48% compared to 70% in those who did not develop NODAT, with NODAT predicting a relative risk of graft loss of 3.72^[54]. In the shorter term, graft survival was shown to be reduced by 17% after 3 years and 34% after 4 years in those with DM compared to those without DM^[55]. In one prospective study, more than 1400 KTR underwent an OGTT at 10 wk post-transplant and were followed for a median of 6.7 years. Impaired glucose tolerance was found to be associated

Table 2 Potential pathogenic mechanisms for drug-induced new-onset diabetes mellitus after transplantation

Immunosuppressive drug	Mechanism for new-onset diabetes after transplantation
Corticosteroids	Increased gluconeogenesis
	Increased insulin resistance
	Reduced glycogenesis
	Decreased insulin release
	Impaired pancreatic beta cell function
Calcineurin-inhibitors (cyclosporine, tacrolimus)	Reduced glucose uptake
	Decreased insulin release
	Reduced insulin gene expression
	Direct pancreatic beta cell toxicity
Sirolimus	Hypertriglyceridemia
	? Decreased pancreatic beta cell proliferation

?: Requires further investigation.

with a 40% greater mortality risk^[56]. This increased risk was not seen with impaired fasting glucose (IFG). In a prospective, single-centre study of 201 consecutive KTR in Norway, the 8-year major adverse cardiac event rate was 7% in those without DM, 21% in those with DM before transplantation, and 20% in those with NODAT^[57]. Perhaps due to low statistical power, NODAT was not associated with mortality in this study. In another single centre cohort of over 1800 KTR, NODAT was associated with a hazard ratio of 1.80 for mortality^[58]. The main cause of mortality is CVD^[58].

An important distinction to be made is if NODAT leads to patient mortality independent of transplant graft function. In one study^[59], an association was detected between NODAT and death with a functioning graft, but there was no impact on graft survival when censored for death. It is easier to demonstrate associations when NODAT is combined with pre-existing DM^[60]. In order to identify the unique contribution of NODAT to mortality, independent of graft function, longer follow-up is required.

NODAT also imposes a significant cost to health care systems. In the United States, NODAT was estimated to cost more than \$12000 in the first post-transplant year, and over \$19000 in the year after this^[41]. This cost is most likely related to the treatment for, and morbidity associated with DM.

Regardless of the implications of NODAT for cardiovascular risk, graft function, or mortality, a diagnosis of NODAT carries great significance for the individual patient. Transplant patients typically require three immunosuppressive medications, to which prophylactic antibiotics, antihypertensive agents, and others are often added. Patients with NODAT are prescribed oral hypoglycemic agents and sometimes insulin in addition to all of these. They are also subject to new dietary restrictions, which will often be superimposed on those mandated by a chronic kidney disease state (such as potassium restriction). Hospitalization may occur for both hyperglycemia and hypoglycemia^[61]. All of these have an important impact on quality of life. Studies assessing this aspect of NODAT implications are few. NODAT has been associated in older studies with

peripheral neuropathy and diabetic nephropathy^[54], as well as more infections^[62], including severe infections^[63].

PREVENTION AND MANAGEMENT OF NODAT

Prevention of NODAT

Some common preventative strategies for NODAT are summarized in Table 3. Preventing NODAT has the potential to prevent many of the short- and long-term complications of NODAT. Screening is an important part of any preventative strategy. Screening for NODAT risk prior to transplantation, as well as the risk for cardiac events, will allow for better informed consent and also help to guide post-transplant management. One recommendation is to obtain a fasting glucose level in all transplant candidates, with a subsequent OGTT performed if IFG is detected^[64]. A pre-transplant OGTT may be justified if the fasting blood glucose (FBG) is as low as 5.1 mmol/L^[65]. Even if the OGTT is normal, a pre-transplant random BG > 6.0 mmol/L is associated with a NODAT risk of over 25%, and > 7.2 mmol/L with a risk exceeding 50%^[66]. Use of OGTT may be justifiable in all transplant candidates if the population is at particularly high risk, such as a multi-ethnic population. More sophisticated testing such as HOMA-IR assessment is not feasible routinely. HbA1c testing has not been evaluated as a pre-transplant screening strategy. If a transplant candidate is determined to be at high risk for NODAT, this should be discussed with the candidate before the transplant, and if appropriate, their proposed post-transplant immunosuppressive strategy discussed as well.

Screening for NODAT has also been employed in the post-transplant setting. Self-testing of blood glucose in the afternoon during the early post-transplant phase has been associated with an increased rate of detection of NODAT^[67]. An OGTT performed at 10 wk post-transplant may help to predict longer-term hyperglycemia^[68]. One difficulty with OGTT in large post-transplant clinics is that it may interfere with CNI blood level measurements, which are strictly timed. It is reasonable to measure fasting glucose at least monthly, and random blood glucose at least twice weekly for the first few months after transplantation. The HbA1c can also be checked. Since the published incidence and prevalence rates published in the literature are too variable to be helpful to individual transplant centres, it behooves every transplant centre to properly estimate its own incidence and prevalence rates, particularly in the early post-transplant period. Making an early diagnosis of NODAT is important because preventative measures can enhance the KTR's chances for a better quality of life and also prolong their graft survival^[12].

As with those at-risk for DM in other populations, lifestyle modification is likely to have a favorable impact on NODAT incidence. Recommendations for weight loss in obese patients prior to transplantation may be beneficial for preventing NODAT, but remains difficult

Table 3 Management strategies for new-onset diabetes after transplantation

Prevention strategies	Management strategies
Identification of risk factors (Table 1) with pre-transplant counseling	Regular blood glucose monitoring with appropriate follow-up
Pre- and post-transplant screening: random blood glucose, fasting blood glucose, 2-h oral glucose tolerance test with appropriate follow-up	Multi-disciplinary care
Lifestyle modification: weight control, diet, exercise (subject to dialysis-imposed restrictions)	Lifestyle modification: weight control, diet, exercise
Rapid corticosteroid reduction or avoidance	Rapid corticosteroid reduction
Selective calcineurin-inhibitor use (<i>e.g.</i> , cyclosporine instead of tacrolimus)	Conversion of cyclosporine to tacrolimus
? Newer immunosuppressive agents (<i>e.g.</i> , alemtuzumab, belatacept)	Oral hypoglycemic agents: metformin, sulfonylureas, meglitinides, dipeptidyl peptidase-4 antagonists (alone and/or in combination)
	Insulin
? Magnesium oxide	Monitoring for complications
? Statins	

?: Requires further investigation.

to enforce due to the predisposition for malnutrition in patients on dialysis. Nonetheless, safe and closely supervised dietary and exercise recommendations for dialysis patients should be encouraged, particularly in those identified to be at higher risk for NODAT. Prompt attention from allied health professionals such as nurse practitioners and dietitians may help prevent NODAT from being established when hyperglycemia is detected.

The role of pharmacotherapy in the prevention of NODAT has also been investigated. Since early post-transplant hypomagnesemia is common, magnesium oxide supplementation has been investigated and has been associated with a reduction in FBG^[69]. The use of statins in the post-transplant period has been associated with a reduced incidence of NODAT^[70]. However, rosuvastatin has not been associated with improved insulin sensitivity in non-diabetic KTR^[71], and so this pleiotropic effect of statins remains to be established^[72]. Although angiotensin-converting enzyme inhibitors have also been associated with reduced NODAT incidence^[70], their beneficial effect has not been demonstrated prospectively. Similarly, metformin has not been tested as a preventative strategy.

Since immunosuppressive medication remains the most readily available tool at the disposal of transplant clinicians to reduce the incidence of NODAT, much attention has been given to the reduction in exposure to existing immunosuppressive drugs such as corticosteroids and tacrolimus. Such reduction is often facilitated through the testing of newer pharmacological therapies. In a large, randomized controlled trial of corticosteroid withdrawal at 7 d post-transplant vs no withdrawal, a trend was noted towards better glycemic control in the withdrawal arm^[73]. However, there was no difference in NODAT rates^[73]. Despite this, fewer patients in the corticosteroid withdrawal arm required insulin for NODAT at 5 years^[73]. On the other hand, a large retrospective study involving more than 25000 KTR reported significant benefits of steroid avoidance at initial hospital discharge when compared to a steroid-containing regimen with respect to NODAT at three years^[74]. Corticosteroid withdrawal has also been shown to be beneficial when combined

with tacrolimus reduction^[75]. Other studies have shown that complete corticosteroid withdrawal has no additional benefit beyond only dose reduction^[40].

Although tacrolimus has a 50% greater association with NODAT than cyclosporine^[10], it is the preferred CNI in many transplant programs for other reasons such as graft function in large clinical trials^[44]. Reduced exposure to tacrolimus has also been associated with a similar incidence of NODAT to cyclosporine monitored using the 2-h instead of trough level^[76]. In the randomized DIRECT study^[77], which also used 2-h cyclosporine monitoring, a marginal increase in the composite safety endpoint of NODAT and IFG (33.6% vs 26.0%, $P = 0.046$) was noted with tacrolimus. Sometimes cyclosporine is switched to tacrolimus late after transplantation for reasons such as the development of other side effects, or rejection. In such instances, the risk of impaired glucose metabolism does not appear to be increased^[78]. In addition, either tacrolimus or cyclosporine may be switched to sirolimus in order to reduce the burden of CNI nephrotoxicity. However, conversion to sirolimus has been associated with worsening insulin sensitivity^[79]. The use of alemtuzumab, which is an anti-lymphocyte induction agent, has been associated with a reduced risk for NODAT^[80]. Belatacept, which is a recently introduced selective costimulation blocker in kidney transplantation, has been associated with a reduced incidence of NODAT compared to cyclosporine when studied as a prespecified endpoint in two Phase III studies^[81]. Both alemtuzumab and belatacept are induction therapies and so cannot be used for this purpose if a higher risk for NODAT is detected post-transplant.

Management of NODAT

Management strategies for NODAT are summarized in Table 3. Even if attempts to prevent NODAT are meticulous, it is likely that NODAT will occur in at least a proportion of KTR. The goals for management at this juncture include adequate glycemic control, perhaps with complete resolution of hyperglycemia without pharmacotherapy, and minimizing the short- and long-term complications of hyperglycemia. At our centre, non-fasting blood tests including glucose measurements

are obtained twice weekly for the first three months and weekly for the next three months. Fasting blood tests including glucose are obtained at least monthly. Our centre also employs an intensive multidisciplinary approach in the post-transplant clinic setting that includes a nurse practitioner with specialized expertise in diabetes prevention and management. This health care professional selectively reviews all clinic patients identified as having NODAT or being at high risk for NODAT. In addition, KTR have access to a dietician and pharmacist at all times in the clinic. Reading material about NODAT is also readily available. While such resource-intensive measures are probably helpful to individual patients, their overall effectiveness at a population level remains to be demonstrated. At other centres, adoption of a healthy diet and exercise program coupled with weight control strategies has demonstrated improvement in glycemic control over 6 mo^[82]. One challenge in this regard will be multi-ethnicity of KTR^[83] and possible language barriers. Collaboration between nephrologists and endocrinologists will help in the delivery of optimal care.

When antihypertensive medication is necessary for blood pressure control, it is reasonable to avoid or minimize the use of beta-blockers and thiazide diuretics in the absence of a compelling indication in those deemed at risk for NODAT. While clinical trials of antihypertensive agents in KTR are especially rare, there have also been no prospective studies of antihypertensive medications in KTR with NODAT as a pre-specified endpoint.

It is tempting for clinicians to alter transplant-related immunosuppression once NODAT has developed, with the view of optimizing glycemic control and perhaps avoiding the use of diabetes-specific medication. Reducing corticosteroid exposure carries an enhanced risk of transplant rejection^[84]. Switching from tacrolimus to cyclosporine once hyperglycemia or NODAT has developed is controversial. Cyclosporine possesses a side effect profile somewhat different from tacrolimus that includes hypertrichosis and gingival hyperplasia. Nonetheless, this conversion has been attempted as rescue therapy from NODAT in small studies, with demonstration that glycemic control can be improved^[85-87]. However, acute rejection remains a risk^[88]. Specific features related to NODAT in patients that can predict a successful conversion have not yet been identified.

If it is clear that lifestyle modification alone will be insufficient to control hyperglycemia, then pharmacotherapy targeting glucose metabolism should be initiated. In the very early post-transplant phase, when corticosteroids are being rapidly tapered, additional pharmacotherapy may not be required if the hyperglycemia is mild. The choice between insulin and oral hypoglycemic agents depends on the severity, timing, and expected duration of hyperglycemia^[49]. Insulin therapy is safe^[50] particularly when graft function is not yet established or is unstable. Since corticosteroids are typically administered in the morning in KTR, a combination of intermediate and short-acting insulin administered several

times during the day and corresponding to mealtimes may be required. In less urgent instances, oral hypoglycemic agents can be commenced without resorting to insulin firsthand.

The choice of oral hypoglycemic agent is dictated by the level of renal function. No agent is specifically contraindicated in KTR, and there are no significant drug interactions with CNIs or other immunosuppressive drugs. Pharmaceutical approaches generally mirror those utilized in the general population, with no studies specific to KTR available. In addition, the target HbA1c for KTR has not been defined^[49]. Metformin improves insulin sensitivity, which is often affected in NODAT. The safety of metformin in KTR with sufficient renal function has been formally demonstrated^[88]. Since the estimated GFR in KTR with well-functioning grafts often exceeds 50 mL/min per 1.73 m², metformin can be safely used for most patients. A theoretical disadvantage to using metformin in KTR would be gastrointestinal upset, to which KTR are already prone by virtue of immunosuppressive medications such as mycophenolate mofetil. However, metformin may counter the post-transplant weight gain associated with corticosteroid administration, but this has not been demonstrated. Keeping these in mind, metformin is often employed as a first-line agent in KTR with NODAT.

Sulfonylureas, which enhance insulin secretion, are also widely used in KTR. Their use is again dictated by level of renal function, and unlike metformin, they may exacerbate post-transplant weight gain. Newer sulfonylureas like glipizide and gliclazide may be used in KTR, with appropriate monitoring for hypoglycemia. Amongst the thiazolidinediones, which are selective agonists of the peroxisomal proliferator-activated receptor gamma, rosiglitazone has been shown to improve glucose tolerance, insulin sensitivity, and even endothelial function in a small group of KTR^[89]. Its short-term efficacy has also been shown by other groups^[90]. Thiazolidinediones should again be used with extreme caution if graft function is severely impaired. Congestive heart failure is a significant concern, and many KTR already have impaired cardiac function. Furthermore, cyclosporine may promote a sodium-retentive state. As a result, thiazolidinediones are usually not used.

Amongst the meglitinides, which are short-acting drugs that induce insulin secretion, repaglinide has been shown to be safe in KTR^[91]. Repaglinide can also be used in severe renal graft dysfunction. Dipeptidyl peptidase-4 (DPP-4) antagonists have been employed in KTR. These drugs inhibit DPP-4, which is responsible for the rapid degradation of numerous substrates including the glucagon-like peptide 1, whose role is to increase pancreatic insulin secretion. Among the DPP-4 antagonists, sitagliptin has been shown to be both safe and efficacious in KTR^[92,93]. A theoretical risk in KTR is an increased risk of infections, to which they may already be prone by virtue of being in an immunosuppressed state.

It is reasonable to employ rationally selected

combinations of two or three of the above drugs before proceeding to insulin therapy. However, the initiation of insulin should not be delayed unnecessarily, since prolonged hyperglycemia may result in hospitalization from intravascular volume depletion and serious comorbidities such as infections. Opportunistic infections of many kinds typically occur in the first 6 mo post-transplant, which corresponds to the phase when NODAT usually occurs.

FUTURE DIRECTIONS

Renal transplant survival has significantly improved over the last 50 years. Death with graft function, often as a result of CVD, has become a major cause of graft loss. As a result, increasing attention is being given to cardiovascular risk factors such as DM, within which NODAT is a significant component. Intensive screening for NODAT should be the norm in all transplant centres. Efforts to combat NODAT have to be balanced against the risks for graft rejection. Large clinical trials of newer immunosuppressive agents in kidney transplantation are few, but the inclusion of NODAT as a prespecified endpoint will help to better understand not only the most important contributing risks for NODAT, but also identify those interventions that are most likely to result in a lower NODAT incidence. Correspondingly, clinical trials that address treatment strategies for diabetes post-transplant have received very little attention. This is indeed unfortunate given the magnitude of NODAT prevalence and its financial implications for society, not to mention the profound impact of NODAT on transplant recipients. Until such trials are performed, multidisciplinary care focused on intensive glucose control in keeping with general population guidelines, along with management of other cardiovascular risk factors should remain the standard.

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Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus

Surapon Tangvarasittichai

Surapon Tangvarasittichai, Chronic Disease Research Unit, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

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Correspondence to: Dr. Surapon Tangvarasittichai, Associate Professor, Chronic Disease Research Unit, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, 99 Moo 9 Tambon Tha Pho, Muang, Phitsanulok 65000, Thailand. surapon14t@yahoo.com

Telephone: +66-08-96388382

Fax: +66-08-55966300

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insulin resistance, dyslipidemia, β -cell dysfunction, impaired glucose tolerance and ultimately leading to T2DM. Chronic oxidative stress, hyperglycemia and dyslipidemia are particularly dangerous for β -cells from lowest levels of antioxidant, have high oxidative energy requirements, decrease the gene expression of key β -cell genes and induce cell death. If β -cell functioning is impaired, it results in an under production of insulin, impairs glucose stimulated insulin secretion, fasting hyperglycemia and eventually the development of T2DM.

Key words: Insulin resistance; Dyslipidemia; Type 2 diabetes mellitus; Oxidative stress

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Core tip: Oxidative stress is underling in the development of cardiovascular disease, type 2 diabetes mellitus (T2DM) and diabetic complications. Increased oxidative stress appears to be a deleterious factor leading to insulin resistance, dyslipidemia, β -cell dysfunction, impaired glucose tolerance and ultimately leading to T2DM.

Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 2015; 6(3): 456-480 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i3/456.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i3.456>

Abstract

Oxidative stress is increased in metabolic syndrome and type 2 diabetes mellitus (T2DM) and this appears to underlie the development of cardiovascular disease, T2DM and diabetic complications. Increased oxidative stress appears to be a deleterious factor leading to

INTRODUCTION

Aerobic life uses oxygen to oxidize (metabolism) food substrates (carbon- and hydrogen-rich) to obtain the heat energy and chemical essential for life. When we oxidize molecules with oxygen, the oxygen molecule itself becomes reduced and forms intermediates.

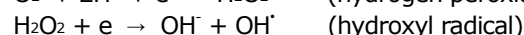
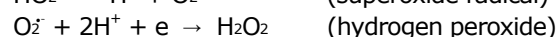
In eukaryotic cells, reactive oxygen species (ROS) are always produced as the consequence of regular physiological metabolism^[1]. These ROS (pro-oxidants) productions are counter-balanced by cellular antioxidant defense mechanisms in the normal physiological conditions. ROS define as diverse chemical that have reactive properties are capable to accommodate or donate electrons (e-) to the broad range of biological molecules. Normally, the production and neutralization of ROS are balance with antioxidants in a living system and does not cause any oxidative damage, determines as physiological state^[2]. The imbalance between these prooxidants and antioxidants in the living organism system to determine as oxidative stress state, brings to cellular disruption and damage^[3]. The free-radical can attack polyunsaturated fatty acids oxidation in physiological systems known as lipid peroxidation. Lipid peroxidation is an autocatalytic free radical mediated destructive process whereby poly-unsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides^[4,5]. By-products of lipid peroxidation such as conjugated dienes and malondialdehyde (MDA) are increased in the patients with obesity, metabolic syndrome and type 2 diabetes mellitus (T2DM). Carbohydrates, lipids, proteins and DNA are the targets of oxidative stress modification biomolecules generally as the principal of ROS induced cellular damage. Therefore, these ROS modified biomolecules are used as oxidative stress markers both *in vivo* and *in vitro* measurement. Recent study suggests that ROS may act as the mechanical link of salt sensitive hypertension, over nutrition and high fat diet, metabolic syndrome and T2DM animal models^[6]. ROS levels are increased in obesity, especially in abdominal obesity which is the major component of metabolic syndrome and it can be reduced by weight loss^[7]. Many studies demonstrated that increased oxidative stress is associated with insulin resistance pathogenesis by insulin signals inhibition and adipokines dysregulation^[8,9]. In animal studies, oxidative stress enhances insulin resistance. The evidence suggested that angiotensin II (Ang II) infused rats required the increased glucose load to maintain normal glucose levels during hyperinsulinemic clamp to stimulate ROS production^[10]. Thus, ROS may also contribute and accelerate the insulin resistance development in insulin-targeted organs of the over nutrition and the excess salt individuals.

In the large general population studies demonstrated that insulin resistance is multifactorial^[11,12] and the genetic component^[11,13,14]. Insulin resistance most often precedes in many years before the onset of T2DM. Insulin resistance and the consequence of declined of insulin secretion are the principle of the T2DM pathogenesis^[11,12,15,16]. The late complications of diabetes have been associated and implicated in their etiology with oxidative stress^[17-19]. The influence of oxidative stress on insulin resistance, dyslipidemia, abnormal lipoprotein production and the pathophysiology of T2DM by using *in vivo*, *in vitro* and animal models data on these effects

were also included in this review.

ROS

Oxygen exists in air known as oxygen molecule (O₂) or dioxygen. Oxygen on the surface of earth appeared in significant amounts approximately 2.5 × 10⁹ years ago. It was created by the photosynthetic activity of plants and microorganisms (blue green algae). Increased atmospheric oxygen concentration was followed by the ozone layer formation in the stratosphere. Both oxygen and ozone layer were filters against the solar ultraviolet radiation reaching surface of the Earth. In eukaryotic cells, ROS is produced as the consequence of the normal aerobic physiological metabolism^[1]. These ROS levels are counter-balanced with the cellular antioxidants in the normal physiological conditions. ROS define as diverse chemical that have reactive properties are capable to accommodate or donate electrons (e-) to the broad range of biological molecules. These species include instability radicals arise from an unpaired e-. Existence of the presence of oxygen and the aerobic organisms on the earth is possible^[20].



However, these molecules are also played an adverse role in the biological systems as oxidative stress. At the steady state of the living systems, oxygen metabolism always produce oxygen-derived free radicals such as superoxide O₂^{•-}, hydroxyl OH[•], alkoxyl RO[•], peroxy RO₂[•], peroxyxynitrite ONOO[•] and oxygen-derived non-radicals such as hydrogen peroxide H₂O₂, hypochlorous acid HOCl and hypobromous acid HOBr. Both free radicals and non-radicals groups are the important factors of the oxidative stress mediated cellular damages^[21]. Normally, the neutralization of ROS productions by cellular antioxidant defense mechanisms are determine as the physiological state and do not cause any oxidative damage^[2]. The imbalance of the ROS production and antioxidants defense system in the living systems caused oxidative stress brings to cellular function disruption and damage^[3].

This imbalance occurs due to over production of ROS and reduction of the antioxidant defense mechanisms. The electron transport chain in mitochondrial, peroxisomes and cytochrome P450 system are the most important sources of ROS production (involves in O₂^{•-} production)^[22]. Moreover, various enzymes can be accelerated ROS production such as cyclooxygenases^[23,24], xanthine oxidase^[25], uncoupled nitric oxide synthases (NOS)^[26-28] and NADPH oxidases^[29]. Drugs such as doxorubicin^[30,31], cisplatin, acetaminophen^[32-34] and nimesulide^[35]. Heavy metals (Fe, Cd, Pb, Hg) as the toxic substances^[36-39], acrolein, chloroform, carbon tetrachloride^[40], tertiary butyl hydroperoxide^[41-44], environmental pollutants (oxides of

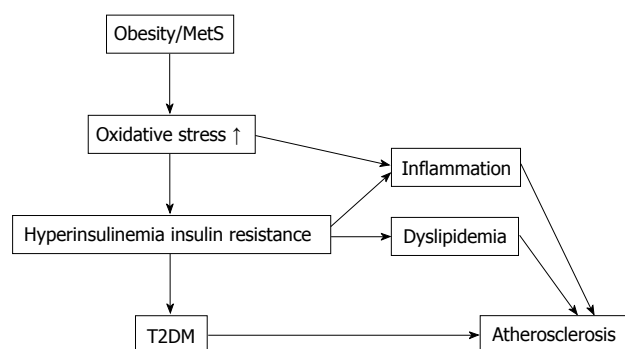


Figure 1 Summarized of obesity and metabolic syndrome elevate in oxidative stress. T2DM: Type 2 diabetes mellitus; MetS: Metabolic syndrome.

nitrogen, SO_2 , CO_2), xenobiotics, UV irradiation and the other factors induce ROS overproduction.

In metabolic disorders assist the increased ROS production in the physiological system such as obesity, insulin resistance and diabetes mellitus^[45-48]. In Figure 1 summarized of obesity and metabolic syndrome elevate in oxidative stress. Superoxide radical ($\text{O}_2^{\cdot-}$), hydroxyl radical (OH^{\cdot}) and hydrogen peroxide (H_2O_2) are the three major ROS in physiological organisms^[49]. Superoxide radical ($\text{O}_2^{\cdot-}$) acts as the parent ROS molecules caused from the one electron reduction of oxygen molecule by electron transport chain enzymes in mitochondrial such as enzymes in cytochrome P450, cyclooxygenase and NADPH oxidase. Various reactions of enzymes and non-enzymes system further convert these ROS molecules to hydroxyl radical (OH^{\cdot}), peroxyxynitrite ion (ONOO^{\cdot}) and hyperchlorous acid (HOCl). For example superoxide dismutase converts $\text{O}_2^{\cdot-}$ to H_2O_2 by the dismutase reaction^[50,51].

Elevated ROS molecules caused the cellular macromolecules damage such as lipids^[52], proteins^[53] and nucleic acids^[54]. In the anti-oxidants system of the living system, possess own antioxidant defense mechanisms^[55] includes enzymes and non-enzyme molecules such as SOD, catalase (CAT) and glutathione peroxidases (GPx). Enzyme SOD catalyzes $\text{O}_2^{\cdot-}$ conversion to H_2O_2 , while CAT converts H_2O_2 to H_2O and O_2 . For reduction of two peroxide molecules use non-enzymatic glutathione (GSH; reduced and oxidized forms), reduced glutathione (GSH) and GPx catalyze to produce oxidized glutathione (GSSG) and water^[56]. Various enzymes play the important combination roles in the series of antioxidant defense systems such as glutathione reductase, glutathione S-transferase, and glutathione disulfide (GSSG).

ROS production is identified as endogenous and exogenous source. UV exposure and xenobiotic agents has been shown to generate these ROS^[57]. In fact, dietary is the major source of these oxidant compounds, especially in animal fat as the source of high lipid peroxides^[58]. ROS may also be derived from the general biochemical reactions in living organism to generate ROS as by-products or end products. In the transition heavy metals such as iron (Fe^{2+}) and copper (Cu^+) are pose the oxidative stress production, especially in

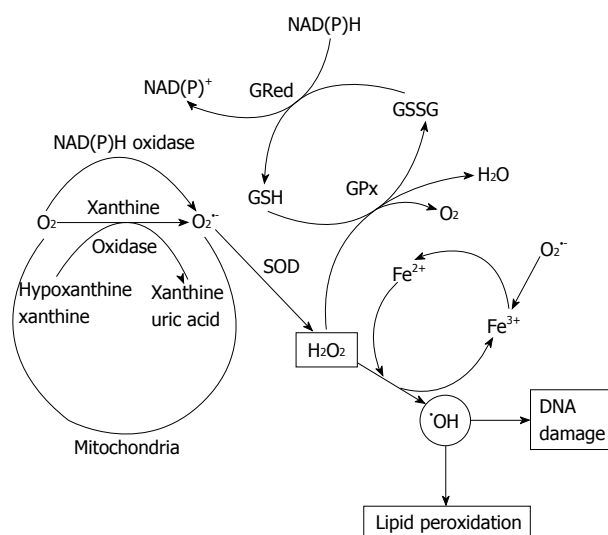
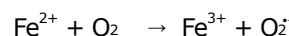


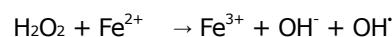
Figure 2 Increased oxidative stress by xanthine oxidase. NADH: Nicotinamide adenine dinucleotide.

Fe^{2+} may cause autooxidation to cause $\text{O}_2^{\cdot-}$ generation and/or interaction with H_2O_2 can generate OH^{\cdot} via the Fenton and Harber Weiss reactions^[59]. Fenton chemical reaction may also causes lipid peroxides generation and propagation^[60].

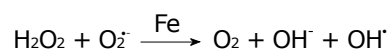
Auto oxidation of Fe^{2+} :



Fenton reaction:



Harber-Weiss reaction:



The major cellular oxidative stress is come from mitochondrial respiration. Heart, brain, kidney, liver and skeletal muscle are the effective oxygen consumption organs is converted oxygen to $\text{O}_2^{\cdot-}$, approximate releasing 0.1%-0.2% while the liver is converted oxygen to $\text{O}_2^{\cdot-}$, approximately releasing 2%^[61]. Electron transport chain complex of the mitochondrial has been sourced to $\text{O}_2^{\cdot-}$ generation and have been estimated upto 107 ROS molecules per mitochondria per day^[62].

In the enzymatic systems of xanthine oxidase generated via xanthine dehydrogenase, which utilize oxygen molecule as e- acceptor during catabolism of xanthine. Xanthine oxidase is the generator of $\text{O}_2^{\cdot-}$, H_2O_2 ^[63] and OH^{\cdot} producer^[64], highly expressed in epithelial, injured and diseased tissues as shown in Figure 2. Xanthine oxidase has been involved to peroxyxynitrite (ONOO^{\cdot}) and nitric oxide (NO) productions through nitrite reduction^[65,66]. Intracellular nitric oxide synthases (NOS) catalyze L-arginine to form citrulline and NO. Endothelial NOS and neuronal NOS are activated by calcium-induced calmodulin binding to produce NO levels^[67]. Inducible NOS (iNOS) has also calmodulin bound molecule. It may rapid and chronic expression in many cell types such as smooth muscle cells, hepatocytes and macrophages. iNOS is induced by the many inflammatory cytokines

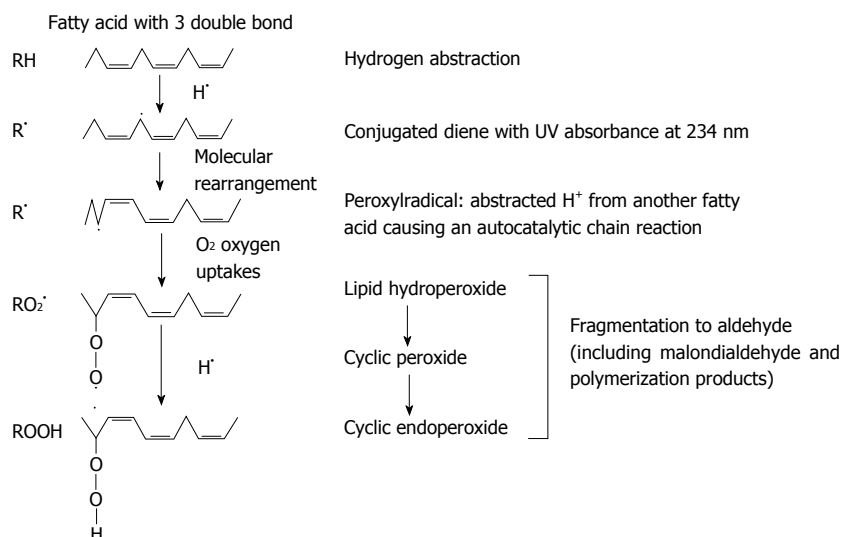


Figure 3 The chain reaction of lipid peroxidation.

[tumor necrosis factor- α (TNF- α), interleukin-6 and growth factors] regulation at the transcriptional level, results in micromolar NO production^[67]. INOS can produce O₂⁻ and OONO⁻ when lower in L-arginine substrate^[68].

LIPID PEROXIDATION

Fats and oils oxidized with characteristic changes in texture, color, taste and odor. This process, known as rancidity, was chemically defined in the 1940s as an autooxidative free-radical chain reaction^[69]. The most powerful oxidant formed in biological systems is hydroxyl radical. It can attack any biological molecule. The initiation step of lipid peroxidation occurred when hydroxyl radicals attack to polyunsaturated fatty acids, to cause the free-radical polyunsaturated fatty acids oxidation in biological systems. Lipid peroxidation is autocatalytic lipid hydroperoxides radical production mediated poly-unsaturated fatty acids in cell membranes destruction and degradation process^[4,5]. Conjugated dienes and MDA, by-products of lipid peroxidation are increased in the circulation of obesity, metabolic syndrome and T2DM patients.

First-peroxidation chain initiation, results from the attack by any species to reduce a hydrogen atom from methylene (-CH₂-) group of polyunsaturated fatty acid or membrane. Because one hydrogen atom contains one electron, reduction leaves an unpaired electron on the carbon of -CH-, double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the other double bond and facilitates its removal. Then, the polyunsaturated fatty acid chains in lipids membrane are sensitive to cause lipid peroxidation. The carbon-centered radical forms a conjugated diene by the molecular rearrangement (Figure 3), which combines with oxygen to form a peroxy radical that able to reduce a hydrogen atom from another fatty acid to start a chain reaction. Peroxidation continues to use up the polyunsaturated fatty acid substrate unless

the chain-breaking antioxidant (vitamin E) agent is added to terminate the chain reaction. The three stages of lipid peroxidation are initiation, propagation and termination. Hydroxyl radical (•OH), alkoxyl radical (RO•), peroxy radical (ROO•), and HO₂• species can abstract the first hydrogen atom of polyunsaturated fatty acid but not H₂O₂ or O₂⁻^[70]. Variety of lipid hydroperoxides and cyclic peroxides are the end products of the chain reaction. Lipid peroxides are stable molecules in the physiological temperatures. Lipid peroxides decomposition is catalyzed by transition heavy metals. For example, iron ion-active complexes present in circulating can participate in the Fenton reaction to promote lipid peroxide decomposition. Hemoglobin and the cytochromes molecules can also facilitate peroxide decomposition, although they do not directly catalyze Fenton chemistry. However, heme proteins can release chelatable iron that can participate in Fenton chemistry^[71]. Ferritin and hemosiderin are effective at stimulating lipid peroxidation and catalase is weakly effective, caused problems to use catalase as a probe for H₂O₂ in lipid peroxidation systems^[72].

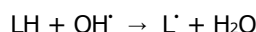
Reduced heavy metal [Fe²⁺, Cu⁺] react with lipid peroxides (LOOH) to alkoxyl radical or Cu⁺ react with LOOH to alkoxyl radical.



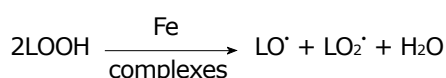
In the reaction oxidized-heavy metals [Fe³⁺, Cu²⁺] slowly react with LOOH to produce alkoxyl and peroxy radicals. Both peroxy and alkoxyl radicals initiate the chain reaction by reducing hydrogen atoms (Figure 3). The fixed oxidation metals ions can affect the rate of lipid peroxidation (Ca²⁺, Pb²⁺ and Al³⁺ ions). Lipid peroxidation accelerates by the iron salts stimulation result in the membrane structure changes and important implications for environmental toxicology^[73].

Rawls *et al*^[74] demonstrated that singlet O₂^{*} is formed during the lipid peroxidation degradation and might contribute to cause more initiation in the chain

reaction. Initiation in the first-chain initiation should be used as lipid peroxide decomposition reactions to start the new chain reaction. Iron ions and ferrous ions are free radicals^[55], can act in electron transfer reactions with oxygen molecule. Then, the presence of iron ions can promote the hydroxyl radicals formation by Fenton reaction. Bielski *et al*^[75] demonstrated that the $\cdot\text{OH}$ radical production in any source can initiate lipid peroxidation reaction.



Superoxide-dependent Fenton reaction (superoxide resulting H_2O_2 and reducing Fe^{3+} to Fe^{2+}) did not demonstrate any substantial involvement of the hydroxyl radical in liposomal peroxidation systems as detected by the scavengers action^[76]. Hydroxyl radicals in the systems can be measured by spin trapping^[77] or deoxyribose degradation measurements^[76] but do not contribute to the lipid peroxidation rate^[76]. The addition of iron ion in any preparations can stimulate peroxidation reaction by lipid hydroperoxide degradation to generate peroxy (LO_2^\cdot) and alkoxy (LO^\cdot) radicals.



The rate constant of the reaction when ferrous ions are reacted as 1.5×10^3 /mol/L per second^[78], which is higher than the rate reaction constant of ferrous ions with H_2O_2 reaction (76 /mol/L per second)^[79]. The iron ions stimulate lipid peroxidation by the lipid degradation reactions from the present of abundant hydroperoxide.

Iron or copper in a biological system attach to biological molecules at the specific location of OH radicals formation to cause lipid, protein and DNA damage. On lipid membrane, the propagation step of lipid peroxidation reactions does not proceed further until the reaction reach the protein portion. Thus, lipid peroxidation *in vivo* causes proteins membrane damage^[80,81]. This damage has more biologically important than those lipids membrane damage. Cells also contain mechanisms for recognizing and removing oxidative modified proteins^[80,81].

OXIDATIVE STRESS

Oxidative stress occurs at the molecular level as the cellular event when increased ROS overwhelm the antioxidant defense capabilities systems. Oxidative stress was defines as the increasing ROS production, vary in intensities, the different cellular locations and may be occurred either acutely or chronically^[82]. Oxidative damage to macromolecules including carbohydrates, proteins, lipids and DNA typically viewed as increased ROS induced cellular damage to cause the irreversible macromolecules modifications. Therefore, the by-products of these oxidative modified biomolecules are used as oxidative stress biomarkers *in vivo* and *in vitro*. Many research studies demonstrated the association of oxidative stress and the pathogenesis

of insulin resistance *via* insulin signals inhibition and adipocytokines dysregulation^[8,9]. Oxidative stress biomarkers included MDA^[83], 4-hydroxy-2-nonenal and isoprostanes species^[84], protein carbonyls, 3-nitrotyrosine, hydroperoxides, protein oxidation products^[85], glycation end products, carbohydrate modifications^[86] and 8-hydroxy-2'-deoxyguanosine (8-OH-dG), an oxidized DNA product^[84].

Assaying lipid peroxidation

The lipid peroxidation contributes to the pathogenesis of atherosclerosis. It is occurred in the blood vessel walls and does not occur from low density lipoproteins (LDL) in circulation^[87,88]. LDL can enter to the blood vessel walls. The modified LDL (oxidized LDL) may escape from the scavenger recognition receptors and back to the circulation. Therefore, this circulating LDL peroxidation is a potentially useful biomarker of lipid peroxidation in circulation. Indeed, this assay is used for the demonstration of *in vivo* antioxidants inhibit the effects of lipid peroxidation^[89,90].

Thiobarbituric acid-reactive substance

MDA from the oxidative polyunsaturated fatty acids (PUFA) degradation is determined by the reaction of thiobarbituric acid (TBA) with MDA to generate the stable end product of MDA-TBA adduct^[91-95]. This MDA free radical has been demonstrated as a causative of the atherosclerosis pathogenesis^[96,97], aging^[98], cancer^[99] and Alzheimer's disease^[100,101]. Serum MDA levels have been used as the lipid peroxidation biomarker and indicator of free radical damage^[37,83,102]. MDA, the three-carbon dialdehyde, can exist in many forms in the aqueous circulation. This method was used the reaction of MDA with TBA and heated under acidic conditions but the TBA can react with many chemical species such as proteins, phospholipids, aldehydes, amino acid and nucleic acids^[103,104]. One MDA molecule reacts with TBA two molecules to form a stable pink to red chromophore that absorbs maximally at 532 nm^[105] or fluorescence detection. This chromophore is termed thiobarbituric acid reacting substances. Elevated MDA levels in T2DM patients are associated with cardiovascular disease risk^[83].

Isoprostanes

The most valuable of lipid peroxidation biomarker in the biological system is the isoprostanes, elevated from the PUFA peroxidation^[106-113]. Isoprostanes identified as free form and the most are esterified to lipids in circulation. Isoprostanes can be analyzed by mass spectrometry techniques, so that can easily be detected in human body fluids^[108,109,112,113]. Isoprostanes appear to turn over rapidly in metabolized and excreted^[108,109]. Isoprostanes and their metabolites detection in urine may be the useful biomarker for lipid peroxidation^[113]. Isoprostanes assay have focused on the F₂-isoprostanes measurement, which elevate from the arachidonic acid peroxidation^[109]. Elevation of F₂-isoprostanes levels have

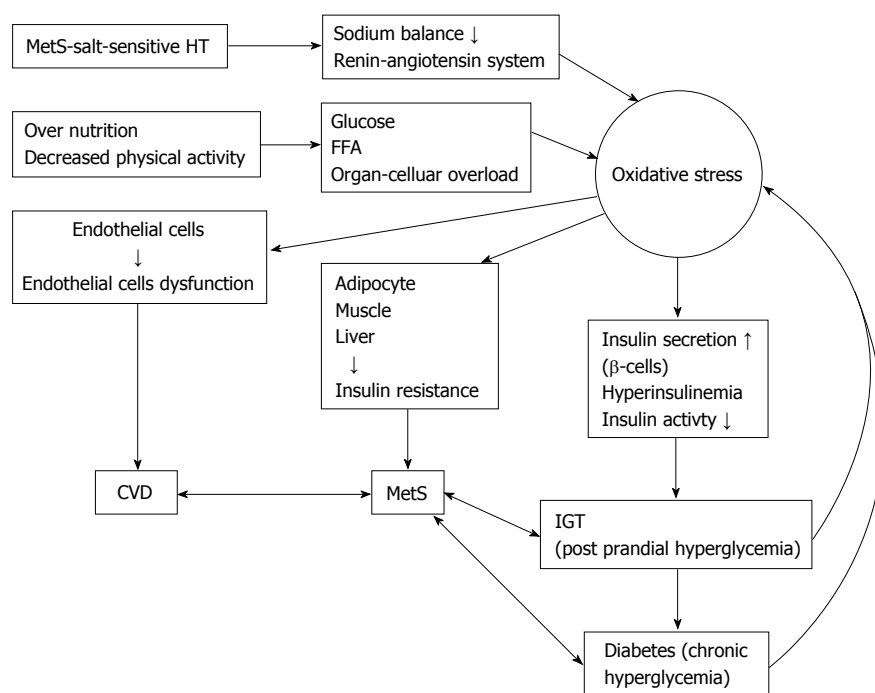


Figure 4 Summarized the increasing reactive oxygen species in obesity, metabolic syndrome and salt sensitive hypertension. FFA: Free fatty acid; MetS: Metabolic syndrome; HT: Hypertension; IGT: Impaired glucose tolerance.

been shown in conditions of the cardiovascular disease, diabetes development^[114,115], cigarette smoking^[111,116,117], hyperhomocysteinaemia^[118] and hypercholesterolaemia^[110,119]. F₂-isoprostane levels have also been shown to decrease by antioxidants supplementation both in animal models and humans subjects^[120-124].

Oxidative stress in metabolic syndrome

The components of metabolic syndrome consist with abdominal obesity, dyslipidemia, hypertension and diabetes^[125,126]. It is the major modern lifestyle complication cause from physical inactivity and overeating and associated with the increased risk of cardiovascular diseases, hypertension and T2DM that summarized in Figure 4.

Over nutrition and oxidative stress: In metabolism of glucose through glycolysis and tricarboxylic acid (TCA) cycle to generate nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂) as the electron donors. In over nutrition, the excessive glucose occur and a large amount of glucose is oxidized in the glycolysis and TCA cycle to increase NADH and FADH₂ generation in electron transport chain of mitochondrial and increased superoxide generation^[127]. The excessive of free fatty acids (FFAs) leads to increase FFA-oxidation and acetyl coenzyme A (CoA) oxidation in TCA cycle generate the NADH and FADH₂ electron donors as glucose oxidation results in mitochondrial ROS overproduction^[127]. Furthermore, NADPH oxidase in the plasma membrane can convert oxygen molecule to superoxide radical and involve in ROS nutrient-based generation. In adipocytes, ROS is generated by in fused

with FFAs, treatment with NADPH oxidase inhibitor can block this ROS generation. This indicates that NADPH oxidase involves in fatty acids ROS generation^[8]. Palmitate can activate diacylglycerol synthesis and protein kinase C (PKC) leading to activate NADPH oxidase^[128]. Thus, over accumulated fat result in the increased fatty acids oxidation and lead to activate NADPH oxidase (in local or remotely cells) to cause ROS over production in over nutrition or obesity (Figure 4). Conversely, calorie restriction may be associated with normal physiological system^[129] and may involve in normal cellular redox state^[130]. In aged animals models treated with antioxidant agents or hypocaloric diets led to ameliorate in oxidative stress status and tissue function^[131,132]. Treatment with resveratrol, a polyphenol reduced atherosclerosis and diabetes development^[133]. These studies demonstrate that nutrition is associated with increased or decreased redox status and over nutrition result to increase oxidative stress to contribute pathogenesis of atherosclerosis, cancer and other diseases.

Oxidative stress in adipose tissue: Increased fat accumulation in human has been associated with oxidative stress biomarkers^[134]. Similarly, obese mice were significantly higher oxidative stress levels in circulation^[8]. Moreover, lipid peroxidation and H₂O₂ levels were increased in adipose tissue^[8]. These mean that adipose tissue may the major source of ROS production and can be released to the circulation potentially affecting various distance organs functions and damage (Figure 4).

Increased NADPH oxidase expression in adipose

tissue associated with increased oxidative stress levels. Increased mRNA expression was found in adipose tissue of obese mice^[8]. Increased ROS generation in lipid accumulation and further elevating ROS generation with FFA treatment were found in 3T3-L1 adipocytes cultured^[8]. These ROS generation processes can be blocked by NADPH oxidase inhibitors, apocynin or diphenyleneiodonium. Many studies suggest that NADPH oxidase induces adipocytes ROS production^[8]. Moreover, obese mice ameliorated hyperinsulinemia, hypertriglyceridemia, hyperglycemia and hepatic steatosis by supplementation with apocynin^[8]. These data demonstrate that NADPH oxidase increase ROS production in obesity and metabolic syndrome may play the important roles in the atherosclerosis, T2DM and cancer pathogenesis. Adipose tissue tries to increase antioxidant enzymes levels to against ROS over production. However, these antioxidant enzymes activity and expression are decreased in adipose tissue^[8,135-137]. Then, increased ROS-production enzymes and decreased antioxidant enzymes may cause oxidative stress in obese and metabolic syndrome.

Oxidative stress and salt-sensitive hypertension:

As in mention above, ROS levels are increased in obesity and can be ameliorated by weight loss^[7]. Obese rats induced by refined sugar or high fat diet leading to ROS overproduction and increase oxidative stress^[6,138]. Many research evidences suggest that metabolic syndrome was associated with the salt-sensitive hypertension. ROS play the roles as mechanical link of metabolic syndrome and salt-sensitive hypertension^[125,126], which itself leads to ROS overproduction^[139-142]. Salt restriction in hypertensive obesity was more effective reduction in blood pressure than in hypertensive non-obesity patients, and weight loss in obesity and salt sensitive hypertensive patients caused the successful of blood pressure reduction^[143]. Salt-sensitive hypertensive patients were significantly more prevalent in metabolic syndrome patients than without metabolic syndrome^[144]. Oxidative stress in abdominal adipocytes due to increase adipocytokines secretion such as TNF- α , angiotensinogen, non-esterified fatty acids^[126]. Interestingly, infused Ang II -rats disturbed sodium balance to cause ROS overproduction in salt-sensitive rats^[139-141]. Moreover, in salt-sensitive hypertensive patients are also increased 8-isoprostane levels^[142]. Thus, ROS may the underling pathogenesis of diseases in metabolic syndrome, obese and non-obese intake excessive salt as the salt-sensitive hypertensive patients.

In high-renin patients (non-modulating salt sensitive hypertension) had elevated the homeostasis model assessment of insulin resistance (HOMA-IR) levels^[145]. In salt-sensitive hypertensive non-obesity patients had significantly lower insulin sensitivity than in non-salt-sensitive hypertensive patients^[146]. Insulin resistance caused salt-sensitive hypertensive obesity and/or metabolic syndrome patients^[125]. Increased renal ROS overproduction may increase the salt sensitive

hypertension^[147]. Then, increased renal oxidative stress may contribute to cause salt-sensitive hypertension development. Moreover, ROS overproduction in vascular endothelial cells suppresses the NO-dependent vasodilation^[148] and may play the role in the salt-sensitive hypertension development.

Oxidative stress in type 2 diabetes

Many research studies demonstrated that T2DM patients have increased ROS production-induced higher oxidative damage in the circulation and also have reduced antioxidant defenses mechanisms^[149-152]. Increased ROS production in T2DM patients is thought to activate many detrimental pathways including hexosamine pathways, advanced glycation end-products (AGEs) formation, and PKC β 1/2^[127]. Hyperglycemia condition can induce oxidative stress by several mechanisms such as glucose autooxidation, polyol pathway, AGE formation and PKC β 1/2 kinase. Elevated free fatty acids, leptin and other circulating factors in T2DM patients may also contribute to cause ROS overproduction. Figure 5 demonstrates the association of increased ROS production with atherosclerosis and sources of ROS generations in T2DM patients.

Glucose autooxidation

Hyperglycemia due to cause increased glucose metabolism leading to increase NADH and FADH₂ overproduction, which are used by the electron transport chain of mitochondria to generate ATP^[153]. NADH overproduction can cause the higher proton gradient production in mitochondria. These electrons are transferred to oxygen to produce higher superoxide^[154]. The NADH dehydrogenase of the complex I ubiquinone oxidoreductase and complex III cytochrome c reductase are the two main site of superoxide production *via* the electron transport chain^[155].

The polyol pathway

Oxidative stress increased in circulation of T2DM patients from the polyol pathway. ROS was generated by two enzymes: (1) Aldose reductase in the reaction use NADPH to change glucose to sorbitol. Sorbitol production is a minor reaction in normal physiological conditions. However, 30%-35% of glucose in T2DM conditions is metabolized by polyol pathway^[156]. In the condition of sorbitol overproduction, the availability of NADPH is reduced this reflect to reduce glutathione regeneration and NOS synthase activity to cause increased oxidative stress^[153]; and (2) Sorbitol dehydrogenase in the second step oxidizes sorbitol to fructose concomitant with NADH overproduction. Increased NADH may be used by NADH oxidases to increase superoxide production^[157] include in mitochondrial over superoxide production.

PKC β 1/2

Many structures and biochemical components changed in the circulation of T2DM patients were caused from PKC β 1/2 activation *via* diacylglycerol leading to cause dysfunction in endothelial contractility and permeability,

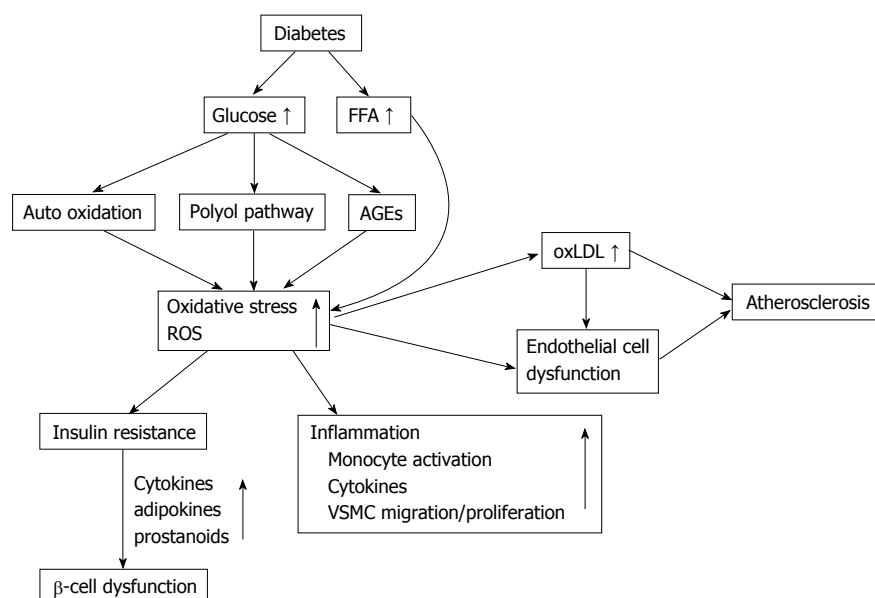


Figure 5 Summarizes the reactive oxygen species associations with atherosclerosis and sources of reactive oxygen species production in type 2 diabetes. oxLDL: Oxidized low density lipoprotein; FFA: Free fatty acids; AGEs: Advanced glycation end-products; VSMC: Vascular smooth muscle cells; ROS: Reactive oxygen species.

hemodynamics (retinal blood flow) changes, extra-cellular matrix protein synthesis, VEGF production and intracellular signaling in the vascular^[128,158,159].

Non-enzymatic glycation

Glycation end-product is the binding of ketone or aldehyde groups of glucose with the free amino groups of proteins leading Schiff bases formation without enzymes, then to form the Amadori product and rearrangements of the structure to the irreversible AGEs in the final^[160,161]. AGEs has been demonstrated in atherosclerotic lesions and their tissue of T2DM patients and increased AGEs levels associated with severity of the diseases^[162]. Moreover, binding of AGEs to specific cell surface receptor for AGE can activate intracellular redox signaling and subsequent to activate the expression of redox-sensitive transcription factors and inflammatory mediator^[163-165].

Inflammation

Oxidative stress is the major factor underlying in the CVD, insulin resistance and T2DM pathogenesis. These may explain by the presence of the inflammation conditions. Now, inflammation recognized as the one manifestation of oxidative stress^[166] and can be generate the inflammatory mediators including adhesion molecules and interleukins to induce oxidative stress^[166]. The concept of atherosclerosis is an inflammatory disease now well established. This chronic inflammation may be involved in the insulin resistance and T2DM pathogenesis^[167]. Recent clinical research indicates that sub-clinical inflammation may impact in the development and progression of diabetic complications^[165,168]. Moreover, excessive FFA and glucose induce inflammation effect through oxidative stress and reduced antioxidants^[169]. Interestingly, the subclinical pro-inflammatory state

observed in many pathogenesis conditions such as atherosclerosis, aging, T2DM and cancer, is caused from mitochondrial ROS overgeneration^[170].

Other sources of oxidative stress in diabetes

Non-esterified FFAs are elevated in T2DM patients^[171]. These excessive FFAs enter the citric acid cycle to generate acetyl-CoA to receive NADH overproduction to cause mitochondrial superoxide over production. In humans, infused FFA has been shown increased lipid peroxidation by elevated isoprostanes marker levels^[172,173]. Adipocytokine, leptin is secreted from the adipocytes to act on the central nervous system to decrease food intake. It reflects all effects on the vascular smooth muscle cells, endothelial cells, macrophages and monocytes^[174]. Leptin levels are increased and associated with cardiovascular disease in T2DM patients^[175-177]. In culture of endothelial cells incubated with leptin to cause ROS production^[178,179].

Antioxidants

Regulation of the cellular redox status is depends on the rate of ROS counterbalance and elimination from the enzymatic and/or non-enzymatic antioxidants. Superoxide is converted by SOD to H₂O₂ and O₂ molecule. There are 3 isoforms of SOD such as cytosolic Cu/Zn SOD (SOD1), mitochondrial Mn-SOD (SOD2) and extracellular SOD (SOD3). Catalase, the heme metalloenzyme is expressed in peroxisomes, mitochondria, cytoplasm and nucleus. H₂O₂ is catalyzed by catalase to oxygen and water^[180]. While glutathione peroxidase the selenoprotein, was found in both intracellular and extracellular. Glutathione peroxidase has a highly sensitive function for lipid peroxides degradation, converses H₂O₂ to water by using the thiol group of glutathione^[181]. Their H₂O₂

detoxification plays the important roles to prevent lipid peroxidation production and regulation of the cellular redox status^[182]. The glutathione system, thioredoxin peroxidase is key enzyme to regulate the cellular levels of thiol/disulfide while the production of antioxidant enzymes is regulated by the redox-cellular transcription factors^[183]. For example, the expressed transcription factor NF-E2 related factor in the cytosolic is interrupted binding with Keap-1 as the responsible to increase oxidative stress and translocate to the nucleus for initiation of the transcription of the various antioxidant enzymes^[184] as the strategy to develop many class of antioxidant, anti-inflammatory, and anticancer agents. Reduction in non-enzymatic antioxidants, thiol glutathione and thioredoxin are the major dysregulation of the cellular redox status^[185]. The cellular redox status is reflected by the reduction of glutathione (GSH), oxidized glutathione (GSSG) ratio (or GSH:GSSG ratio), ascorbic acid, tocopherols and methionine and cysteine amino acids. Exogenous herbal antioxidants compounds in dietary foods include flavanoids, anthocyanins and polyphenolics act as ROS scavenging^[186,187]. The direct interaction of ROS with non-enzymatic antioxidants is based on chemical structure properties. In free radicals participate in 1e- oxidation while non-radical species was 2e- oxidation. For example, $O_2^{\cdot-}$ and OH^{\cdot} radicals react with the ascorbic acid and thiols. While the OH^{\cdot} more activity and instability react with methionine and tocopherols. H_2O_2 and the non-radical may react with thiols and methionine, and the $OONO^{\cdot}$ discriminate to react with thiols, ascorbic acid, tocopherols and methionine^[188].

Oxidative stress induces insulin resistance

Oxidative stress plays the major role in the association with the insulin resistance pathogenesis by insulin signals disruption and adipocytokines dysregulation^[8,9]. In rat models, oxidative stress enhances insulin resistance. The evidence suggested that Ang II infused rats required the increased glucose infusion to maintain euglycemia during hyperinsulinemic clamp to stimulate ROS production^[10]. For this example, Ang II-infused rats were caused insulin resistance from the suppression on insulin-induced glucose uptake in skeletal muscle and increased in oxidative stress biomarkers in this animal experiment. In experimental model, superoxide dismutase and tempol can reduce the insulin resistance. Many evidences indicated that ROS overproduction may induce insulin resistance and confirmed by the supplementation of antioxidant tempol to cause insulin resistance amelioration in Ren-2 transgenic rats^[189]. Insulin-target organs of the obese and diabetic KKAY mice were stimulated and caused ROS over production (skeletal muscle, liver and adipose tissue)^[8] and to cause insulin resistance in these organs. High fat-fed mice found ROS overproduction in liver and adipose tissue of these obese mices to induce insulin resistance^[190]. Many research studies suggested that antioxidant agents decreased plasma insulin, glucose, triglycerides levels and ameliorate insulin resistance in KKAY mice

with no weight loss^[8]. Antioxidant coenzyme Q10 supplementation can ameliorate the increased insulin levels in circulation of SHR/cp rats^[191]. As mention above, in over nutrition, the excessive glucose occur and a large amount of glucose is metabolized in the glycolysis and TCA cycle leading to increased NADH and $FADH_2$ production in electron transport chain of mitochondrial and increased superoxide production^[127]. In aged animals models treated with antioxidant agents or hypocaloric diets led to ameliorate in oxidative stress status and tissue function^[131,132].

Insulin resistance

In general population, insulin resistance precede in many years before onset of T2DM and it is also multifactorial^[11,12] such as genetic component^[11,13]. Insulin resistance and reduction in insulin production are the major characteristics of the T2DM pathogenesis^[11,12,14-16]. Modern lifestyle, physical inactivity, abdominal obesity and excessive of adipokines can cause insulin resistance^[11,15]. In early stage, normal glucose tolerance is preserved by compensation hyperinsulinemia. About 25% of non-diabetic subject cause insulin resistance in the same ranges that found in T2DM patients^[12]. Insulin resistance continuous increases and/or decreases in insulin secretory compensation responses, the deterioration into impaired glucose tolerance occurred. Increased glucose, FFA and insulin levels lead to ROS overproduction, increased oxidative stress and activate stress transduction factor pathways. This can cause insulin activity inhibition and secretion to accelerate the onset of T2DM as shown in Figure 6.

Oxidative stress has been demonstrated the implication and association in the late complications of diabetes mellitus^[17,18] as in the schematic of Figure 5. Many studies have demonstrated ROS overproduction and increased oxidative stress to insulin resistance^[192-194]. Both *in vitro* studies and in animal models demonstrated that α -lipoic acid (LA), antioxidant agent increase insulin sensitivity^[194-196]. In clinical trials, supplementation with vitamin C, vitamin E, glutathione increases insulin sensitivity in both insulin-resistance and T2DM patients^[197,198]. LA act as insulin sensitizer agent, it increased insulin sensitivity about approximately 25% and approximately 20% higher than metformin and rosiglitazone, respectively^[199,200]. Oral supplementation with LA formulation for 6 wk decreased circulating fructosamine levels^[201] and increase insulin sensitivity^[202] in T2DM patients and the other studies have confirmed 2.5 mmol/L of LA to cause GLUT4 activation and translocation^[203-205].

Because insulin resistance occurred before chronic hyperglycemia development^[12], that difference from insulin resistance in the pre-diabetic state result from oxidative stress activation by increased glucose levels. However, obesity demonstrated the strong association with insulin resistance. In this regard, the mediator of oxidative stress-induced insulin resistance of the pre-diabetic state might be from the adipocyte-derived

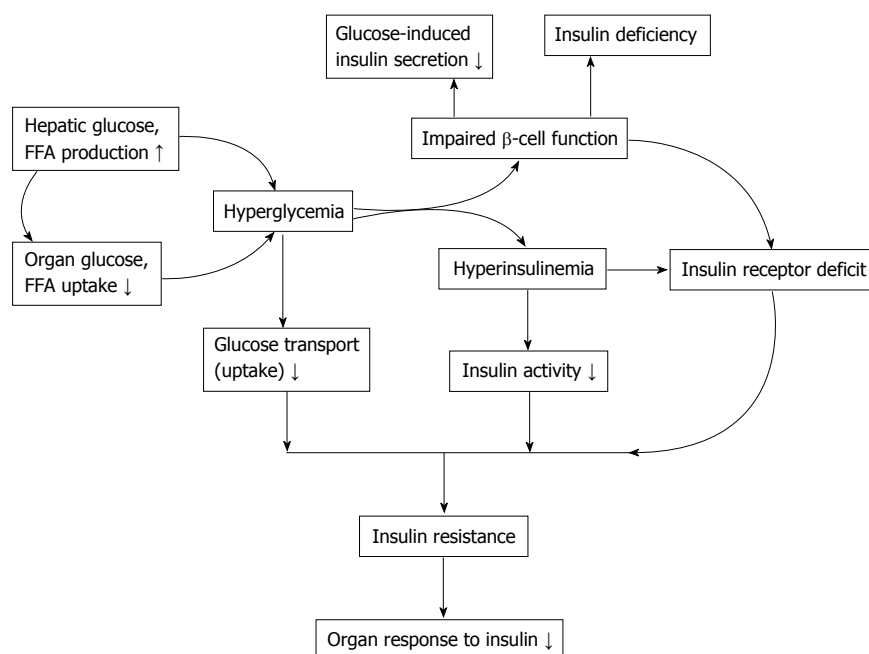


Figure 6 Insulin resistance development and consequence of β -cell dysfunction. FFA: Free fatty acid.

factor such as $\text{TNF-}\alpha$ ^[206], leptin^[207], FFAs^[208-210] and resistin^[211]. However, the FFAs elevations are associated with insulin resistance and obesity^[208,209,212]. Many studies found that increased FFA levels decrease insulin sensitivity, as in the Randle hypothesis^[210] and insulin-signaling inhibition^[212]. The increased fasting FFA levels are significantly correlated with decreased reduced/oxidized glutathione ratio in T2DM patients^[190]. Elevated FFA concentrations cause mitochondrial dysfunction such as uncouplers of oxidative phosphorylation in mitochondria^[213] and increased superoxide production^[214]. These caused the exacerbated situation from FFAs induce oxidative stress and reduce intracellular glutathione caused impaired endogenous antioxidant defenses^[190,215,216]. Supplementation with glutathione improves insulin sensitivity and β -cell function by the restoration of redox status in T2DM patients and healthy subjects^[217].

FFA mediated the nuclear factor- κB (NF- κB) activation, as the consequence of FFAs increased ROS overproduction and glutathione reduction^[216,218-220] and also linked to FFA-activated PKC- θ ^[221] to caused NF- κB activation^[222]. Vitamin E supplementation inhibits the FFA-induced NF- κB activation^[216] indicated that FFAs act as pro-inflammatory agent effects the alteration of the cellular redox status.

The HOMA-IR was proposed by Matthews *et al*^[223] that can be used to estimate insulin resistance and insulin sensitivity in individuals. HOMA-IR is easy to calculate and no more laborious technique. HOMA-IR method derives from the mathematic calculation from fasting plasma insulin and glucose concentrations.

Oxidative stress and β -cells dysfunction

Increased circulating glucose levels stimulate the β -Cells

function by sensing and secreting of insulin in appropriate amount^[224] and as the target of oxidative stress. The processes are complex and depend on many factors^[16]. The critical glucose metabolism in mitochondrial is the importance linking stimulus the insulin secretion^[224-226]. Therefore, mitochondria damage and markedly blunt insulin secretion is also occur by the ability of oxidative stress (H_2O_2)^[226]. Many studies in T2DM patients have suggested that chronic exposure to high glucose and/or high FFA levels impaired β -cells function and β -cells dysfunction^[16,227]. Because β -Cells are lower in antioxidant enzymes levels (superoxide dismutase, catalase and glutathione peroxidase) and higher sensitive to oxidative stress^[228]. Oxidative stress exposure to β -cells activated the increased p21 cyclin-dependent kinase inhibitor production, decreased insulin mRNA, ATP and calcium flux reductions in mitochondria and cytosol to cause apoptosis^[226]. Glucose or methyl succinate can stimulate insulin secretion and inhibit by response to K^+ within 30 min^[226]. The results indicate that mitochondria in β -cells involved in the processes of glucose induced insulin secretion are affected by increased oxidative stress. Lipid peroxidation, oxidative stress products exposed to islets, inhibited insulin secretion and also caused glucose oxidation^[229]. Conversely, antioxidants can protect β -cell against the toxicity of oxidative stress, AGEs production and inhibit NF- κB activation^[230-234]. These antioxidants are N-acetyl cysteine (NAC), α -phenyl-tert butylnitrone, aminoguanidine and zinc. Recent research study evaluated β -cells function after over expression of glutamine. Hexosamine over production resulted from the deterioration of insulin signaling of glucose-stimulated insulin secretion. Fructose-6-phosphate amidotransferase is the rate-limiting enzyme increase in hexosamine pathway^[235], coincident with increased

H₂O₂ production^[235] that can ameliorate by NAC supplementation.

β-cells glucose-induced toxicity

West^[19] demonstrated that insulin secretion in T2DM patients improved by the reduction of hyperglycemia with diet, insulin or sulfonylureas. On the other hand, in healthy normal, high glucose infused as a clamp reduces insulin secretion^[236]. In the study of long term culture of HIT-T15 and/or βTC-6 cells demonstrated that increased glucose levels cause decreased insulin secretion, insulin mRNA and decreased binding of transcription factors^[237,238]. Thus, glucose toxicity, the concept of the condition of hyperglycaemia itself can decrease insulin secretion which implies the irreversible damage to cellular components of β-cells^[239]. Generally in β-cells, excessive glucose oxidation and metabolism will always cause to ROS over production. Superoxide dismutase and catalase are normally as the detoxified antioxidant enzymes. β-Cells are low amount of these antioxidant enzymes and also low in glutathione peroxidase, a redox-regulating enzyme^[240]. Then, hyperglycaemia condition leads to increase ROS production and accumulation in β-cells and subsequent of cellular components damage. Pancreas duodenum homeobox-1 is an insulin promoter activity regulator was loss leading to β-cell dysfunction^[240]. Supplementation with NAC and/or aminoguanidine can ameliorate the glucotoxic effects on insulin gene activity^[230], reduced insulin levels and increased insulin mRNA and insulin sensitivity^[230].

β-cells lipid-induced toxicity

Lipotoxicity to β-cells concept, elevation of non-esterified fatty acids concentrations in diabetic and non-diabetic obese patients, result of the enhanced adipocyte lipolysis. In the presence of the excessive fatty acid oxidation in β-cells is caused increased long-chain acyl CoA accumulation leading to inhibit β-cells function^[241]. This process is as an integral part of the normal insulin secretory function. This long-chain acyl CoA can inhibit the insulin secretory function by opening β-cell K⁺-sensitive ATP channels. In the second mechanism, in long-term culture of β-cells formulas with FFAs can effect the potential reduction on mitochondrial membrane and uncoupler proteins-2 over expression to cause the K⁺-sensitive ATP channels opening which lead to decreased ATP production and insulin secretion^[242,243]. Third mechanism, β-cells apoptosis might possess from triglyceride or fatty acid induced ceramide synthesis and/or nitric oxide production. Thus, impaired insulin secretion and β-cell dysfunction strongly associated with the FFA-stimulated ROS overproduction^[244].

β-cells combined glucose/lipid toxicity

Elevation of glucose and FFA levels are the major characteristic of T2DM patients. This combination is the major β-cells toxicity and require the maximize protection. In culture cells of islets or HIT cells were

exposed to high concentrations of glucose and FFA levels. There was decrease in insulin-gene activity and insulin mRNA^[245]. In the study of islets co-culture with high glucose and palmitate levels caused impaired insulin signaling of the glucose-stimulated insulin secretion^[244]. Recent studies have confirmed that β-cells lipotoxicity is the concurrent status as the amplifying effect mediated by glucose toxicity in hyperglycemia condition^[246,247].

Dyslipidemia

Insulin resistance and T2DM are characterized by dyslipidemia one major risk factor for cardiovascular disease. Lipid triad is the complex metabolic milieu associated with dyslipidaemia^[248] comprise with hypertriglyceridemia, low levels of high-density lipoprotein cholesterol (HDL-C) and the appearance of small, dense, LDL (sdLDL) - and caused excessive post prandial lipemia^[249,250]. Diabetic dyslipidemia caused from the disturbance of lipid metabolism, an early event cardiovascular complications development and was preceded in T2DM patients by several years^[249-253]. Indeed, insulin resistance status in both with and without T2DM patients was display qualitatively similar lipid abnormalities^[250]. The different components of diabetic dyslipidemia are closely linked to each other metabolically^[249-253] and are initiated by the elevation of triglyceriderich very LDL (VLDL) from hepatic over production^[249,251]. It is the key importance mechanisms to elucidate the over production of VLDL involved in diabetic dyslipidemia^[249].

In insulin resistance state, decrease insulin function and lack of insulin inhibits lipolysis leads to increase FFAs generation of and lower lipoprotein lipase activity. This occurs after meal consumption, generates a chylomicron remnant rich in TG^[254], caused elevated hepatic FFAs and VLDL TG-rich particles secretion. These processes affects HDL-C metabolism through the interchange with TG-rich lipoproteins *via* cholesteryl ester transfer protein to produce HDL particles containing high TG concentrations. These HDL-TG particles were hydrolyzed with hepatic lipase to TG and HDL. This HDL becomes smaller and less antiatherogenic activity, easily to remove from the circulation by the kidneys. Moreover, insulin resistance in T2DM patients associated with endothelial dysfunction led to increase risk of CVD^[255]. The most atherogenic subfractions of sdLDL are elevated in circulation of obesity individuals, as a key feature in association with elevated triglyceride and low HDL cholesterol. Elevated sdLDL concentrations are also founded in abdominal obesity subjects and demonstrated greater myocardial risk. The mechanisms are related to excess accumulation of abdominal adipose tissues, elevated total cholesterol and LDL-C and related to high saturated-fat consumption, weight gain and obesity.

Dyslipidemia is commonly occurred in T2DM patients and might play the major role in accelerated

macrovascular atherosclerotic disease and increased CVD risk in T2DM patients^[256]. Dyslipidemia in T2DM patients as lipids triad is characterized by increased insulin levels, hypertriglyceridemia, low HDL-C levels and increased sdLDL-particles (independent of LDL-cholesterol) and increased TG-rich remnant lipoprotein (TGRs) concentrations^[257,258]. In this manner, low HDL-C levels associated with hyperinsulinemia or insulin resistance and insulin signaling for insulin-mediated glucose disposal^[259] characterized by higher fasting plasma glucose and insulin levels. Then, these major changes associated with the insulin resistance syndrome are increased TGRs and decreased HDL-C levels. Thus, in dyslipidemia, using the lipoprotein concentration ratios are associated with insulin resistance and increased CVD risk conditions. Lipoprotein ratios might be useful to identify insulin resistance individuals even different in fasting glucose or insulin levels. Obesity, metabolic syndrome, and T2DM may also show the same dyslipidemia characteristic^[12,257,259] and measuring TG, HDL-C, TC/HDL-C and TG/HDL-C ratio in circulation may also use as insulin resistance estimation. For example, these TG, HDL-C, TC/HDL-C and TG/HDL-C ratio are independently associated with insulin levels, insulin resistance and CVD risk^[258,260,261].

Lipoprotein ratios: In description above, the major change is increased TGRs and decreased HDL-C levels are associated with insulin resistance syndrome. Insulin plays the important role in TG metabolism, in normal condition TGRs particles reduces synthesis by the distinct pathways when compared with VLDL particles synthesis^[249,258]. Insulin fails to suppress VLDL particles synthesis^[262]. Insulin resistance is significantly associated with increased lipid synthesis in the liver, increased FFAs flow to the liver and decreased VLDL particles clearance resulting in increased VLDL levels in the circulation^[251]. Thus, dyslipidemia (as lipoprotein ratios) may associate with insulin resistance and increased CVD risk. On this basis, waist circumference, LDL-C, TG levels, insulin resistance and the CVD risk are estimated^[263]. The major features of dyslipidemia are determined by hypertriglyceridemia, low HDL-C levels and slightly high or normal LDL-C levels with altered composition. Hypertriglyceridemia is indicate as elevated atherogenic chylomicron and VLDL remnant and associated with increased CVD risk^[264,265]. These phenomenons demonstrated the problems of VLDL and HDL levels but not the LDL levels and concurrent with increased insulin levels. Low HDL-C level is associated with the hyperinsulinemia and/or insulin resistance and insulin signaling for insulin-mediated glucose disposal^[259]. All of these features are associated with coronary heart disease risk in obesity, metabolic syndrome and T2DM patients. The TC/HDL-C, TG/HDL-C ratios and non-HDL-C (as TC - HDL-C) were used as surrogate markers for insulin levels and insulin resistance estimation. In Tangvarasittichai *et al*^[258] study suggests that TC/HDL-C, TG/HDL-C ratios and non-

HDL-C can be used as markers of insulin levels, insulin resistance and CVD risk factor^[258,263]. The highest % sensitivity and % specificity cut-off points corresponding to the TC/HDL-C, TG/HDL-C ratios and non-HDL-C are 3.58, 2.48 and 130.4, respectively^[258]. Because of TC/HDL-C, TG/HDL-C ratios and non-HDL-C are easily calculated and ordered with every lipid profiles available to the clinician and no costs addition. The cut-off value of these ratios in Tangvarasittichai *et al*^[258] study was lower than the results from Western populations^[266-268]. Then, insulin resistance was significantly predicted by these markers. For atherosclerotic risk assessment in obesity, metabolic syndrome and T2DM patients requires more attention to lipid screening.

Development of T2DM from insulin resistance

Insulin resistance often occurs with T2DM but is insufficient for the T2DM development. β -cells dysfunction are important event for the T2DM development and progression. In early stage of insulin resistance, β -cells increase the secretory function try to compensate and control hyperglycemia. In Pima Indian population study caused acute insulin response dysfunction or decreased β -cell responses was found during the normal glucose tolerance state in individuals who eventually progressed from normal glucose tolerance to impaired glucose tolerance or T2DM when compared with individuals who persisted in the state of normal glucose tolerance^[269]. There was evidence of early defects in glucose disposal by decreased insulin sensitivity before the development of glucose intolerance state, although output of circulating glucose did not increase until the progression from impaired glucose tolerance to T2DM revealed. Interestingly, individuals who demonstrated transient glucose intolerance but were able to recover and to reach normal glucose tolerance and did not show the early secretory defect observed in progressed individuals^[269]. β -cells failure or dysfunction occurred as the results of the combination of increased oxidative stress, glucose and lipids accumulation to cause glucotoxicity and lipotoxicity to β -cells to progress increased apoptosis and loss of the insulin granule secretory components expression^[270].

T2DM

The World Health Organization updated the prevalence of T2DM estimated by the year 2025 those 30.3 million people in the United States and total of 380 million people worldwide will be diagnosed as DM^[271]. By the year 2050, those 45.6 million Americans will be diagnosed as DM^[272]. T2DM is associated with obesity, sedentary lifestyle and lack of exercise in the aging population. There are a number of gene abnormalities related to T2DM, that showed significant differences exist in the abnormalities gene associated with T2DM among the various ethnic populations, such as African Americans, Asians and Europeans^[273,274]. The contribution of any one of these genes to T2DM is small and total

Table 1 Type 2 diabetes mellitus and glucose levels for diagnostic criteria¹

Glucose management test	Range	Diagnosis
Fasting plasma glucose (mg/dL) (at least 8 h fast)	≥ 126	Diabetes mellitus
	100-125	Impaired fasting glucose
	≤ 99	Normal
2-h oral glucose tolerance test of 75 g glucose load (mg/dL) WITH	≥ 200	Diabetes
Random screening with common symptoms of diabetes (polyuria, polydipsia, weight loss, etc.)	140-199	Impaired glucose tolerance
	≤ 139	Normal
Hemoglobin A1c (%)	≥ 6.5	Diabetes
	5.7-6.4	Prediabetes/high risk
	≤ 5.7	Normal

¹All tests in diabetes range must be repeated after 24 h, to be confirmed diagnosis.

aggregate of all described genes accounts for < 15% of the predisposition^[273,275]. It is typically diagnosed in patients older than 30 years with overweight or obesity and positive in family history of T2DM. However, insulin resistance may occur and develop in many years before diagnosed as T2DM^[276]. Figure 7 summarized the etiology of the T2DM pathogenesis.

Patients are diagnosed as T2DM when plasma glucose levels reach at the diagnostic criteria (Table 1). These T2DM patients are at high risk for microvascular complications (e.g., nephropathy, retinopathy and neuropathy) and macrovascular complications (e.g., peripheral vascular disease, cerebrovascular disease and cardiovascular disease). T2DM patients with good controlled plasma glucose levels demonstrated to delay the progression of microvascular and macrovascular complications^[271,277].

MANAGEMENT OF DYSLIPIDEMIA AND HYPERGLYCEMIA IN T2DM PATIENTS

Fasting serum lipids profile should be determined annually in T2DM patients as in the recommendation by the American Diabetes Association (ADA)^[278]. ADA recommended for the satisfied lipids profile level as low-risk by LDL-C < 100 mg/dL (2.6 mmol/L), triglycerides < 150 mg/dL (1.7 mmol/L) and HDL-C > 50 mg/dL (1.3 mmol/L)^[276].

Treatment

Lifestyle interventions: The American Diabetes Association and the American Heart Association recommend that increased physical activity and lifestyle modifications should be advised for all T2DM patients^[278,279]. Combination with such interventions included nutrition therapy or supplementation, weight loss and non-smoking. These have been help T2DM patients to receive better controlled their lipid concentrations. Nutrition interventions and supplementations should be designed according to the condition of T2DM individuals such as diabetes status,

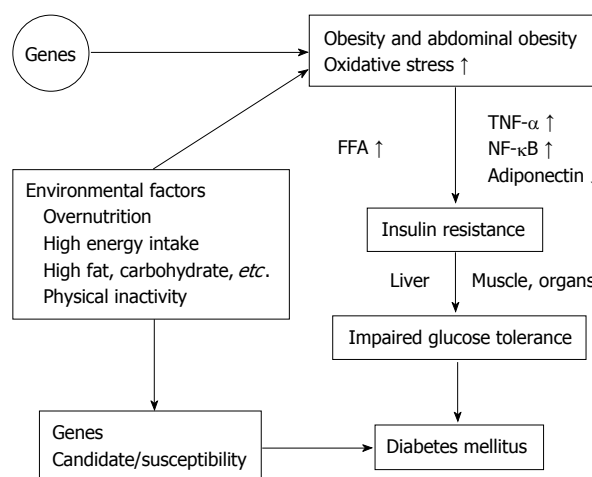


Figure 7 Summarized the etiology of the type 2 diabetes mellitus pathogenesis. FFA: Free fatty acid; NF-κB: Nuclear factor-κB; TNF-α: Tumor necrosis factor α.

age, other comorbidities and avoidance to intake transfat, saturated fat, cholesterol and should increase intake of fiber (fiber in oats, legumes, citrus), omega-3 fatty acids and plant stanols/sterols^[278]. Glycemic control can also modify circulating triglycerides levels, especially in T2DM patients with hypertriglyceridemia and poor glycemic control^[278].

Pharmacological interventions of dyslipidemia

There are many pharmacological classes available for dyslipidemia treatment.

Statins: Statins inhibit enzyme 3-hydroxy-3-methylglutaryl CoA reductase suppress cholesterol synthesis and increase number and activity of LDL-receptor. Statins are effective drug for lowering LDL-cholesterol, raising HDL-C and reducing TG levels. There are seven pharmaceutical forms of statins including lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin and pitavastatin available in the market. Statins also have the other pharmacodynamic actions such as vascular inflammation reduction, immune suppression, improved endothelial function, platelet aggregability, enhanced fibrinolysis, antithrombotic action, increase neovascularization in ischemic tissue and stabilization of atherosclerotic plaques^[280].

Fibrates

Fibrates control the lipid metabolism by mediated through peroxisome proliferator-activated receptors-α activation, stimulation of β-oxidation of fatty acids in peroxisomes and mitochondria to cause lowering fatty acid and triglycerides levels in circulation. The first drug of this class is Clofibrate. Eventually, the revolution in lipid-lowering drugs research discover of many other fibrate drugs such as fenofibrate, bezafibrate, gemfibrozil and ciprofibrate. These drugs demonstrated the adverse effect to cause hepatomegaly and tumor formation in the liver of rodents. Then, they had restricted for the widely

use in humans. Gemfibrozil and fenofibrate are Food and Drug Administration (FDA)-approved for lipid lowering drugs due to milder effect on peroxisome proliferation.

Nicotinic acid

Long term study of the coronary drug project demonstrated that niacin is the effective drug to increase HDL-C levels and reduced CVD events^[281] in a non-diabetic subjects. Niacin cause adverse effects on the glycemic control levels in T2DM patients. In high doses treatment with niacin may increase blood glucose levels. The modest doses of 750-2000 mg/d of niacin are significantly increased HDL-C levels and decreased LDL-C, triglyceride levels and accompanied with modest changes in glucose levels for diabetes therapy^[282,283]. However, there is no evidence for the CVD outcomes reduction with niacin supplementation in T2DM patients.

Antihyperglycemic drugs: The standard care for T2DM patients is mainly in controlled blood glucose levels by using glycemic lowering drugs and concomitant with controlled diet and increased physical activity. With proper controlled and managed these contributors such as circulating glucose levels, hemoglobin A1c, lifestyle modifications, these can be effectively controlled and reduced the progression and complications disease. In general, only approximately 50% to 60% of T2DM patients have achieved their glycemic goals^[284]. There are many reasons for poor control of T2DM including medication efficacy, adverse effects, access to medications and health care education, poor adherence, lack of lifestyle changes and no physical activity. Now a day, more pharmacologicals for T2DM treatment have been approved for use. There are 12 classes of antihyperglycemic drugs FDA-approved in the United States^[285] such as sulfonylureas, meglitinides, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, biguanides, sodium glucose transporter 2 inhibitors, α -glucosidase inhibitors, amylin analogues and glucagon-like peptide-1 (GLP-1) receptor agonists. These are insulin analogues. Metformin is one of the most commonly prescribed medications for T2DM management. Metformin treatment ameliorate the insulin resistance especially in liver and skeletal muscle but less effect in adipose tissue^[286,287], decreased inflammatory response, improved glycemic control^[288,289] and enhance β -cell function in T2DM patients by increased insulin sensitivity and glucotoxicity reduction^[290]. Metformin reduces fatty acid oxidation in adipose tissue^[291], increased GLUT4 translocation in muscle and adipose tissues by activated enzyme adenosine monophosphate kinase and reduced gluconeogenesis in liver^[292-295]. There are many developed non-conventional drugs to improve glycemic control such as Cycloset is used together with diet and exercise to treat type 2 diabetes. Cycloset is not for treating type 1 diabetes. Welchol is a non-absorbed, polymeric form, lipid-lowering and glucose-lowering agent for oral administration. Welchol is a high-capacity bile acid-binding molecule. Afrezza Inhalation Powder is

the FDA approved the inhalation form of insulin. The new drug is not a substitute for long-acting insulin and use as the combination with conventional long-acting insulin drug for both types of diabetes and many drugs are in the late clinical trials state.

There are new medications and treatments were identified from the FDA, they are in the clinical trials or waiting for approval treatment in dyslipidemia, obesity and T2DM^[296]. Recent research study reports that metformin treatment cause metabolic effects to increase GLP-1 concentration in the circulation^[297,298]. GLP-1 is an incretin generated from the transcription product of the proglucagon gene. Incretin is a signaling polypeptide contained with 30-amino acid. GLP-1 secretion by ileal L-cells is not depend on the presence of nutrients in the small intestine and responsible for stimulated insulin secretion to limit glucose elevations with the higher efficacy at high glucose levels^[276,299]. Elevated GLP-1 secretion might possibly cause increased glucose absorption in the distal segments of small intestine.

Incretins are the gastrointestinal hormone secreted from the intestine and stomach responsible for oral food intake and stimulated the secretion of insulin during meals in healthy peoples^[276]. Two major incretin molecules are (1) GLP-1; and (2) Glucose-dependent insulinotropic peptide known as gastric inhibitory polypeptide (GIP) and to neutralize stomach acid to protect the small intestine and no therapeutic efficacy in T2DM. GLP-1 has lower glucose levels by stimulated insulin production and increased glucose metabolism in adipose tissue and muscle. GLP-1 promote the pancreatic β -cells proliferation, reduce apoptosis, increase cardiac chronotropic, inotropic activity, decreases glucagon secretion, reduces glucose production, increase appetite suppression for food intake reduction and slow gastric emptying^[271,276,299]. GLP-1 is degraded by enzyme DPP-4 and this enzyme does not inhibit by metformin^[298]. The prevention of GLP-1 degradation by DPP-4 is one method to increase the effects of GLP-1. DPP-4 inhibitor drugs inhibit the glucagon secretion which in turn increases secretion of insulin to decrease blood glucose levels and decreases gastric emptying. The FDA-approved the DPP-4 inhibitor drugs including sitagliptin (Januvia), alogliptin (Nesina), saxagliptin (Onglyza), linagliptin (Tajenta), anagliptin, vildagliptin, teneligliptin, gemigliptin and dutogliptin. The adverse effects are dose-dependent to cause headache, vomiting, nausea, nasopharyngitis, hypersensitivity and other conditions. Other side effects of exenatide (GLP-1 agonist) note for abdominal pain, acid stomach, diarrhea, altered renal function, weight loss, dysgeusia, belching and cause pruritus, urticaria and rash reactions at the injection site.

CONCLUSION

In this present review has described the detrimental effects from chemicals and biochemicals reaction, metals, medications, over nutrition, obesity and diseases in oxidative stress, insulin resistance development and

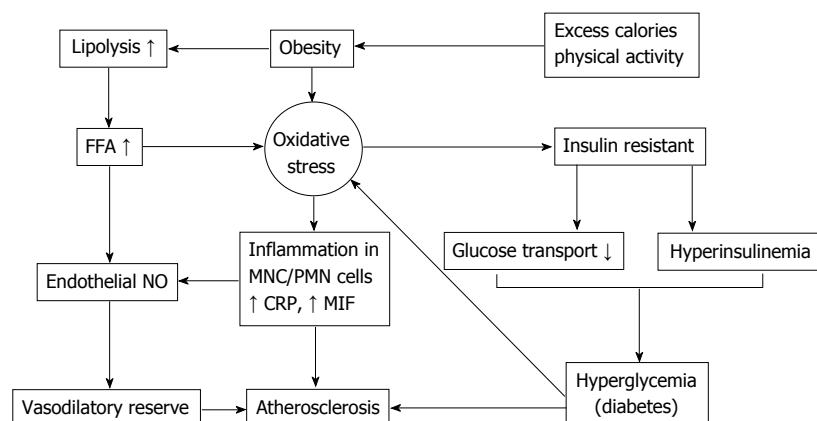


Figure 8 Connection between life style, oxidative stress, insulin resistance, inflammation and atherosclerosis. FFA: Free fatty acid; NO: Nitric oxide; MNC: Mononuclear cells; PMN: Polymorpho nuclear cells; CRP: C-reactive protein; MIF: Migration inhibitory factor.

the progression of T2DM and the progression of diabetic complications and organ dysfunctions. Oxidative stress played underlying associated with the pathogenesis of diseases, leading to increases risk of insulin resistance, dyslipidemia, elevated blood pressure, metabolic syndrome, inflammation and endothelial dysfunction. This reviewed support the oxidative stress contribution of the multifactorial etiology of oxidative stress and insulin resistance in the whole body. ROS act as the signal transduction factor and plays the important role in oxidative stress-mediated downstream signaling pathways and enhances the cell death. Furthermore, risk for several chronic diseases development associated with oxidative stress and metabolic syndrome including T2DM, hypertension, arthritis, congestive heart failure, chronic renal failure, cancer and Alzheimer's. These diseases may be substantially reduced by dietary modifications, increased physical activity and antioxidant drugs ameliorated oxidative stress. The therapeutic approaches target on oxidative stress may delay or prevent the progression and onset of diseases. Then, antioxidants supplementation may curtail the progression and onset of the metabolic disease complications. Antioxidant interventions, an importance goal of future clinical investigations should be implementation and to improve oral bioavailability targeted to the oxidant overproduction site. Lifestyle change remains the best prevention and therapeutic approach to oppose the increasing epidemic of cardiovascular diseases, obesity, hypertension, dyslipidemia and T2DM. Finally, the connection between oxidative stress, insulin resistance, dyslipidemia, inflammation, life style, atherosclerosis and diabetes as demonstrated in the schematic in Figure 8.

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Birth defects in pregestational diabetes: Defect range, glycemic threshold and pathogenesis

Rinat Gabbay-Benziv, E Albert Reece, Fang Wang, Peixin Yang

Rinat Gabbay-Benziv, E Albert Reece, Fang Wang, Peixin Yang, Department of Obstetrics, Gynaecology and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD 21201, United States

E Albert Reece, Peixin Yang, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201, United States

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Correspondence to: Peixin Yang, PhD, Department of Obstetrics, Gynaecology and Reproductive Sciences, University of Maryland School of Medicine, BRB11-039, 655 W. Baltimore Street, Baltimore, MD 21201,

United States. pyang@fpi.umaryland.edu

Telephone: +1-410-7068402

Fax: +1-410-7065747

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Abstract

Currently, 60 million women of reproductive age (18-44 years old) worldwide, and approximately 3 million American women have diabetes mellitus, and it has been estimated that this number will double by 2030. Pregestational diabetes mellitus (PGD) is a significant public health problem that increases the risk for structural birth defects affecting both maternal and neonatal pregnancy outcome. The most common types of human structural birth defects associated with PGD are congenital heart defects and central nervous system defects. However, diabetes can induce birth defects in any other fetal organ. In general, the rate of birth defects increases linearly with the degree of maternal hyperglycemia, which is the major factor that mediates teratogenicity of PGD. Stringent prenatal care and glycemic control are effective means to reduce birth defects in PGD pregnancies, but cannot reduce the incidence of birth defects to the rate of that is seen in the nondiabetic population. Studies in animal models have revealed that PGD induces oxidative stress, which activates cellular stress signalling leading to dysregulation of gene expression and excess apoptosis in the target organs, including the neural tube and embryonic heart. Activation of the apoptosis signal-regulating kinase 1 (ASK1)-forkhead transcription factor 3a (FoxO3a)-caspase 8 pathway causes apoptosis in the developing neural tube leading to neural tube defects (NTDs). ASK1 activates the c-Jun-N-Terminal kinase 1/2 (JNK1/2), which leads to activation of the unfolded protein response and endoplasmic reticulum (ER) stress. Deletion of the *ASK1* gene, the *JNK1* gene, or the *JNK2* gene, or inhibition of ER stress by 4-Phenylbutyric acid abrogates diabetes-induced apoptosis and reduces the formation of NTDs. Antioxidants, such as thioredoxin, which inhibits the ASK1-FoxO3a-caspase 8 pathway or ER stress inhibitors, may prevent PGD-induced birth defects.

Key words: Pregestational diabetes; Birth defects; Glycemic threshold; Diabetic embryopathy; Range of defects

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Core tip: Pregestational diabetes is a rising problem with gravid impact on adverse pregnancy outcomes. This review concentrates on diabetes-induced birth defects and the underlying mechanism of diabetic embryopathy derived from animal studies. The main defects associated with pregestational diabetes are in the cardiovascular and central nervous systems, and are linearly related to maternal glycemic control. Animal studies reveal oxidative stress and stress kinase signalling-induced apoptosis as key factors in pathogenesis. However, many questions remain unanswered, and the rate of congenital defects in human diabetic pregnancies is still high. The cause of diabetic embryopathy warrants further investigations.

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INTRODUCTION

Major structural or genetic birth defects affect approximately 3% of births^[1], and are the leading cause of infant mortality in the United States^[2]. The pathogenesis of most birth defects is unknown^[3,4]. Pregestational diabetes (PGD) is one of the leading known causes with up to a nine-fold increase in birth defects, compared with the rate seen in nondiabetic pregnancies^[5]. Although the risk for birth defects in PGD pregnancies can be significantly reduced by appropriate pregestational counselling and meticulous control of maternal glycemic levels^[6], it cannot be equalized to the background risk partially because at least 40% of diabetic pregnancies are usually unplanned^[6,7].

The pathophysiology of maternal diabetes induced birth defects is complex, however, clearly relates to maternal glucose levels. The mechanism is not entirely understood, but animal studies have shown it to be associated with decreased cell proliferation and increased cell apoptosis due to high oxidative stress. The second major change seen in animal models of PGD is altered gene expression causing deviation from the normal developmental process.

PGD can affect almost any organ. However, most congenital defects associated with diabetes occur in the cardiovascular, central nervous and musculoskeletal systems^[8-10].

In this review we will discuss how PGD increases the risk of birth defects, the range of defects seen in diabetic

pregnancies and the relationship of these defects to maternal glucose control. As the risk of birth defects is well established in type 1 and type 2 diabetes mellitus, while it is still controversial in gestational diabetes^[11], we will focus this review on PGD only.

RANGE OF DEFECTS

PGD is associated with a wide range of anomalies in almost any fetal organ. Based on available literature, women with PGD have 2- to 9-fold higher risk for having babies with birth defects, with a prevalence of birth defects of 2.7%-18.6%, compared with the healthy population^[5,12-17], having a prevalence of birth defects of 2%-3%.

Although any birth defect can be associated with PGD, more anomalies are seen in the cardiovascular, central nervous and skeletal systems^[9,10,18]. Rare abnormalities almost exclusively associated with diabetes include caudal regression syndrome with femoral shortening and sacral agenesis. Caudal regression syndrome consists of a spectrum of congenital anomalies of the lower spine and hips, is associated with genitourinary and lower limb defects, and is one of the few syndromes in which the presence of maternal diabetes has always to be considered as the cause, if not confirmed^[19]. Although very rare, some studies found this syndrome to be 200-600 times more prevalent in infants of diabetic mothers compared with nondiabetic mothers^[16]. Be that as it may, it can still be seen also in non-diabetic mothers as described in a case report by Versiani *et al*^[20] who reported 2 cases of caudal dysplasia sequence - one woman had PGD while the other had gestational diabetes.

Previous studies assessed the prevalence of congenital anomalies in the diabetic population. Eidem *et al*^[21] Studied the risk of congenital anomalies related to type 1 diabetes. They compared major congenital anomalies (excluding minor anomalies as defined by the EUROCAT system) between women with pregestational type 1 diabetes and controls. All women registered at the Norway national delivery registry from 1999 to 2004 were included. Anomalies were registered in 5.7% (91/1583) offspring of women with type 1 diabetes, compared with 2.9% in the background population. The risk for cardiovascular anomalies was 3 fold higher in the diabetic group (3.2% vs 0.94%, respectively) with ventricular septal defect (VSD) (12/1583) and patent ductus arteriosus (10/1583) being the most prevalent birth defects in the cardiovascular system and the most common compared with all other birth defects.

Game *et al*^[22] used the same EUROCAT classification to describe birth defects in 18 population-based EUROCAT registries of congenital anomalies, which included births between 1990 to 2005, and compared 669 diabetes cases with 92976 non-diabetes cases. The authors showed significantly increased odds ratios for neural tube defects (anencephaly and encephalocele, but not spina bifida) and several subgroups of congenital heart defects. Other birth defects found in increased odds ratios in

infants of pregnancies complicated by pregestational diabetes were anotia, omphalocele and bilateral renal agenesis. Multiple congenital anomalies were present in 13.6% of the diabetes cases and 6.1% of nondiabetes cases. The odds ratio for caudal regression sequence was very high (26.4, 95%CI: 8.98-77.64), but only 17% of all caudal regression cases occurred in pregnancies complicated by PGD.

Most recently, Correa *et al*^[23] reviewed the association of type 1 and type 2 PGD with 39 birth defects using the National Birth Defects Prevention Study. The authors examined databases from 10 birth defects surveillance systems in the United States looking for isolated (one or more major defects in same organ system or with known sequence) or multiple birth defects (2 or more major, unrelated defects) from births between 1997 to 2003. They found an association between PGD and 11 cardiac defects and 7 non cardiac defects.

Galindo *et al*^[12] prospectively followed 126 women with pregestational diabetes in a single tertiary centre in Spain. They reported 17 offspring with congenital anomalies (13.5%). Although chromosomal abnormalities are not generally associated with PGD, one fetus had trisomy 21 and atrioventricular septal defects. Eight fetuses were listed with major birth defects (6.3%), 35.3% of fetuses had cardiovascular defects, and genitourinary defects accounted for another 23.5%. They noted positive correlation between high levels of haemoglobin A1c (HbA1c) > 7% at early pregnancy and major congenital anomalies. Despite this study including only small number of women, the prospective nature of it allows strength in addressing its results.

Wender-Ozegowska *et al*^[16] studied a group of 198 diabetic women and found 17 pregnancies that resulted in infants with birth defects (8.6%). The most common defects were in the cardiovascular system (5.5%), with 7/198 (3.5%) of cases being atrioventricular septal defects (3 cases of VSD and 3 cases of atrial septal defect). Wren *et al*^[17] focused their research on cardiovascular birth defects. They followed 609 diabetic pregnancies and found that 3.6% of the women delivered infants with cardiovascular defects, the most common being transposition of great arteries, truncus arteriosus and tricuspid atresia. Janssen *et al*^[18] studied 1511 PGD cases, with congenital birth defects prevalence of 7.2%. The most common defects occurred in the cardiovascular system, NTDs, cleft lip/palate and skeletal defects.

Although many studies discuss diabetes associated congenital anomalies, the leading type of birth defects and the exact rate of diabetes associated birth defects are difficult to determine. The literature varies widely in what is considered the "leading" type of anomaly, as well as the rate of anomalies, because of suboptimal coding of the diabetic pregnancies according to White's classification, differences in anomaly coding and reporting bias. Moreover, the recognition of a birth defect may not always occur in the immediate neonatal period, thus, may not be entered into a registry. On the other

hand, over documentation of diabetes related birth defects, compared with birth defects in the nondiabetic population, may occur because of the known association of defects to diabetes, or the relatively high transfer of infants of diabetic pregnancies to neonatal intensive care units, both leading to a more thorough neonatal examination.

Based upon the available data, it seems that the major organ systems affected by PGD are the cardiovascular and the central nervous systems, which could be related to their embryonic origin in the neural crest. However, PGD can increase the rate of any other birth defects described above. Therefore, all pregnant women with PGD are advised to undergo a detailed anatomical scan and full fetal echocardiography to screen for possible birth defects, regardless of their having type 1 or type 2 diabetes mellitus or glycemic control at the time of pregnancy.

GLYCEMIC THRESHOLD FOR BIRTH DEFECTS

Periconceptional HbA1c is used as a surrogate marker for glycemic control, and is almost linearly related to PGD-induced birth defects. Previous studies have shown that stringent glucose control prior to or at early pregnancy, during organogenesis, can significantly reduce the incidence of birth defects^[12,24-29]. However, there are still questions to be answered. Hanson *et al*^[25] studied 532 type 1 PGD women and compared their malformation rate to 222 nondiabetic women. The rate of malformations did not differ significantly between the diabetic and the control groups (4.3% vs 2.4%) although significant difference was found in levels of first trimester HbA1c. The median value of HbA1c was 7.7% in the diabetic and 5.3% in the control group ($P < 0.001$). However, when higher levels of HbA1c were evaluated (HbA1c greater than 10.1% - equal to 8 SD above the normal mean control value), there was statistically significant higher occurrence rate of congenital malformation ($P < 0.01$).

Wender-Ozegowska *et al*^[16] tried to determine the cut-off for first trimester glycemic levels for prediction of congenital anomalies. They used whole day glycemic profile as well as HbA1c to assess glycemic control. Their cohort included 198 diabetic pregnancies and 4700 nondiabetic pregnancies. The rates of malformation were 8.6% and 3.6%, respectively. They determined the cut-off of value of HbA1c that could predict congenital malformations as 9.3% (measured up to 16 wk).

Other studies demonstrated the connection between poor glycemic control before or early at pregnancy: Ylinen *et al*^[29] found that the mean HbA1c for women whose infants had congenital malformations was higher than that measured for women with healthy infants prior to 15 gestational weeks (9.5% vs 8.0%, respectively). Mironiuk *et al*^[27] compared an occurrence rate of congenital malformations in newborn to mothers with

type 1 diabetes ($n = 170$), newborns of healthy mothers ($n = 26368$) and mothers with GDM ($n = 56$). They demonstrated that type 1 diabetes was associated with congenital malformations (11.2%, 1.8% and 2.2%, respectively) and that the risk of major birth defects was directly proportional to the level of maternal blood glucose control during the first trimester. These studies all support the idea that lack of glycemic control leads to congenital malformations, but do not indicate what HgA1c value should be maintained to reduce the risk for infant anomalies. Moreover, Shields *et al.*^[30] tried to find cut-off value associated with congenital anomalies but failed to do so, and Lucas *et al.*^[31] proved the association of PGD with congenital anomalies only when HgA1c was combined with other factors such as diabetic classification (White's classification) and maternal vascular complications. The Atlantic-Diabetes in Pregnancy (DIP) trial compared PGD pregnancy outcomes in the same population after a change in pre-pregnancy care policy and improved glycemic control throughout pregnancy. In the Atlantic DIP study, the first trial conducted from 2005-2007, included 104 diabetic pregnancies (87% type 1 PGD). The authors reported two pregnancies with birth defects that had HgA1c values of 6.6% and 5.4% at early pregnancy. The second trial, conducted from 2008-2010, included 168 pregnancies, with more women affected by type 2 PGD (81/168, 48%) and lower HgA1c values (7.3% compared to 6.9%); however, despite of presumed better prenatal care and the lower HgA1c - rate of malformation reported was not changed between the trials. The authors did not report the type of birth defects seen in either of the two studies^[32,33].

Other studies have demonstrated a linear relationship between HgA1c and major congenital defects. Greene *et al.*^[34] showed an increase in PGD-induced birth defects correlating to the level HgA1c. Their risk for major malformation was 3.0% when HgA1c taken at first trimester was less than or equal to 9.3% and 40% with HgA1c was greater than 14.4% (RR = 13.2; 95%CI: 4.3-40.4). Todorova *et al.*^[35] studied 124 pregnancies complicated by pregestational diabetes. The mean values of HgA1c were significantly higher in pregnancies complicated by fetal malformations ($n = 19/15.3\%$) than those values measured in pregnancies without fetal malformations [9.01% (SD ± 1.53) vs 8.06% (SD ± 1.64) $P = 0.022$, respectively].

In conclusion, it is clear that glycemic control is associated with a reduced risk of congenital anomalies. However, the recommended threshold of HgA1c for pregestational diabetic women planning pregnancy is still not known. Reaching a consensus as to what HgA1c level a diabetic pregnant woman should strive for has remained elusive for many reasons. First, differing definitions for major and minor anomalies, and taking into account the various forms of diabetes, limit the ability to derive conclusions from the published data. Second, PGD pregnancies can have the same spectrum of anomalies as nondiabetic pregnancies which makes

it difficult to differentiate PGD-induced anomalies from others encountered in nondiabetic pregnancies. Third, most studies use HgA1c to reflect the level of glycemic control, but HgA1c only measures a woman's average glycemic control over a 3-mo period. Therefore, it does not necessarily reflect a woman's level of glycemic control during organogenesis and embryogenesis. Moreover, because it is an average measurement, the same level of HgA1c may reflect completely different glycemic patterns, one being constant around the average, and the other with larger fluctuations lower and above from the mean HgA1c value^[35]. The effects of such fluctuations (*i.e.*, episodes of acute hyperglycemia and hypoglycemia with "normal" HgA1c) are yet to be determined. Fourth, given the complicated metabolic nature of diabetes, coupled with metabolic changes that are normal during pregnancy, other factors may influence the developing embryo. Finally, lower prenatal detection rates of fetal anomalies (due to obesity, lack of prenatal care, *etc.*) in diabetic women may lead to statistic skewing when studying infants with congenital anomalies born to PGD mothers^[36,37].

DOES DIABETES TYPE AFFECT THE RATES OF CONGENITAL ANOMALIES?

Diabetes can be classified by the mechanism (type 1, type 2 or gestational diabetes) or alternatively, by White's classification^[38]. Rates of diabetes, both type 1 and types 2 are increasing all over the world^[39,40] causing an increase in the incidence of maternal diabetes in pregnancy^[41]. Although hyperglycemia is a common mechanism for teratogenicity, differences in disease characteristics, such as age of onset, ethnicity, obesity and duration of disease, may affect the disease impact on the perinatal outcome and the rate of congenital anomalies. Current literature does not specify the risk for anomalies for any type of diabetes separately, as some studies include only type 1 diabetes^[25,42], others only type 2 diabetes^[32] and only a few compare the outcomes between them^[43,44].

Gonzalez-Gonzalez *et al.*^[44] retrospectively compared the outcomes of type 1 diabetes ($n = 904$), type 2 diabetes ($n = 516$), gestational diabetes ($n = 3188$) and control ($n = 115996$) deliveries in Ontario, Canada. Congenital anomalies were most frequently observed in women with type 1 diabetes and gestational diabetes, with risk approaching 1.5- to 2-times that of controls. They found a total of 44 anomalies (6.1%) among all women with diabetes, most commonly in the cardiovascular system. Adjusted OR for anomalies was 3.5, 1.7, 2.5 and 1.9 for type 1, type 2, gestational diabetes and control deliveries, respectively. Peticca *et al.*^[45] found similar results in a multicentre study in Italy. They prospectively compared 504 type 1 PGD to 164 type 2 PGD pregnancies. The rate of birth defects was significantly higher in women with type 1 diabetes (5.9%), compared with in women with type 2 diabetes

(2.0%). Difficulties in glycemic control, increased disease severity and frequent episodes of ketoacidosis and hypoglycaemia were postulated as explanations for this difference. Other studies have found the opposite results^[46,47]. Clausen *et al.*^[47] compared 389 type 1 and 146 type 2 diabetics delivering in the United Kingdom. Pregnancies affected by type 2 diabetes had worse perinatal outcomes with congenital abnormalities (12.3% in type 2 vs 4.4% in type 1; $P = 0.002$) accounting for most of this difference. In 2005, Clausen *et al.*^[47] showed similar results with almost doubled number of congenital anomalies among pregnant women with type 2 diabetes (6.6%) compared to type 1 diabetes (2.9%)^[46]. Both studies cited a delay in antenatal care, suboptimal glycemic control prior to conception, inadequate folate supplementation and maternal obesity, as possible reasons to explain the worse outcomes in pregnancies complicated by type 2 diabetes. Another explanation could be the tendency toward oral glycemic agents in type 2 diabetes, which may lead to less than optimal glycemic control compared to the use of insulin only in type 1 diabetes. Conversely, Roland *et al.*^[48], showed a high rate of congenital anomalies in women with type 1 diabetes and type 2 diabetes, compared with the nondiabetic population, with no significant difference between them. Jensen *et al.*^[43] found similar results.

Over the past years, there has been a great improvement in perinatal outcomes for women with type 1 diabetes. However, with rising incidence of obesity and type 2 diabetes, it seems that type 2 diabetes has become a more prominent concern. Women with type 2 diabetes tend to be under less stringent control of their glucose values, either because they use oral glycemic agents, have lower compliance with management strategies or have a lower prevalence of pre-pregnancy counselling, as sometimes type 2 diabetes is regarded as a "lesser" problem compared with type 1 diabetes. Moreover, lack of folate supplementation, obesity with lower ultrasound detection rates and other demographic differences between the women with type 1 or type 2 diabetes may account for the differences showed by different studies comparing the types of diabetes. There is no doubt that pre-pregnancy care and good glycemic control is equally important in type 2 and type 1 diabetes.

PATHOGENESIS OF DIABETES INDUCED BIRTH DEFECTS

Most data regarding the pathophysiology of diabetes associated birth defects originates from animal studies. Our research group, as well as others, have shown that maternal diabetes triggers multiple cellular stress responses and subsequent aberrant signal transduction pathways^[49-51]. All these may contribute to gene dysregulation and apoptosis in the affected organs of the developing embryo leading to structural birth defects. Studies from animal models have shown that maternal

diabetes increases the production of cellular reactive oxygen species and simultaneously impairs endogenous cellular antioxidant capacity, leading to an overall oxidative stress in the embryo^[50,52-55]. Maternal diabetes also appears to increase the expression of inducible nitric oxide synthase (iNOS)^[56], whose enzymatic activity catalyzes the reaction of superoxide with nitric oxide to produce reactive nitrogen species. Work in animal models indicates that reactive nitrogen species create a severe form of oxidative stress, nitrosative stress, which is responsible for the activation of cellular stress signalling. Our laboratory has shown that over expression of an antioxidant enzyme, superoxide dismutase 1 (SOD1), in SOD1 transgenic mice, mitigates maternal diabetes-induced oxidative stress and reduces embryonic malformations in diabetic pregnancies^[57-59]. Likewise, studies indicate that eliminating the *iNOS* gene in *iNOS* knockout mice reduces the incidence of embryonic malformations caused by maternal diabetes^[60]. Therefore, our work and that of others has shown that oxidative and nitrosative stress mediates the teratogenicity of maternal diabetes in the developing embryo.

In our studies, maternal diabetes-induced oxidative stress activates the c-Jun-N-terminal kinase 1/2 (JNK1/2)^[49,50,61-63]. Deletion of either *JNK1* or *JNK2* gene ameliorates maternal diabetes-induced NTDs formation^[49,50], supporting the hypothesis that activation of the cellular stress kinases, JNK1/2, mediates the adverse effect of maternal diabetes on neural tube closure. In the absence of JNK1 or JNK2, maternal diabetes-induced apoptosis in the neuroepithelial cells are blunted^[49,50]. Thus, JNK1/2 activation induced by maternal diabetes transmits the pro-apoptotic signal emanating from oxidative stress under diabetic conditions^[49,50].

JNK1/2 belongs to the mitogen-activated protein (MAP) kinase family, whose activation follows a three-tier cascade: MAP three kinases activate MAP kinase kinases, which in turn trigger JNK1/2 phosphorylation. Subsequent studies have revealed the upstream kinases in diabetic embryopathy, including apoptosis signal-regulating kinase 1 (ASK1)^[64]. ASK1 is a three kinase that leads to JNK1/2 activation. We have observed that *ASK1* gene deletion abolishes maternal diabetes-induced JNK1/2 activation, as well as activation of four major transcription factors downstream of JNK1/2. Similar to our findings in the *JNK1* and *JNK2* gene deletion studies, *ASK1* gene deletion blocks maternal diabetes-induced apoptosis in the developing neural tube, and consequently reduces the number of embryos with NTDs^[64]. Thus, the ASK1-JNK1/2 pathway, which is activated by oxidative stress, appears to play a causal role in the induction of diabetic embryopathy.

Recently, we have worked to understand how cellular ASK1-JNK1/2 kinase signalling relays its pro-apoptotic signals to the nucleus^[64]. We have observed that ASK1 activation increases the activity of Forkhead transcription factor 3a (FoxO3a), and that FoxO3a

induces TNFR1 associated death domain (TRADD)^[64]. TRADD up-regulation triggers caspase 8 activation, which, in turn, activates the caspase cascade and leads to apoptosis^[64]. Both germline deletion and conditional deletion of the *FoxO3a* gene significantly reduce maternal diabetes-induced apoptosis and NTDs formation, underscoring the potential importance of FoxO3a in the induction of diabetic embryopathy^[64]. We have successfully inhibited the whole stress pathway, ASK1-JNK1/2-FoxO3a-TRADD-caspase 8, using an endogenous ASK1 inhibitor, thioredoxin. Thioredoxin treatment ameliorates NTDs formation in embryos of diabetic dams and cultured embryos^[64]. These studies reveal the ASK1 initiated stress signalling with the activity of a transcription factor and pro-apoptotic gene expression.

We and others have shown that JNK1/2 activation leads to endoplasmic reticulum (ER) stress^[50]. Under ER stress, the unfolded protein response (UPR) is activated, and prolonged UPR activation induces apoptosis^[65,66]. Maternal diabetes *in vivo* and high glucose *in vitro* induce ER stress and activate the UPR pathway^[50]. Treatment with an ER chemical inhibitor, 4-Phenylbutyric acid, reduces high glucose-induced JNK1/2 activation, neuroepithelial cell apoptosis and NTD formation^[50]. Deletion of either the *JNK1* or the *JNK2* gene abrogates maternal diabetes-induced UPR and ER stress^[50]. These findings support a reciprocal causation between JNK1/2 and ER stress in diabetic embryopathy. Other studies have found that activation of the inositol-requiring enzyme 1 alpha, one of the major UPR arms, activates ASK1 and subsequently leads to JNK1/2 activation and apoptosis^[67,68], which support our hypothesis that ASK1 plays a major role in diabetic embryopathy.

CONCLUSION

While the risk for major congenital defects has been well established in women with pregestational diabetes, many questions remained unanswered. For example, it is still unknown why some organs display vulnerability to glucose teratological effects more than others, or why meticulous control of maternal blood glucose does not reduce the rate of birth defects to the background risk of non-diabetic population, or why some women with pregestational diabetes will have normal pregnancies even when their levels of HgA1c are way above the accepted threshold for birth defects.

The key to understanding the true relationship between PGD and birth defects lies in a basic understanding of the pathogenesis of congenital defects. Based on our research, the oxidative stress-triggered ASK1 pathway mediates the pro-apoptotic effect of maternal diabetes and high glucose *in vitro* by inducing pro-apoptotic gene expression and causing UPR and ER stress. The endogenous ASK1 inhibitor, thioredoxin, and the ER stress inhibitor, a Food and Drug Administration approved drug, may be potential therapeutics against maternal diabetes-induced structural birth defects.

We believe further research to evaluate glucose teratological effects on different embryonic organs, *via* animal models that can encompass the diversity seen in human diabetes, is needed. Research should utilize type 1 and type 2 models, as well as models of chronic and acute glycemia and how these two conditions may affect organogenesis. Our hope is that, by combining all known clinical data with current and future basic and translational science studies, we will be able to establish better ways to care for the diabetic mother and prevent the mal-effects of maternal diabetes on the developing embryo.

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Diabetic retinopathy - ocular complications of diabetes mellitus

Martin M Nentwich, Michael W Ulbig

Martin M Nentwich, Michael W Ulbig, Department of Ophthalmology, Ludwig-Maximilians University, 80336 Munich, Germany

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Correspondence to: Dr. Martin M Nentwich, Department of Ophthalmology, Ludwig-Maximilians University, Mathildenstr 8, 80336 Munich,

Germany. martin.nentwich@med.uni-muenchen.de

Telephone: +49-89-440053811

Fax: +49-89-440054569

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Abstract

In industrialized nations diabetic retinopathy is the most frequent microvascular complication of diabetes mellitus and the most common cause of blindness in the working-age population. In the next 15 years, the number of patients suffering from diabetes mellitus is expected to increase significantly. By the year 2030, about 440 million people in the age-group 20-79 years are estimated to be suffering from diabetes mellitus worldwide (prevalence 7.7%), while in 2010 there were 285 million people with diabetes mellitus (prevalence 6.4%). This accounts for an increase in patients with diabetes in industrialized

nations by 20% and in developing countries by 69% until the year 2030. Due to the expected rise in diabetic patients, the need for ophthalmic care of patients (*i.e.*, exams and treatments) will also increase and represents a challenge for eye-care providers. Development of optimized screening programs, which respect available resources of the ophthalmic infrastructure, will become even more important. Main reasons for loss of vision in patients with diabetes mellitus are diabetic macular edema and proliferative diabetic retinopathy. Incidence or progression of these potentially blinding complications can be greatly reduced by adequate control of blood glucose and blood pressure levels. Additionally, regular ophthalmic exams are mandatory for detecting ocular complications and initiating treatments such as laser photocoagulation in case of clinical significant diabetic macular edema or early proliferative diabetic retinopathy. In this way, the risk of blindness can considerably be reduced. In advanced stages of diabetic retinopathy, pars-plana vitrectomy is performed to treat vitreous hemorrhage and tractional retinal detachment. In recent years, the advent of intravitreal medication has improved therapeutic options for patients with advanced diabetic macular edema.

Key words: Laser photocoagulation; Diabetic macular edema; Diabetic retinopathy; Intravitreal injection; Prevention

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Core tip: Diabetic retinopathy is a potentially blinding complication of diabetes mellitus. In patients with diabetes, regular retinal exams are essential. While laser photocoagulation is effective, if performed in time, advanced stages of diabetic retinopathy need to be treated by vitreo-retinal surgery and have limited visual prognosis. Even though new therapeutic options such as intravitreal medical therapy and sutureless pars-plana vitrectomy have improved ophthalmic care of patients

with diabetes, interdisciplinary care of these patients is essential. Good metabolic and blood pressure control is indispensable for reducing the risk of ophthalmic complications.

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INTRODUCTION

Diabetic retinopathy is a potentially blinding complication of diabetes mellitus. Reasons for loss of vision are diabetic maculopathy and complications of proliferative diabetic retinopathy (PDR) such as vitreous hemorrhage, tractional retinal detachment, and neovascular glaucoma. By 2030 developing countries will face an increase by 69% and industrialized countries by 20% of the number of patients with diabetes compared to 2010^[1]. For Africa more than 18 million, according to some estimations even 24 million, diabetic patients are predicted for the year 2030^[1,2].

Probability of retinal complications increases with increasing duration of disease. In up to 50% of patients with type 1 diabetes and 30% of those with type 2 diabetes potentially vision-threatening retinal changes develop over time, while early retinal changes are not noticed by the patients^[3].

Diabetic retinopathy is the most common micro-vascular complication of diabetes mellitus and affects between 3%-4% of people in Europe, while the relative risk for developing diabetic retinopathy is higher in type 1 diabetes compared to type 2^[4-6]. Diabetes mellitus is responsible for about 15% of all cases of legal blindness (best corrected visual acuity less than 0.02) in Germany^[7]. It is the main cause of blindness within the working-age population in industrialized nations^[4].

While retinal changes are rarely seen in patients with type 1 diabetes before adolescence, about one third of patients have signs of diabetic retinopathy at time of initial diagnosis of diabetes mellitus. The risk of PDR is higher in type 1 diabetes than in type 2, while diabetic macular edema is more commonly found in type 2 diabetes (prevalence after 15 years of disease: type 1 vs type 2 = 15% vs 25%)^[8].

Kramer *et al*^[9] reported in a recent study, that in patients with type 1 diabetes progression of diabetic retinopathy and development of nephropathy each increase the risk for incidence of the other. This association was independent of established risk factors for micro-vascular complications and the authors suggested a shared etiologic basis of these two complications of diabetes mellitus^[9]. Another group also found proliferative diabetic retinopathy to be an independent marker of long-term nephropathy in patients with type 1 diabetes^[10].

Other studies indicated association of the presence of diabetic retinopathy and increased overall-mortality and cardiovascular events both in type 1 and type 2 diabetes^[11].

Therefore an interdisciplinary approach of physicians, endocrinologists, and ophthalmologists is needed for optimal care.

PATHOGENESIS OF DIABETIC RETINOPATHY

Micro-angiopathy due to hyperglycemia in patients with diabetes mellitus results in vascular leakage, which causes diabetic macular edema on one hand, and capillary occlusion on the other hand. Capillary occlusion then again causes retinal ischemia and increased levels of vascular endothelial growth factor (VEGF) which are responsible for the development of neovascularization and the proliferative stage of diabetic retinopathy.

More recently new pathways which may be involved in the pathogenesis of diabetic retinopathy have been identified, such as inflammation, nerve growth factor autophagy and epigenetics. A detailed discussion of all these pathways would go beyond the scope of this mini-review about clinical aspects of diabetic retinopathy, however some aspects should be addressed.

Biochemical alterations such as oxidative stress, activation of protein kinase C and formation of advanced glycation end products have been detected as a response of the retina to hyperglycemia^[12]. Also kinin B1 and B2 are thought to increase vascular permeability, infiltration of leukocytes and inflammation. Especially kinin B1, which is almost non-existent in normal tissue, is upregulated in the retina of diabetic patients. These findings may be important for developing new therapeutic strategies aiming at antagonizing kinin receptors or at inhibiting kallikreins^[13].

Recent investigations showed that the whole retinal neurovascular system is impaired by diabetes mellitus resulting in loss of neurovascular coupling, neurodegeneration and neuroinflammation, which can be detected even before the advent of vascular damage^[14]. Clinically, reduced dark adaption, impaired colour and/or contrast vision and visual field defects are found during functional examinations of diabetic patients^[15].

Diabetic retinopathy tends to deteriorate during hormonal changes such as adolescence and pregnancy^[16].

CLASSIFICATION AND PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

During fundoscopy, which should be performed after dilation of the pupil (mydriasis) to allow visualization of the entire retina, presence and grade of diabetic retinopathy can be assessed clinically. Typical changes seen in early diabetic retinopathy (non-PDR) are micro-



Figure 1 Non-proliferative diabetic retinopathy. Wide-field fundus photo of a 65-year-old female patient (right eye) showing several retinal hemorrhages.



Figure 2 Non-proliferative diabetic retinopathy. Color fundus photo of a 51-year-old male patient with micro-aneurysms and lipid exudates.



Figure 3 Proliferative diabetic retinopathy with neovascularization at disk.

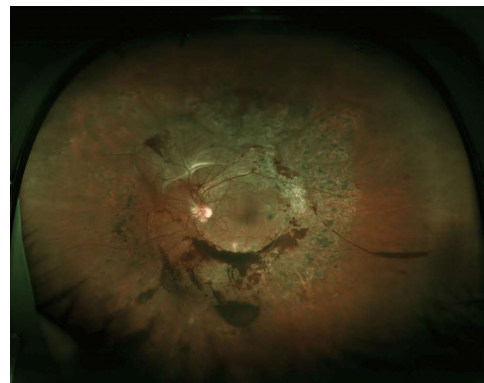


Figure 4 Advanced proliferative diabetic retinopathy with neovascularization and limited vitreous hemorrhage.

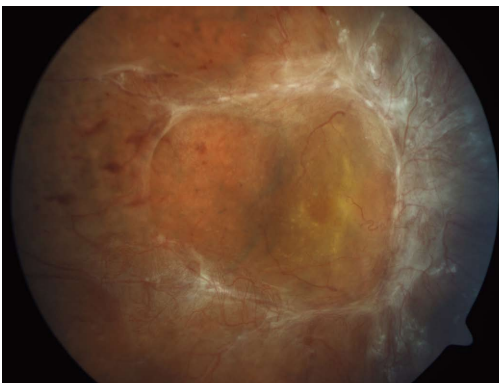


Figure 5 Advanced proliferative diabetic retinopathy with tractional retinal detachment.

aneurysms, retinal hemorrhages, and exudates (Figure 1). In the beginning, these alterations are often found slightly temporal to the central area of the macula (Figure 2). These are caused by deranged vascular integrity and loss of pericytes. In the later course of disease, intra-retinal micro-vascular anomalies may develop, which represent dilated and therefore during fundoscopy visible retinal capillaries, and indicate possible risk of neovascularization and proliferative diabetic retinopathy.

Proliferative diabetic retinopathy is caused by increased intraocular VEGF-levels due to retinal ischemia because of capillary occlusion of retinal vessels. Proliferation may

grow at the optic disc (neovascularization at the disk) or elsewhere in the retina (neovascularization elsewhere) into the vitreous (Figure 3). These newly formed vessels leak on fluorescein angiography and may cause vitreous hemorrhage and finally tractional retinal detachment (Figures 4 and 5). Tractional retinal detachment results in separation of the neurosensory retina from the retinal pigment epithelium. Detached sections of the retina cause relative visual-field defects (scotoma) and loss of visual acuity in cases with macular involvement.

Further, neovascularization on the iris in the anterior segment of the eye may evolve as anterior segment sequel of ischemia (Figure 6). These new blood vessels grow towards the anterior chamber angle and may obstruct the trabecular meshwork, thus increasing outflow resistance of aqueous humor. This results in increased intraocular pressure followed by optic atrophy (neovascular glaucoma).

CLASSIFICATION AND PATHOPHYSIOLOGY OF DIABETIC MACULOPATHY

Diabetic maculopathy may develop in the non-proliferative and in the proliferative stage of diabetic retinopathy. While complications of untreated proliferative diabetic

Table 1 Types of diabetic maculopathy^[19]

Focal	Localized edema Lipid exudates Intraretinal hemorrhages Focal hyperfluorescence in late fluorescein angiography
Clinically significant without foveal thickening (non-center-involving) (sight-threatening)	Edema within 500 µm around the foveola Exudates within 500 µm around the foveola accompanied by edema
Clinically significant with foveal thickening (center-involving)	Edema ≥ 1 optic-disk diameter within one optic-disk diameter around the foveola Ill-defined edema, which may be cystoid Exudates Intraretinal hemorrhages Origin of leakage often not clearly identifiable by fluorescein angiography
Tractional	Due to vitreous traction to the fovea Thickened posterior hyaloid membrane OCT visualizes vitreal traction
Ischemic maculopathy (occlusion of the perifoveal capillaries)	Loss of vision without any clearly visible cause on fundoscopy Fluorescein angiography needed for diagnosis Difficult to diagnose by fundoscopy only Edema may be present or absent

OCT: Optical coherence tomography.

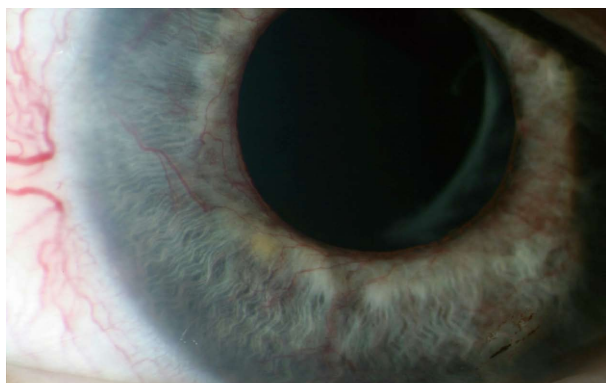


Figure 6 Neovascularization of the iris. These neovascular vessels may block the trabecular meshwork and cause neovascular glaucoma.

retinopathy, such as vitreous hemorrhage and tractional retinal detachment involving the macula may cause the most severe loss of vision in diabetic retinopathy, diabetic maculopathy is the main cause of visual impairment in patients with type 2 diabetes^[17].

Diabetic macular edema is caused by a disruption of the inner blood-retinal barrier, formed by the retinal vascular endothelium, due to hyperglycemia, increased levels of growth-factors, inflammation and cytokines. This also causes impairment of pericytes and leads to consecutive exudation of fluid, proteins and lipids by para cellular and trans cellular transport mechanisms^[18]. Clinically, areas of thickened retina are often demarcated by surrounding yellowish lipid exudates (Figure 7). Diabetic macular edema may be limited or affect a large retinal area and it may also involve the macula (center-involving) or spare the central area (non-center-involving) (Figure 8).

Traction caused by vitreous attachment to the fovea may result in macular thickening, as well. Vitreous traction can excellently be documented by high-resolution optical coherence tomography (OCT, spectral-

domain OCT). Vitrectomy is needed in patients with symptomatic tractional macular edema in order to release traction by surgical removal of the vitreous.

Apart from edema, diabetic maculopathy may also present as occlusion of perifoveal capillaries resulting in distinct visual impairment in the absence of edema on fundoscopy. In these cases, fluorescein angiography is needed to confirm the diagnosis by visualizing capillary occlusion in around the fovea.

Table 1 summarizes the different types of diabetic maculopathy (adapted from Nentwich *et al.*^[19]).

CLINICAL DIAGNOSIS

Dilated funduscopy is the most important clinical investigation in patients with diabetes during screening examinations by an ophthalmologist. Dilation of the pupil is necessary in order to enable a proper stereoscopic view, which is needed for evaluation of macular edema, and to allow visualization of the peripheral retina.

Fluorescein angiography was introduced by Novotny *et al.*^[20] in the 1960s into the clinical ophthalmic practice and allows evaluation of the retinal vascular status. Fluorescein, a fluorescent dye, is injected intravenously and distributes throughout the body. In the eye, fluorescence is activated by blue light of 490 nm. During fluorescein angiography vascular leakage, capillary occlusion, ischemic areas of the retina and neovascularization can be seen (Figure 9A and C). It provides information on the area of leakage as well as of the location of non-perfused parts of the retina. In patients with suspected diabetic maculopathy, fluorescein angiography is mandatory for excluding ischemic maculopathy and guiding possible focal laser photocoagulation.

Wide-field fundus photography (2-laser wavelength nonmydriatic 200° ultra-wide-field scanning laser ophthalmoscopy) has been introduced recently. Wide-field images and traditional color fundus photography

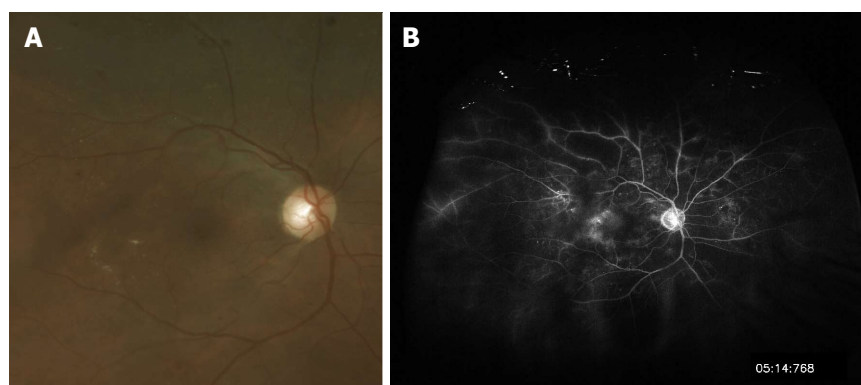


Figure 7 Clinically significant diabetic macular edema without involvement of the fovea. A: Fundus photo; B: Fluorescein angiogram depicting leakage of perifoveal retinal blood vessels.



Figure 8 Center-involving diabetic macular edema with subfoveal edema and numerous lipid exudates.

in mydriasis seem to correlate with regard to the classification of diabetic retinopathy and visualization of diabetic macular edema according to recent studies (Figure 10A)^[21-23]. This wide-field imaging technique can be combined with fluorescein angiography and provides information about peripheral retinal ischemia or peripheral neovascularization (Figure 10B)^[24].

Probably the most important new imaging technique, which was introduced in ophthalmologic practice, is OCT. Recently, resolution and recording speed have been greatly improved by spectral-domain OCT technique (SD-OCT). OCT provides data on retinal volume and configuration of the macular region. In diabetic macular edema SD-OCT is a very valuable examination technique comparing follow-up visits with baseline data, as modern OCT-devices use eye-tracking for finding the same imaging position during follow-up examinations (Figure 11)^[25].

IMPORTANCE OF PREVENTION

In early diabetic retinopathy, laser photocoagulation can be used effectively for preventing loss of vision^[26]. However, early diabetic retinopathy is not noticed by patients because of the absence of visual loss in early diabetic retinopathy. Thus, regular retinal exams with dilation of the pupil are necessary in patients with

diabetes mellitus in order to detect sight-threatening changes timely and enable ophthalmologists to perform treatment.

Patients with type 1 diabetes should undergo retinal exams starting at the age 11 and/or after 5 years of diagnosis on a yearly basis. In case of retinal changes, shorter follow-up intervals are recommended.

In type 2 diabetes, the first retinal exam should be performed immediately after first diagnosis of diabetes, because the previous duration of the disease is unknown. Annual follow-up examinations are recommended in the absence of retinal changes, otherwise shorter intervals are recommended.

Pregnancy bears an increased risk of worsening of diabetic retinopathy due to hormonal changes. During pregnancy diabetic retinopathy may start in about 10% of cases and may worsen in an even higher percentage in case of preexisting diabetic retinopathy at time of conception^[27]. In case of proliferative diabetic retinopathy before or shortly after conception, pan-retinal laser photocoagulation should be performed as retinal changes may worsen in one out of two women in this population. This risk can be reduced by half by thorough retinal laser photocoagulation. Therefore all females with diabetes, who are planning to get pregnant, should undergo fundoscopy prior to conception and then every three months during pregnancy to enable early intervention, if needed. Additionally good metabolic control should be achieved before conception^[28].

On the other hand, the presence of diabetic retinopathy is no indication for cesarean section per se, as no study has indicated so far, that Valsalva-maneuvers during vaginal delivery bear an increased risk of vitreous hemorrhages^[29].

The German Diabetes Society recommends scheduling of follow-up examinations depending on the grade of diabetic retinopathy as summarized in Table 2 (Adapted from^[30]).

These recommended examination intervals will result in an increased work-load for ophthalmologists due to the rising number of diabetic patients. Studies examined the effect of an extension of follow-up intervals and found no increased risk of progression to sight-

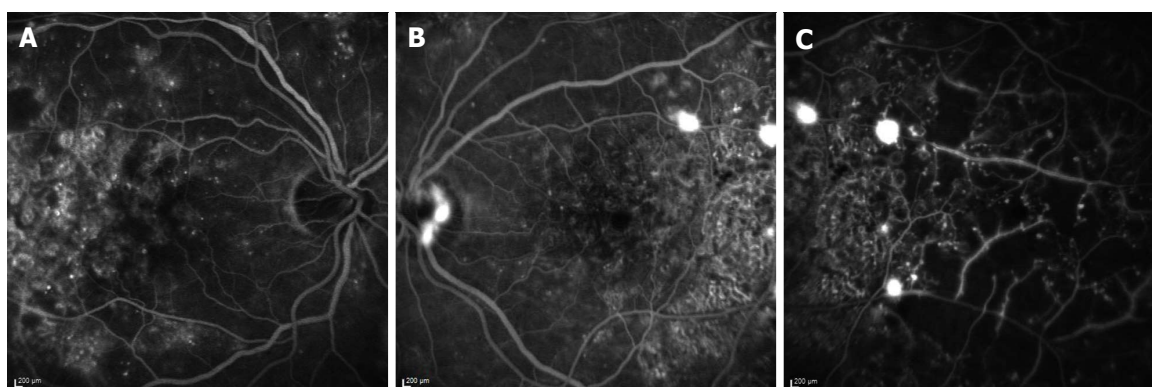


Figure 9 Fluorescein angiogram of a 49-year-old female patient. A: Fluorescein angiogram of the right eye 50 s after intravenous injection of fluorescein dye. Here, leaking micro-aneurysms in the macula can be seen; B: Fluorescein angiogram of the left eye 25 s after intravenous injection of fluorescein dye. Leakage from neovascular blood vessels causes spots of increased fluorescence at the optic disk and temporal to the fovea; C: Fluorescein angiogram of the temporal part of the left eye 30 s after intravenous injection of fluorescein dye. Areas of retinal non-perfusion can be seen as reason for neovascularization.

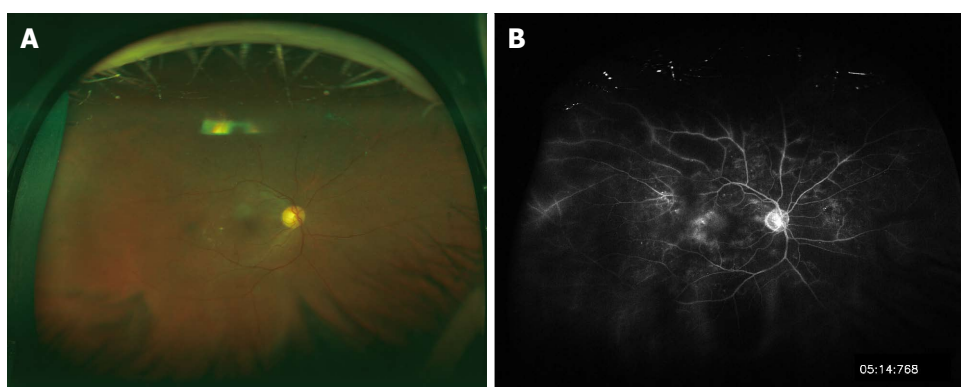


Figure 10 Wide-field picture of the right eye of a 65-year-old female patient. A: On scanning-laser-ophthalmoscope-Imaging some micro-aneurysms and lipid exudates can be seen; B: Fluorescein angiogram shows leakage from micro-aneurysms and extensive areas of retinal non-perfusion.

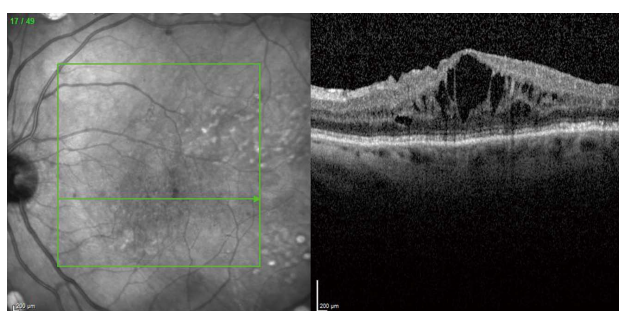


Figure 11 Spectral-domain optical coherence tomography of a female patient with center-involving diabetic macular edema. On the left side of the picture, an infra-red image shows the exact location of the OCT-scan on the right. The OCT-scan visualizes intraretinal edema with thickening of the fovea. OCT: Optical coherence tomography.

threatening diabetic retinopathy in patients with good metabolic control (HbA1c < 8%) and no known diabetic retinopathy^[31-35]. By adequate selection of patients, the number of necessary screening examinations could be reduced by 40% according to one study and even by 59% in case a special mathematical algorithm is used^[31,33]. However, feasibility of these strategies remains to be proven in routine clinical practice and until then

ophthalmic screening intervals as indicated above are recommended.

GENERAL MEDICAL THERAPY AND INTERVENTIONS

Blood glucose and blood pressure control

Good metabolic and blood-pressure control are essential for successful ophthalmic care of patients with diabetes.

In type 2 diabetes, a reduction of HbA1c from 7.9% to 7.0% resulted in a decline in the frequency of laser treatments needed^[36]. In type 1 patients, improved blood glucose control with a reduction of HbA1c values from 9.1% to 7.1% reduced the risk of developing diabetic retinopathy within 6.5 years by 76%, the risk of progression of diabetic retinopathy by 54%, and the risk of developing proliferative diabetic retinopathy by 47%^[37]. Therefore an HbA1c level of about 7% should be aimed for from the ophthalmologist's point of view^[19], for the individual patient a bespoke treatment regime may be needed.

Rapid improvement of metabolic control may result in temporary worsening of diabetic retinopathy ("early-worsening") in patients with long-lasting disease and

Table 2 Recommended timing of retinal examinations in patients with type 2 diabetes^[41]

Patient characteristics	Timing of retinal examination
Initial diagnosis of type 2 diabetes	Soon
No diabetic retinopathy	Once a year
Presence of symptoms such as	During the next few days
Loss of vision	
New difficulties during reading	
Altered color perception	
New, moving dark spots in the eye	
Presence of diabetic retinopathy	Depending on the severity of retinopathy, e.g., every 3-6 mo

high HbA1c levels. Thus, retinal exams should be performed every three months during the first year after initiation of an improved anti-diabetic treatment^[5,38]. However, in the long-term, positive effects of good metabolic control outweigh these initial problems^[37,39]. Also a slow decrease of blood sugar levels, which would be a therapeutic challenge, does not have any advantages in the long term and is not recommended from the ophthalmologist's point of view^[19].

Additionally, optimizing blood pressure helps to reduce the necessity of laser photocoagulation and the risk of loss of vision^[40]. While some studies suggested a protective effect of ACE inhibitors, according to the results of studies available to date, reduction of blood pressure itself seems to be more important than the type of blood pressure lowering medication^[41-43]. Levels of about 140/80 mmHg should be aimed for.

Acetylsalicylic acid and smoking

Acetylsalicylic acid has neither been shown to have any positive effect on diabetic retinopathy or diabetic macular edema, nor to be harmful for patients with diabetic retinopathy. Therefore acetylsalicylic acid should be recommended for cardiovascular reasons in patients with early non-proliferative diabetic retinopathy, in case there are no contraindications^[44].

Data on the effect of smoking on diabetic retinopathy is heterogeneous. While some studies did not find an association of smoking with diabetic retinal changes, others identified smoking in a multiple regression analysis as risk factor for any grade of diabetic retinopathy^[45-47]. However, results of a recent experimental study suggest that nicotine expedites diabetes-induced retinal changes^[48]. Additionally, patient characteristics such as selective mortality among smokers and patients with proliferative diabetic retinopathy at baseline may provide some explanation for the apparently absent association, reported in some studies^[47]. Cessation of smoking should therefore be recommended from the ophthalmologist's point of view.

OPHTHALMIC THERAPEUTIC OPTIONS

Diabetic macular edema

Until the advent of anti-VEGF medication, focal and grid laser photocoagulation was the standard of care

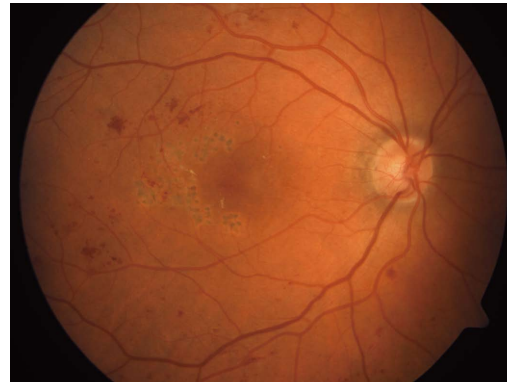


Figure 12 Color fundus photo of a 53-year-old male patient several months after focal laser photocoagulation because of clinical significant diabetic macular edema. While the laser scars and some remaining dot-hemorrhages are visible, lipid exudates and retinal edema have disappeared.

for clinical significant diabetic macular edema (CSME) as well as for diffuse diabetic macular edema.

In patients with focal CSME without involvement of the fovea, focal laser photocoagulation of microaneurysms and localized areas of leakage reduces the relative risk of moderate loss of vision by 50% (from 24% to 12%) as demonstrated by the ETDRS Group (Figure 12)^[49]. In case of persisting areas of leakage two to three months after initial focal laser photocoagulation, repeated laser treatment should be considered^[26].

Apart from the direct effect by coagulating microaneurysms, focal laser photocoagulation is thought to increase oxygenation of the retina by improving diffusion of oxygen from choroidal vessels and to reestablish the blood-retinal barrier by thermal stimulation of the retinal pigment epithelium and of endothelial cells of retinal capillaries^[50].

Grid laser photocoagulation of diffuse diabetic macular edema with involvement of the fovea ("center-involving") has a limited functional prognosis^[49]. Therefore, grid laser photocoagulation has been replaced by intravitreal anti-VEGF therapy, which provides superior functional results^[51].

VEGF is associated with a breakdown of blood-retinal barrier causing leakage and retinal edema^[52]. Intravitreal application of anti-VEGF medication enables high local concentrations in the vitreous and a low systemic exposure. However, repeated intravitreal injections of anti-VEGF medications are necessary in patients with center-involving diabetic macular edema. The average number of injections is around 7 in the first year of treatment and 4 in the second year as indicated by recent studies^[53,54]. Intravitreal anti-VEGF therapy is generally safe with regard to side-effects of medication, although the incidence of systemic thromboembolic events varies among trials^[55]. As the intravitreal application of medication is a surgical procedure, it is associated with the possible risk of postoperative infection, which is called endophthalmitis in case of severe infection of the inner eye and its internal structures. For this reason several recommendations of ophthalmologic societies



Figure 13 Wide-field picture of the right eye of a 48-year-old male patient after pan-retinal laser photocoagulation because of proliferative diabetic retinopathy. The peripheral laser scars can be seen in the picture, while neovascularization have regressed.



Figure 14 Proliferative diabetic retinopathy with extensive fibro-vascular membranes.

have been published concerning prevention of post-injection endophthalmitis. Recent studies reported very low rates of post-injection endophthalmitis of less than 1 in 8000 injections performed in an operating theater setting after diligent disinfection of the conjunctiva with povidone-iodine, using sterile gloves and wearing face-masks^[56,57]. Other studies report somewhat higher rates of infectious endophthalmitis after intravitreal injection, both after procedures performed in an office-setting and in a theater-setting^[58]. The effect of meticulous povidone-iodine prophylaxis on the conjunctival bacterial load and contamination of needles used for injection prophylaxis has been shown in several studies^[59-62].

Apart from anti-VEGF medication intravitreal steroids have been evaluated for the treatment of diabetic macular edema. Compared to anti-VEGF medication, steroids have additional anti-inflammatory effects and sustained-release devices can lengthen the intervals between re-treatments^[63]. In Europe, a non-absorbable implant containing 190 µg fluocinolone acetonide has been approved as second-line treatment of chronic diabetic macular edema not responding to other therapeutic options. Also approval of an absorbable dexamethasone implant for the treatment of diabetic macular edema is expected by the end of 2014. While

intravitreal steroids are effective in reducing diabetic macular edema, patients need to be informed about possible progression of cataract and increased intraocular pressure^[64-67].

Proliferative diabetic retinopathy

In early proliferative diabetic retinopathy, pan-retinal laser photocoagulation is effective in reducing the risk of visual loss, while surgery needs to be performed in very advanced proliferative diabetic retinopathy^[68]. The suggested screening intervals of patients with diabetes are intended to enable the treating ophthalmologist to detect proliferation amenable for laser treatment early. Pan-retinal laser photocoagulation should be performed when retinal neovascularization is detected during fundoscopy or *via* fluorescein angiography in order to prevent complications of proliferative diabetic retinopathy such as vitreous hemorrhage or tractional retinal detachment (Figure 13)^[68]. Pan-retinal laser photocoagulation aims at eliminating non-perfused parts of the retina, thus reducing ischemia, intravitreal VEGF-levels, and the stimulus for proliferation. The efficacy of pan-retinal laser photocoagulation in reducing the risk of loss of vision has already been shown more than 30 years ago^[68]. However, patients need to be informed about side-effects of pan-retinal laser treatments such as constriction of visual fields and reduced dark-adaptation due to loss of rod function. These side-effects may also interfere with the patients' driving ability.

Pars-plana vitrectomy is performed in advanced cases of proliferative diabetic retinopathy, the presence of extensive tractional membranes, vitreous hemorrhage, and tractional retinal detachment (Figure 14). About ten years ago trans conjunctival sutureless 23 gauge vitrectomy started to replace conventional 20 gauge technique and offers comparable safety and efficacy as well as reduced surgery-times and faster rehabilitation of patients^[69].

Combination of diabetic macular edema and proliferative diabetic retinopathy

In patients with diabetic macular edema and proliferation, treatment of macular edema should be performed before pan-retinal laser photocoagulation in order to prevent worsening of macular edema due to peripheral pan-retinal laser photocoagulation.

CONCLUSION

In patients with diabetes, regular retinal exams are essential. While laser photocoagulation is effective, if performed in time, advanced stages of diabetic retinopathy need to be treated by vitreo-retinal surgery and have limited visual prognosis. Even though new therapeutic options such as intravitreal medical therapy and sutureless pars-plana vitrectomy have improved ophthalmic care of patients with diabetes, interdisciplinary care remains essential. Good metabolic and blood pressure control is indispensable for reducing the risk of

ophthalmic complications.

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Tyrosine isomers and hormonal signaling: A possible role for the hydroxyl free radical in insulin resistance

Gergő A Molnár, Esztella Zsóka Mikolás, István András Szijártó, Szilárd Kun, Eszter Sélley, István Wittmann

Gergő A Molnár, Esztella Zsóka Mikolás, István András Szijártó, Szilárd Kun, Eszter Sélley, István Wittmann, 2nd Department of Medicine and Nephrological Center, University of Pécs, H-7624 Pécs, Hungary

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Correspondence to: István Wittmann, MD, PhD, Professor of Medicine, Head of the Department, 2nd Department of Medicine and Nephrological Center, University of Pécs, Pácsi Str. 1, H-7624 Pécs, Hungary. istvan.wittmann@aok.pte.hu
 Telephone: +36-72-536050

Fax: +36-72-536051

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can attack macromolecules, such as lipids, nucleic acids or amino acids. Phenylalanine (Phe) can be enzymatically converted to the physiological para-tyrosine (p-Tyr); however, a hydroxyl free radical attack on Phe may yield meta- and ortho-tyrosine (m- and o-Tyr, respectively) in addition to p-Tyr. Hence, m- and o-Tyr may be regarded as markers of hydroxyl free radical-induced damage. Their accumulation has been described; *e.g.*, this accumulation has been found in the urine of patients with diabetes mellitus (DM) and/or chronic kidney disease, in cataract lenses, in vessel walls, in irradiated food and in amniotic fluid, and it may serve as an indicator of oxidative stress. The use of resveratrol to treat patients with type 2 DM led to a decrease in the urinary excretion of o-Tyr and concomitantly led to an improvement in insulin signaling and insulin sensitivity. Literature data also suggest that m- and o-Tyr may interfere with intracellular signaling. Our group has shown that erythropoietin (EPO) has insulin-like metabolic effects on fat cells in addition to its ability to promote the proliferation of erythroid precursor cells. We have shown that the supplementation of cell culture medium with m- and o-Tyr inhibits erythroblast cell proliferation, which could be ameliorated by p-Tyr. Additionally, *in vivo*, the o-Tyr/p-Tyr ratio is higher in patients with renal replacement therapy and a greater need for EPO. However, the o-Tyr/p-Tyr ratio was an independent determinant of EPO-resistance indices in our human study. The o-Tyr content of blood vessel walls inversely correlates with insulin- and acetylcholine-induced vasodilation, which could be further impaired by artificial oxidative stress and improved by the use of antioxidants. In rats that receive o-Tyr supplements, decreased vasorelaxation is detected in response to insulin. Additionally, o-Tyr supplementation led to the incorporation of the unnatural amino acid into cellular proteins and caused a decrease in the insulin-induced phosphorylation of endothelial nitric oxide synthase. Our data suggest that m- and o-Tyr may not only be markers of oxidative stress; instead, they may also be incorporated into cellular proteins, leading to resistance to insulin, EPO and acetylcholine.

Abstract

Oxidative stress processes play a major role in the development of the complications associated with diabetes and other diseases *via* non-enzymatic glycation, the hexosamine pathway, the polyol pathway and diacylglycerol-protein kinase C. Oxidative stress may lead to the production of hydroxyl free radicals, which

Key words: Acetylcholine; Insulin resistance; Hormone resistance; Oxidative stress; Para-tyrosine; Ortho-tyrosine; Meta-tyrosine; Erythropoietin

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Core tip: Hydroxyl-free radical-derived amino acids, such as meta- and ortho-tyrosine (m- and o-Tyr, respectively) are regarded as free radical markers, but they may also be taken up and incorporated into blood vessel walls, erythroid precursors and endothelial cells. These pathological amino acids can induce vascular insulin and acetylcholine, as well as cellular erythropoietin resistance.

Molnár GA, Mikolás EZ, Szijártó IA, Kun S, Sélley E, Wittmann I. Tyrosine isomers and hormonal signaling: A possible role for the hydroxyl free radical in insulin resistance. *World J Diabetes* 2015; 6(3): 500-507 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i3/500.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i3.500>

OXIDATIVE STRESS IN THE OLD PERSPECTIVES

Oxidative stress plays a role in the pathogenesis of many diseases, such as diabetes mellitus (DM), chronic kidney disease (CKD) and inflammatory diseases, as well as in the development of the complications associated with these diseases. Oxidative stress, free radicals and reactive oxygen species (ROS) are tightly connected to DM in several ways. Hyperglycemia may lead to a shift in cellular metabolism toward the polyol pathway, which leads to an oxidative shift in the NADPH/NADP ratio. NADPH is in turn required for antioxidant defense, *e.g.*, for the reduction of oxidized glutathione by glutathione reductase. Furthermore, hyperglycemia activates the diacylglycerol-protein kinase C intracellular signaling pathway, which can activate NADPH oxidases; this oxidation leads to the translocation of nuclear factor κ B into the nucleus and then to the transcription of proinflammatory cytokines, which results in increased oxidative stress. Additionally, hyperglycemia increases the rate of non-enzymatic glycation, which produces advanced glycation end-products (AGEs) that can bind to their receptors of AGE (RAGE), and the AGE-RAGE interaction also leads to inflammation and oxidative stress. DM involves the enhancement of not only non-enzymatic glycation but also enzymatic glycation *via* the hexosamine pathway; this enhanced glycation may also result in a proinflammatory response and oxidative stress. However, this interplay is complex; the ROS arising from oxidative stress may also contribute to the activation of the abovementioned pathways and reactions and can thus generate a vicious circle^[1,2].

DETECTION OF OXIDATIVE STRESS

The study of oxidative stress processes is therefore important, albeit somewhat cumbersome. Per their definition, free radicals are highly reactive molecules with a very short lifetime; therefore, detecting these molecules requires spin traps and an electron spin resonance device^[3,4], but the sensitivity of this method is usually too low for many diseases. Because of the high reactivity of free radicals, they can react with macromolecules and yield oxidation products, some of which are more chemically stable molecules. These products may include lipid peroxidation products (such as malonyldialdehyde derivatives), nucleobase products (such as 8-hydroxydeoxyguanosine), so-called advanced oxidation products or amino acid derivatives^[5,6].

DETECTION OF HYDROXYL FREE RADICALS BY STABLE L-PHENYLALANINE DERIVATIVES

L-Phenylalanine (Phe) is a highly abundant essential amino acid in proteins of the human body, and Phe plays a role in forming peptides and proteins; Phe also gives rise to the semi-essential amino acid L-para-tyrosine (p-Tyr) and its derivatives, such as 3,4 dihydroxy-phenylalanine (DOPA), and derivatives thereof^[7]. p-Tyr is mainly produced *via* the enzymatic reactions catalyzed by the Phe hydroxylase enzyme, especially in the kidney and liver^[8]. p-Tyr synthesis becomes impaired in patients with renal failure (*e.g.*, CKD); therefore, the serum levels of p-Tyr in these patients are lower than those in patients/controls with normal renal function [CKD: 28 (26-34), DM + CKD: 32 (29-39) μ mol/L vs controls: 56 (36-57) μ mol/L]^[9]. However, other isomers of tyrosine, namely meta- and ortho-tyrosine (m-Tyr and o-Tyr, respectively) also exist in humans. These amino acids cannot be formed enzymatically in humans; instead, they are stable products of the reaction between the hydroxyl free radical and Phe. Additionally, p-Tyr may be formed non-enzymatically, *via* the hydroxyl radical, but the enzymatically produced p-Tyr is much more abundant than the free radical-derived p-Tyr. Therefore, p-Tyr is regarded as the physiologic isoform, whereas m- and o-Tyr are considered to be free radical markers^[10-14].

All four aromatic amino acids (p-Tyr, m-Tyr, o-Tyr and Phe) can be readily detected in the nanomolar range *via* their autofluorescence and reverse-phase high performance liquid chromatography^[9,15-22], as well as by gas chromatography combined with mass spectrometric detection^[23] or by ultra-performance liquid chromatography combined with mass spectrometry^[24].

ABUNDANCE OF THE HYDROXYL FREE RADICAL MARKERS M- AND O-TYR

In vitro measurements^[25] and *in silico* calculations^[26] have shown that in free radical reactions, the three isoforms (p-,

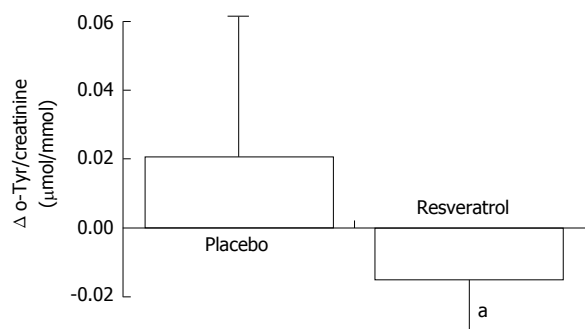


Figure 1 Changes in urinary ortho-tyrosine:creatinine excretion after resveratrol treatment. For each participant, the value measured at baseline was subtracted from that measured at week 4 (*i.e.*, Δ o-Tyr:creatinine ratio), and then the resulting values were averaged within the respective groups. The values are the means, with the standard deviations represented by vertical bars. ^aMean values were significantly different ($P = 0.043$). Comparisons were performed using ANOVA and Bonferroni post hoc tests; $P < 0.05$ was regarded as statistically significant. Republished with permission of Cambridge University Press from Brasnyó *et al.*^[18]. o-Tyr: Ortho-tyrosine.

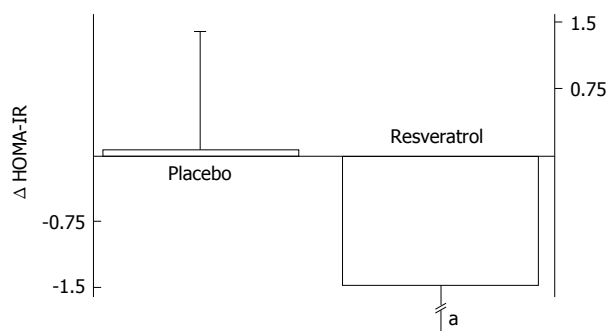


Figure 3 Decrease in homeostasis model of assessment for insulin resistance upon resveratrol treatment. For each participant, the value measured at baseline was subtracted from that measured at week 4 (*i.e.*, Δ HOMA-IR), and then the resulting values were averaged within the respective groups. Values are the means with the standard deviations represented by vertical bars. ^aMean values were significantly different for the resveratrol group vs the placebo group ($P = 0.044$), comparisons were performed using ANOVA and Bonferroni post hoc tests; $P < 0.05$ was regarded as statistically significant. Republished with permission of Cambridge University Press from Brasnyó *et al.*^[18]. HOMA-IR: Homeostasis model of assessment for insulin resistance.

m- and o-Tyr) are not produced stoichiometrically (*i.e.*, 1:1:1), and their concentrations and ratios may vary from tissue to tissue *in vivo*. These isoforms, along with others, have been detected in cataractous lenses by our group and others^[16,27], in the brain after acute oxidative stress^[28], in the serum/plasma of diabetic patients with/without CKD by our group^[9], after the administration of thyroid hormone^[29], after ischemia-reperfusion injury^[14], in the hair of the *Homo tirolensis* and mummies^[30], in irradiated food^[31], in urine specimens^[9,24] and in amniotic fluid^[32]. In earlier publications, we have shown that m-Tyr, o-Tyr and DOPA accumulate in the non-water soluble proteins of cataractous lenses during aging and in patients with DM^[16], and we also showed that the urinary excretion of o-Tyr increases in patients with type 2 DM and/or CKD [CKD: 122 (94-183), DM: 641 (272-499), DM+CKD: 403 (258-651) nmol/d vs controls: 24 (0-35) nmol/d]^[9]. Using the so-called fractional excretion values,

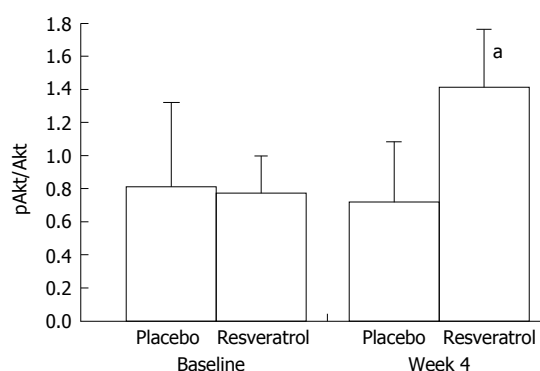


Figure 2 Increase in protein kinase B phosphorylation in platelets upon resveratrol treatment. Values are the means, with the standard deviations represented by vertical bars. ^aMean values were significantly different for baseline vs week 4 within the resveratrol group ($P = 0.032$). Comparisons were performed using ANOVA and post-hoc tests; $P < 0.05$ was regarded as statistically significant. Republished with permission of Cambridge University Press from Brasnyó *et al.*^[18]. pAkt: Protein kinase B phosphorylation.

which show the renal handling of o-Tyr, we have found active urinary secretion or *in loco* renal synthesis of o-Tyr in the kidney of diabetic patients [DM: 125 (69-140), DM + CKD: 112 (69-187)% vs controls: 8 (4-12)%]^[9].

In patients with stroke, the urinary excretion of o-Tyr is associated with the total urinary albumin excretion; immunoreactive albumin excretion; and most significantly, urinary non-immunoreactive albumin excretion^[17].

DECREASED URINARY EXCRETION OF O-TYR IS ASSOCIATED WITH AN IMPROVEMENT IN INSULIN RESISTANCE IN TYPE 2 DIABETES

In a human study, we demonstrated the protective effect of the polyphenolic compound resveratrol in patients with type 2 DM; a short-term administration of resveratrol led to a decrease in urinary o-Tyr excretion (Figure 1), an increase in the phosphorylation of the insulin signaling molecule protein kinase B (or Akt), (Figure 2) and an amelioration of the calculated marker of insulin resistance (homeostasis model assessment-insulin resistance) (Figure 3)^[18].

ARE M- AND O-TYR JUST MARKERS OR ALSO MAKERS?

All of the abovementioned papers focused on m- and o-Tyr as markers of hydroxyl free radical damage. However, the last of our abovementioned studies (on the *in vivo* effects of resveratrol) raised the possibility that these molecules may not only be markers; instead, they may also play a role in the development of pathological states^[18]. Independent of our results, Ruggiero *et al.*^[33,34] showed that m- and o-Tyr can inhibit tumor cell growth *via* influencing the mitogen-activated protein kinase/extracellular signal regulated kinase (ERK) and

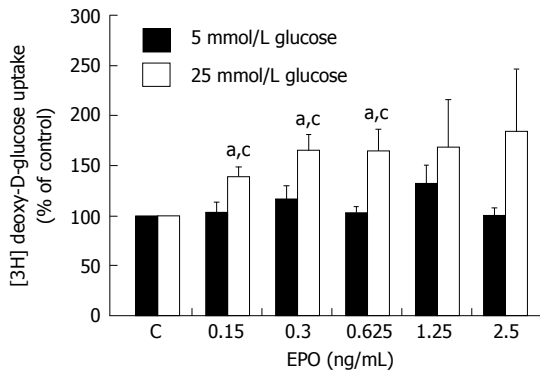


Figure 4 Effects of treatment with r-mo-erythropoietin on the rate of [3H]-deoxy-D-glucose uptake in 3T3-L1 adipocytes cultured in either normal (5 mmol/L) or high (25 mmol/L) glucose medium (insulin: $n = 8$ and $n = 6$; r-mo-erythropoietin: $n = 6$ and $n = 5$, respectively). ^a $P < 0.05$ vs control in the same medium; ^c $P < 0.05$ between the two media; Comparisons with control were performed using a one-sample *t*-test; pairwise comparisons between 5 and 25 mmol/L glucose-cultured cells were performed using a paired samples *t*-test. Republished with permission of Georg Thieme Verlag KG Stuttgart, New York from Mikolás *et al.*^[37]. EPO: Erythropoietin.

the signal transducer and activator of transcription (STAT) pathway. In plants, m-Tyr inhibits cell growth and plant root growth and may be considered a natural herbicide^[35,36]. This finding raises the possibility that the unnatural isoforms, m- and o-Tyr, might affect cellular function in mammals and plants and may interfere with hormonal signaling.

INSULIN-LIKE EFFECT OF ERYTHROPOIETIN ON GLUCOSE METABOLISM

In a subsequent study, we have shown that under hyperglycemic circumstances, erythropoietin (EPO) exerts insulin-like effects on the uptake of isotope-labeled glucose by 3T3-L1-type fat cells (Figure 4) and can lead to the translocation of glucose transporters (GLUTs) from their intracellular pools to the membrane (Figure 5). EPO also improves glucose metabolism in streptozotocin-induced diabetic rats^[37].

M- AND O-TYR ARE INCORPORATED INTO CELLULAR PROTEINS AND LEAD TO ERYTHROPOIETIN RESISTANCE

In further studies, we showed that the administration of m- and o-Tyr to erythroblasts inhibited erythroblast proliferation in a time- and concentration-dependent manner. Increasing doses of p-Tyr could overcome the inhibition by m- and o-Tyr, suggesting potential competition among the structural isoforms (Figure 6).

Erythroblasts grown in cell culture medium supplemented with m- or o-Tyr incorporated the Tyr isoforms into their cellular proteins (Figure 7).

Supplementing erythroblast cells with o- or m-Tyr inhibited the EPO-dependent increase in the rate of

phosphorylation of STAT5 and ERK1/2 (Figure 8). This finding indicates that the unnatural isoforms, o- and m-Tyr, can be incorporated into cellular proteins and interfere with the hormonal signaling of EPO^[21].

This finding is consistent with a clinical observation of our group that the ratio of the pathological o-Tyr to the physiological p-Tyr (o-Tyr/p-Tyr ratio) is higher in patients who receive renal replacement therapy compared with controls. Additionally, the o-Tyr/p-Tyr ratio in the blood was higher in the individuals receiving hemodialysis and EPO replacement than in those patients receiving hemodialysis who did not require EPO replacement or in patients receiving peritoneal dialysis and requiring markedly lower EPO doses. Furthermore, the plasma o-Tyr/p-Tyr ratio was an independent predictor of the EPO dose and EPO-resistance indices in these patients. This finding is another indirect demonstration that o-Tyr may interfere with EPO signaling and lead to EPO resistance^[22].

VASCULAR INSULIN RESISTANCE ACCORDING TO THE O-TYR CONTENT OF THE VESSEL WALL

In a further set of experiments, we analyzed the o-Tyr levels in the blood vessel walls of rodents and found that the o-Tyr content of blood vessels decreases toward the peripheral vasculature (*i.e.*, thoracic aorta > abdominal aorta > femoral artery) (Figure 9A). The o-Tyr content could be increased by treatment with hydrogen peroxide and aminotriazole or by aortic banding, and it could be inhibited using superoxide dismutase and catalase (Figure 9B).

We have shown that vascular segments with higher o-Tyr content show lower vasorelaxation in response to insulin; *i.e.*, the insulin-induced vasorelaxation is lowest in the thoracic aorta, higher in the abdominal aorta and the highest in the femoral artery (Figure 10).

Furthermore, the insulin-induced relaxation could be increased by an antioxidant (superoxide dismutase and catalase) treatment in the thoracic aorta. By contrast, pro-oxidant therapy further diminished the vasorelaxation in an ERK1/2-dependent manner^[19].

VASCULAR ACETYLCHOLINE RESISTANCE ACCORDING TO THE O-TYR CONTENT OF THE VESSEL WALL

In the same set of experiments, the vasorelaxation in response to acetylcholine was also tested, and we also found an inverse relationship between the vessel wall o-Tyr content and the vasorelaxation in response to acetylcholine; *i.e.*, the blood vessels with high o-Tyr content (see Figure 9A) show less vasorelaxation in response to Ach (Figure 11, previously unpublished data).

Based on these experiments, we subjected rats

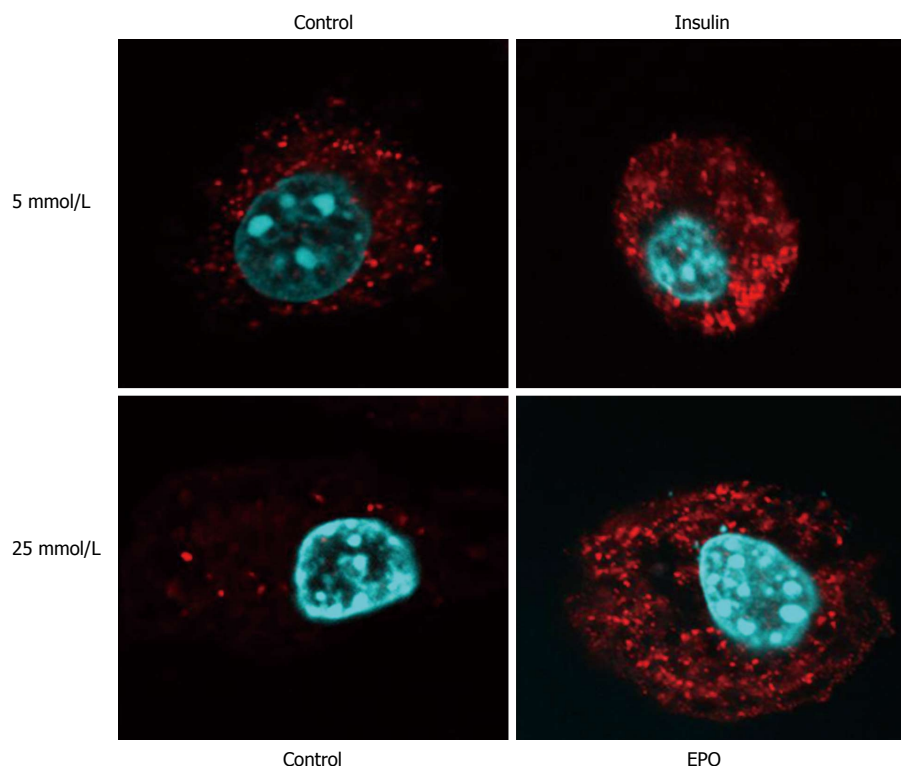


Figure 5 GLUT4 translocation (red fluorescence) after a 30 min treatment with insulin (400 nmol/L) or r-mo-erythropoietin (40 ng/mL) in 3T3-L1 adipocytes cultured in high glucose (25 mmol/L) or normal glucose (5 mmol/L) medium compared with that in untreated cells (Control). Nuclei are shown with blue fluorescence. The GLUT4 translocation was apparent after both the erythropoietin (EPO) and insulin treatments, whereas it was not present in untreated cells. Representative images are shown from $n = 3$ independent experiments. Republished with permission of Georg Thieme Verlag KG Stuttgart, New York from Mikolás *et al.*^[37].

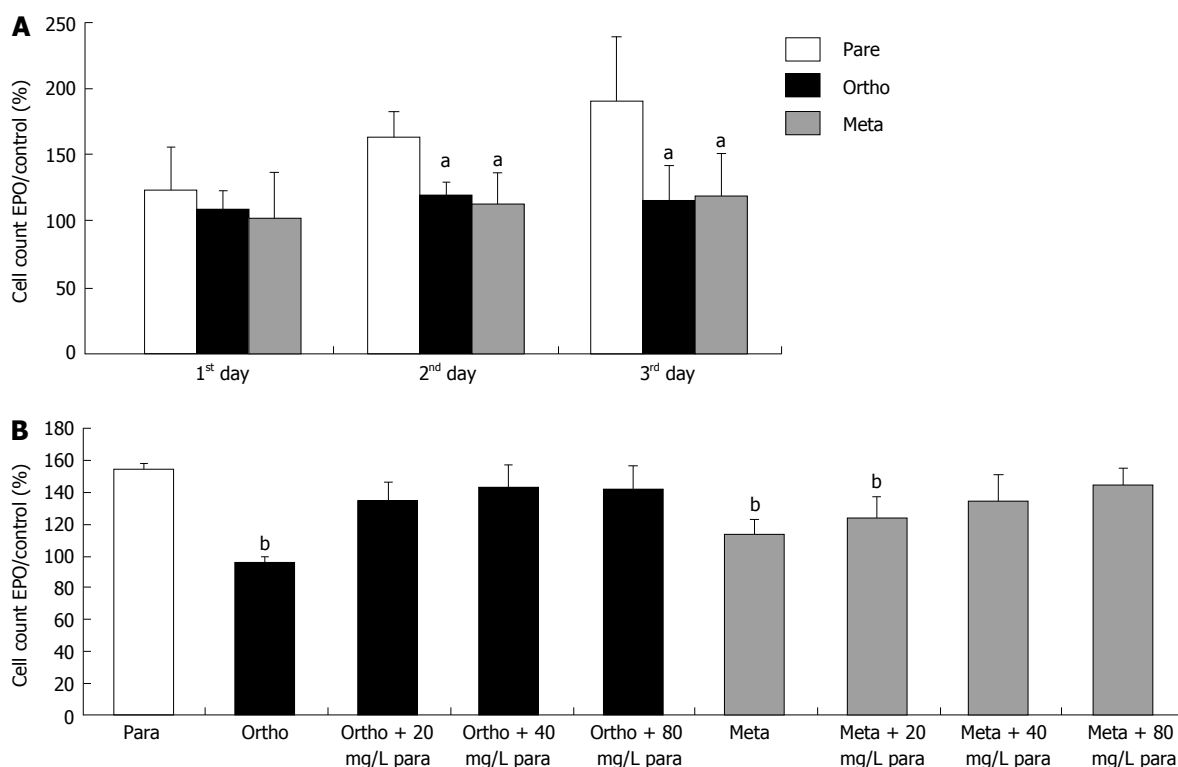


Figure 6 Effect of para-, ortho-, or meta-tyrosine supplementation on the proliferation of cells cultured in medium with or without erythropoietin. A: The time-dependent increase in cell count in medium supplemented with p-Tyr (open bars), o-Tyr (black bars) or m-Tyr (grey bars) ($^aP < 0.05$ vs p-Tyr cultured cells on the same day; $n = 10$); B: The alterations in the cell counts after incubation for 3 d in medium containing o- or m-Tyr and the indicated additional amount of para-tyrosine ($^bP < 0.001$ vs p-Tyr-containing medium; $n = 5$). The results are expressed as the ratio of the protein content of erythropoietin (EPO) and non-EPO (control) cells ($^aP < 0.05$ vs p-Tyr cultured cells; $n = 10$). Comparisons were performed using ANOVA and Bonferroni's post-hoc test. Republished with permission of S Karger AG, Basel from Mikolás *et al.*^[21]. p-Tyr: Para-tyrosine; o-Tyr: Ortho-tyrosine; m-Tyr: Meta-tyrosine.

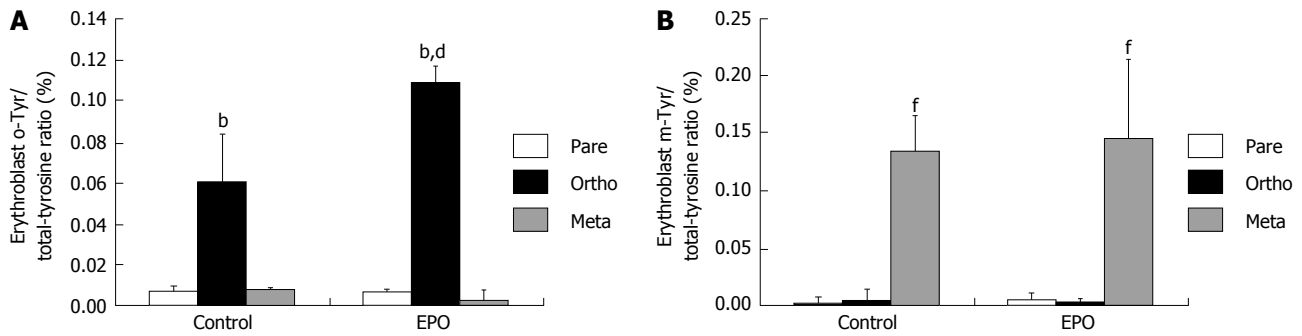


Figure 7 Relative meta- and ortho-tyrosine content (*i.e.*, ratios of meta- and ortho-tyrosine/total tyrosine) of the cellular proteins of TF-1 cells (**A** and **B**, respectively). ^b $P < 0.001$ vs p- and m-Tyr cultured cells; ^{b,d} $P < 0.001$ vs o-Tyr cultured control cells; ^f $P < 0.001$ vs p- and o-Tyr cultured erythroblasts; $n = 10$. Comparisons were performed using ANOVA and Bonferroni's post-hoc test. Republished with permission of S Karger AG, Basel from Mikolás *et al*^[21]. p-Tyr: Para-tyrosine; o-Tyr: Ortho-tyrosine; m-Tyr: Meta-tyrosine.

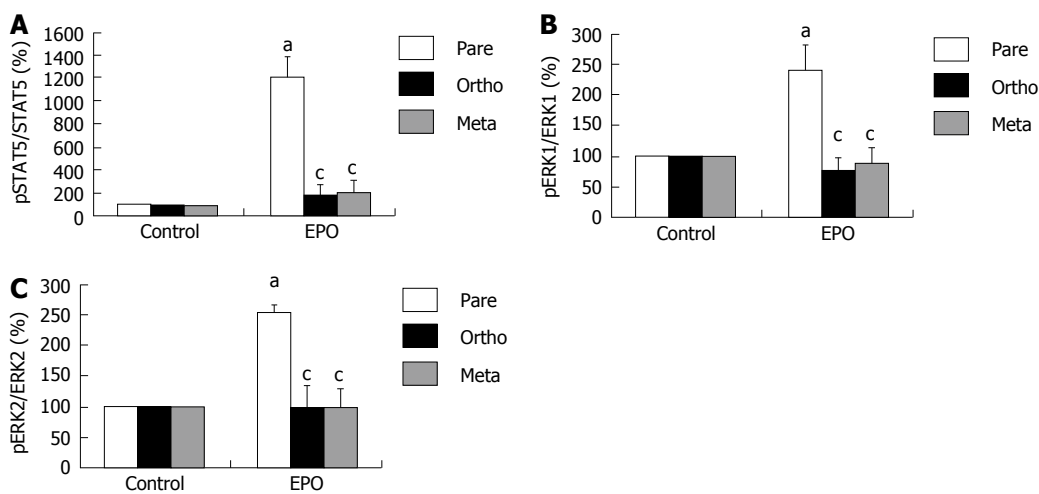


Figure 8 Densitometric analyses of the phosphorylation of signal transducer and activator of transcription 5 (**A**; $n = 3$); densitometric analysis of extracellular signal regulated kinase 1 (**B**; $n = 4$) and extracellular signal regulated kinase 2 (**C**; $n = 4$). The data are expressed as the percent of untreated (control) cells. ^a $P < 0.05$; erythropoietin (EPO) vs control (one-sample *t*-test); ^c $P < 0.05$ vs para EPO (one-way ANOVA). Republished with permission of S Karger AG, Basel from Mikolás *et al*^[21].

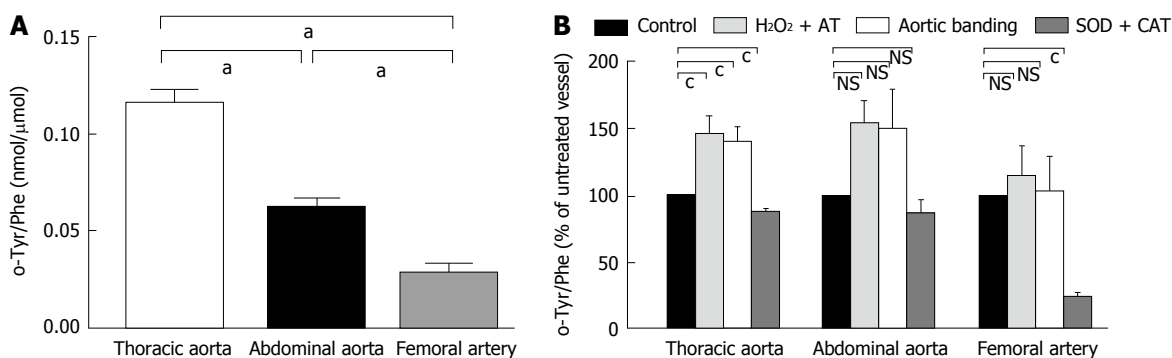


Figure 9 The ortho-tyrosine levels of consecutive arterial segments isolated from rats (**A**). There were significant differences (^a $P < 0.05$) in the o-Tyr levels among the thoracic aorta ($n = 7$), abdominal aorta ($n = 7$), and the femoral artery ($n = 9$) of rats. The changes in the o-Tyr levels in the consecutive arterial segments of rats after H₂O₂ + AT treatment (*i.e.*, high oxidative state), SOD + CAT treatment (*i.e.*, low oxidative state), and aortic banding were compared with those of the control vessels (**B**). Control: untreated, control vessels (thoracic: $n = 7$; abdominal: $n = 7$; femoral: $n = 9$); H₂O₂ + AT: hydrogen peroxide- and aminotriazole-incubated vessels (aminotriazole is an inhibitor of catalase) (thoracic: $n = 5$; abdominal: $n = 5$; femoral: $n = 4$); SOD + CAT: superoxide dismutase- and catalase-incubated vessels (thoracic: $n = 5$; abdominal: $n = 5$; femoral: $n = 5$); aortic banding (thoracic: $n = 5$; abdominal: $n = 4$; femoral: $n = 4$). Data are the mean \pm SEM. The o-Tyr levels are relative to the phenylalanine (Phe) levels (Panels A and B) and are expressed as the percentage of the control vessels (Panel B). ^c $P < 0.05$; NS, $P > 0.05$ (ANOVA). Republished with permission of Informa Healthcare from Szijártó *et al*^[19]. o-Tyr: Ortho-tyrosine; NS: Non-significant; SEM: Standard error of the mean.

to vehicle or o-Tyr supplementation for 4 wk. By the end of the 4 wk, we showed decreased vasorelaxation in response to insulin in the o-Tyr-supplemented rats

compared with the controls. Additionally, the endothelial cells that received o-Tyr supplementation incorporated o-Tyr into their cellular proteins. In these cells, the

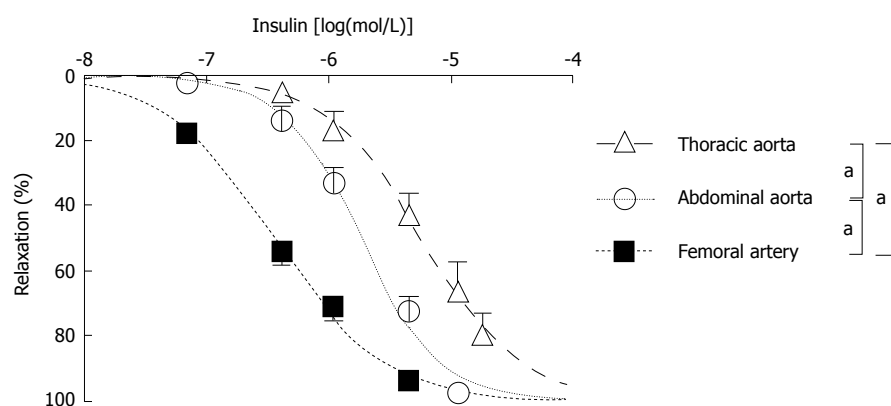


Figure 10 The dose-response curve of the insulin-induced relaxation of consecutive arterial segments isolated from rats. The relaxation in response to insulin was the greatest in the femoral artery ($n = 7$) and was less pronounced in the abdominal aorta ($n = 7$) and even less in the thoracic aorta ($n = 7$). The relaxation is depicted as a function of the logarithm of the insulin dose. Data are the mean \pm SEM. $^aP < 0.05$; NS, $P > 0.05$ (Extra sum-of-squares F test). Republished with permission of Informa Healthcare from Szijártó *et al.*^[19]. NS: Non-significant; SEM: Standard error of the mean.

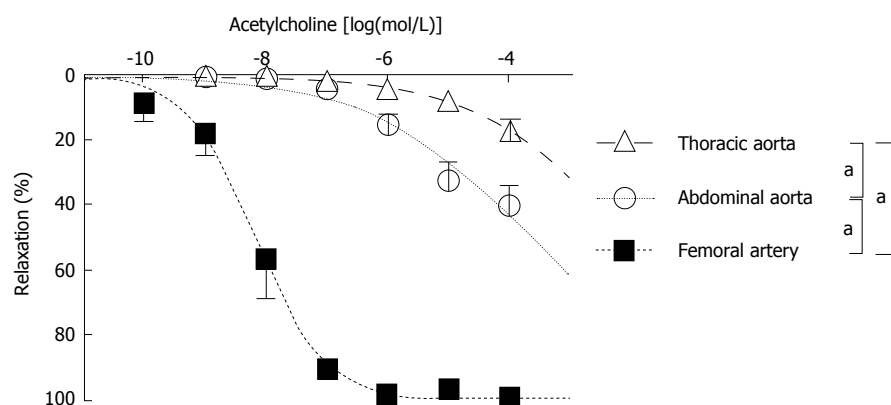


Figure 11 Acetylcholine-induced vasorelaxation in the abdominal aorta and femoral artery. Data are the means \pm SEM. $^aP < 0.05$; NS, $P > 0.05$ (Extra sum-of-squares F test). NS: Non-significant.

insulin-induced increase in endothelial nitric oxide synthase phosphorylation was diminished compared with that in the control cells^[20] (data not shown).

CONCLUSION

Our results, which are consistent with previous findings, indicate that m- and o-Tyr are valuable markers of oxidative stress and other types of stress in patients or experimental animals with DM. Furthermore, the results suggest that these unnatural amino acids may also perform a pathogenic role, *i.e.*, interfere with the signaling of three distinct hormones: insulin (vascular and perhaps metabolic signaling), acetylcholine and erythropoietin (also has metabolic effects). This inhibition may be even more pronounced in patients who have high levels of the pathological amino acids m- or o-Tyr (*e.g.*, in DM) and simultaneously have lower levels of physiological p-Tyr (*e.g.*, patients with impaired kidney function). This finding, together with the effect in which p-Tyr overcomes the inhibitory effect of m- and o-Tyr, raises the possibility that the physiological amino acid p-Tyr could be a therapeutic tool in hormone resistance in states with increased oxidative stress (*e.g.*,

in DM).

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Interplay between Rab27a effectors in pancreatic β -cells

Mami Yamaoka, Toshimasa Ishizaki, Toshihide Kimura

Mami Yamaoka, Toshimasa Ishizaki, Toshihide Kimura, Department of Pharmacology, Oita University Faculty of Medicine, Oita 879-5593, Japan

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Correspondence to: Toshihide Kimura, Associate Professor, Department of Pharmacology, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama, Yufu, Oita 879-5593, Japan. t-kimura@oita-u.ac.jp

Telephone: +81-97-5865722

Fax: +81-97-5865729

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Abstract

The small GTPase Rab27a is a member of the Rab family that is involved in membrane trafficking in various kinds of cells. Rab27a has GTP- and GDP-bound forms, and their interconversion regulates intracellular signaling pathways. Typically, only a GTP-bound GTPase binds its specific effectors with the resulting downstream signals controlling specific cellular functions. We previously

identified novel Rab27a-interacting proteins. Surprisingly, some of these proteins interacted with GDP-bound Rab27a. The present study reviews recent progress in our understanding of the roles of Rab27a and its effectors in the secretory process. In pancreatic β -cells, GTP-bound Rab27a regulates insulin secretion at the pre-exocytotic stages *via* its GTP-specific effectors such as Exophilin8/Slac2-c/MyRIP and Slp4/Granuphilin. Glucose stimulation causes insulin exocytosis. Glucose stimulation also converts Rab27a from its GTP- to its GDP-bound form. GDP-bound Rab27a interacts with GDP-specific effectors and controls endocytosis of the secretory membrane. Thus, Rab27a cycling between GTP- and GDP-bound forms synchronizes with the recycling of secretory membrane to re-use the membrane and keep the β -cell volume constant.

Key words: Insulin; Exocytosis; Endocytosis; Rab27a; IQGAP1; Coronin 3; Glucose; Small GTPase

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Core tip: The small GTPase Rab27a is a member of the Rab family that is involved in membrane trafficking in pancreatic β -cells. GTP-bound Rab27a regulates insulin secretion at pre-exocytotic stages *via* its GTP-specific effectors such as Exophilin8/Slac2-c/MyRIP and Slp4/Granuphilin. Glucose stimulation causes insulin exocytosis. Glucose stimulation also converts Rab27a from its GTP- to its GDP-bound form. GDP-bound Rab27a interacts with GDP-specific effectors and controls endocytosis of the secretory membrane. Thus, Rab27a cycling between GTP- and GDP-bound forms synchronizes with the recycling of secretory membrane to re-use the membrane and keep the β -cell volume constant.

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INTRODUCTION

Diabetes mellitus is defined as chronic hyperglycemia due to relative insulin deficiency. Impairment of secretory activity in pancreatic β -cells plays an important role in the pathogenesis of this disease. In particular, decreased output in the early phase of glucose-induced insulin release precedes the onset of type 2 diabetes mellitus^[1,2]. Insulin secretion from pancreatic β -cells is finely tuned for glucose homeostasis by multiple physiological factors such as nutrients, hormones and neurotransmitters. Some of these factors induce or enhance insulin release whereas others decrease it, thereby enabling sophisticated glucose regulation. One characteristic of this regulation in pancreatic β -cells is the control of secretion by several nutrients. Glucose, the most important insulin secretagogue, stimulates insulin secretion mainly by the generation of ATP *via* glucose metabolism, although there appear to be other signaling pathways involved that have not been fully identified.

The insulin secretory process comprises insulin synthesis and its packaging into secretory granules, granule transport in the cytoplasm, interaction with the cell membrane, exocytosis as a result of an increase in cytoplasmic Ca^{2+} , endocytosis and retrograde transport. The present study reviews recent progress in our understanding of the roles of the small GTPase Rab27a and its effectors in the secretory process.

SMALL GTPASE CYCLE

Small GTPases are GTP-binding proteins with molecular masses ranging from 20 to 30 kDa. These proteins, comprising Ras, Rho, Rab, Ran and Sar/Arf, are expressed in almost all eukaryotic cells and participate in a wide variety of cellular functions including proliferation, cytoskeletal rearrangement and intracellular transport^[3]. Small GTPases have GTP- and GDP-bound forms, and their interconversion regulates intracellular signaling pathways (Figure 1). Typically, only the GTP-bound small GTPase binds its specific effectors and the resulting downstream signals control specific cellular functions^[4]. Therefore, GTP- and GDP-bound small GTPases are considered as active and inactive forms, respectively. The activation of small GTPases is modulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and GDP-dissociation inhibitors (GDIs). Small GTPases localize in the cytosol as a GDP-bound form under unstimulated conditions. Cell stimulation recruits GDP-bound small GTPases to the vicinity of the plasma membrane and converts them to the GTP-bound form through the action of GEFs^[4,5]. These GTP-bound forms interact with their specific effectors and transduce signals. GAPs promote the intrinsic GTPase activity of small GTPases and induce the conversion of the GTP- to the GDP-bound form^[4,6]. GDIs form a complex with GDP-bound small GTPases and induce their intracellular redistribution from the

plasma membrane to the cytosol^[7-9].

RAB27A

The Rab family, which consists of more than 60 members, regulates membrane trafficking^[10-12]. Rab27 is a Rab-family member that controls vesicle transport in various kinds of cells^[13]. There are two isoforms of Rab27; Rab27a and Rab27b. Mutations in Rab27a have been reported to cause Griscelli syndrome^[14]. This disorder results in pigment dilution in the skin and the hair. The same symptoms are observed in ashen mice with a natural mutation in Rab27a^[15]. To date, three forms of Griscelli syndrome have been reported. Mutations in Myosin Va^[14,16-18], Rab27a^[14,15], and its effector Slac2-a/melanophilin^[19,20] cause type 1 (GS1), type 2 (GS2), and type 3 (GS3) Griscelli syndrome, respectively. These proteins play an important role in melanosome transport in melanocytes. Peripheral melanosome distribution is regulated by the molecular motor protein myosin Va. GTP-bound Rab27a localizes on the surface of melanosomes where it interacts with Slac2-a/melanophilin. Since Slac2-a/melanophilin binds myosin Va, this complex functions as a linker protein between the melanosome and the motor protein^[10,21,22]. GTP-bound Rab27 also interacts with another effector protein Slp2-a, thereby regulating docking of the melanosome to the plasma membrane^[23].

Griscelli syndrome also results in immunodeficiency^[14,17]. Exocytosis of granzyme-A-containing granules is decreased in ashen mice cytotoxic T lymphocytes^[24,25]. Although both Rab7 and Rab11 are also present on the surface of these granzyme-A-containing granules, Rab27a is the main regulator of granule exocytosis^[26]. Indeed, GTP-bound Rab27a interacts with phosphatidylinositol 4,5-bisphosphate (PIP₂) *via* its effector protein Munc13-4 and regulates the docking of the granules to the synapse. These results suggest that Rab27a regulates the secretion of lytic granules in cytotoxic T lymphocytes.

Rab27b, a closely related isoform of Rab27a, also participates in intracellular transport and secretion. Rab27b and its effector proteins including Slac2-c and Slp4-a are expressed in parotid acinar cells^[27]. In these cells, GTP-bound Rab27a interacts with both Slac2-c and Slp4-a. Amylase release was inhibited when streptolysin O-permeabilized cells were treated with anti-Slac2-c antibody^[28]. Amylase release was also inhibited when the Rab27b/Slp4-a complex was dissociated with a GST-Slp4-a-linker^[29]. Isoproterenol (IPR) stimulation promoted intracellular redistribution of Slac2-c from the cytosol to a luminal site^[30]. Since Slac2-c potentially interacts with Myosin Va, it is possible that the Rab27b/Slac2-c complex regulates F-actin-dependent intracellular transport of secretory vesicles. In contrast, the intracellular distribution of Slp4-a was not changed in the presence or absence of IPR stimulation^[30]. The Rab27b/Slp4-a complex may regulate docking of the secretory vesicles to the membrane^[31].

Amylase is also released from pancreatic acinar

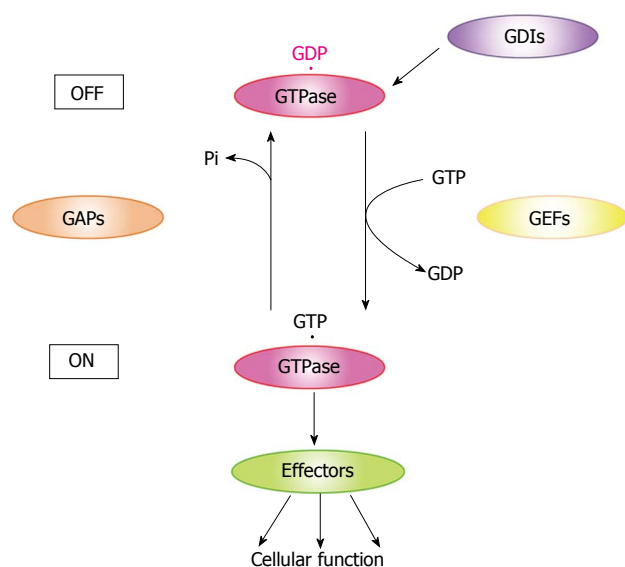


Figure 1 Small GTPase GTP/GDP cycle. Small GTPases localize in the cytosol as a GDP-bound form under unstimulated conditions. Cell stimulation recruits GDP-bound small GTPases to the vicinity of the plasma membrane and converts these proteins to the GTP-bound form through GEF activity. The GTP-bound form interacts with its specific effectors and thereby transduces signals. GAPs promote the intrinsic GTPase activity of small GTPases and induce conversion of the GTP- to the GDP-bound form. GDIs form a complex with GDP-bound small GTPases and induce their intracellular redistribution from the plasma membrane to the cytosol. GEFs: Guanine nucleotide exchange factors; GAPs: GTPase-activating proteins; GDIs: GDP-dissociation inhibitors.

cells in which Rab27b is localized on the surface of zymogen granules^[32]. Rab27b-Q78L, a constitutively active Rab27b mutant, enhances amylase release. In contrast, Rab27b-N133I, a dominant negative mutant, inhibits amylase release. These results suggest that Rab27b regulates amylase release in pancreatic acinar cells. Exophilin7/Slp1/JFC1 is a GTP-bound Rab27a interacting protein. The number of zymogen granules was increased in the pancreatic acinar cells of fasted Exophilin7/Slp1/JFC1-KO mice^[33]. Moreover, amylase release was promoted in pancreatic acinar cells from Exophilin7/Slp1/JFC1-KO mice that were treated with carbamylcholine chloride or cholecystokinin-8, which mimic fed conditions. Thus, Rab27b may be involved in amylase release *via* Exophilin7/Slp1/JFC1 in pancreatic acinar cells.

Rab3a, which has the highest homology to Rab27, is highly expressed in synaptic vesicles^[27]. In PC12 cells, Rab27a and Rab3a are co-localized on the surface of dense-core granules^[34]. Silencing of both Rabs caused a significantly greater decrease in the number of these granules docked to the plasma membrane compared to silencing of either Rab alone. Since, Rab27a and Rab3a interact with the same effector proteins, these Rabs must cooperatively regulate the docking step of dense-core granules. In contrast, each of these Rabs has a specific function in pancreatic β -cells^[35]. Rab3a-GAP inhibited the dot-like distribution of Rab3a in the insulin-secreting β -cell line MIN6. In contrast, the distribution of Rab27a was not changed in Rab27a-GAP expressing

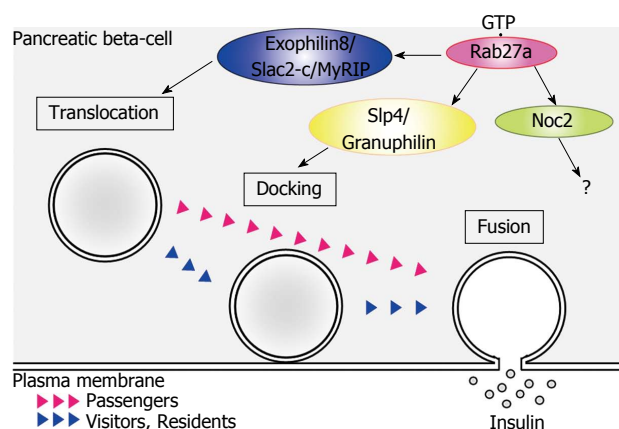


Figure 2 Schematic model of GTP-bound Rab27a function in pancreatic β -cells. Insulin-containing granules transported in the cytoplasm attach to the inner surface of the cell membrane (docking). The contents of both docked and undocked granules are eventually released following elevation of intracellular Ca^{2+} levels. GTP-bound Rab27a regulates insulin secretion by modulating the granule transport and docking steps *via* its effector proteins. Residents are granules that are pre-docked to the plasma membrane before fusion. Passengers are granules that fused without stably docking. Visitors are granules that remain near the plasma membrane for some time before fusion.

cells. Furthermore, whereas Rab3a is localized on insulin granules as a GTP-bound form, the Rab27a on these granules is present as both GTP- and GDP-bound forms. Moreover, these Rabs direct unique kinetic and functional properties of the exocytic pathway.

RAB27A IN PANCREATIC β -CELLS

Rab27a is highly expressed in pancreatic β -cells and is involved in insulin secretion^[36]. In ashen mice, blood glucose levels after a glucose load were higher, and insulin secretion in response to high glucose was lower than that in control mice. Interestingly, the decrease in insulin secretion was specific to glucose stimulation. These data suggest that Rab27a signaling in pancreatic β -cells is glucose-specific.

Insulin granules that are synthesized in pancreatic β -cells are eventually released by exocytosis *via* a series of stages (Figure 2). Granules that are transported in the cytoplasm attach to the inner surface of the cell membrane (docking). The contents of both docked and undocked granules are eventually released following elevation of intracellular Ca^{2+} levels. The granules are categorized into three types^[37,38]. Residents are granules that are pre-docked to the plasma membrane before fusion. Passengers are granules that fused without stably docking. Visitors are granules that remain near the plasma membrane for some time before fusion. GTP-bound Rab27a regulates insulin secretion by modulating the transport and docking steps of insulin granules *via* its effector proteins (Table 1)^[39-41]. To date, Exophilin8/Slac2-c/MyRIP, Slp4/Granuphilin, Exophilin7/Slp1/JFC1 and Exophilin1/Rabphilin3A are known to act as GTP-dependent Rab27a effectors in pancreatic β -cells. Exophilin8/Slac2-c/MyRIP possesses a potential

Table 1 GTP-bound Rab27a effectors in pancreatic β -cells

GTP-bound Rab27a effectors	Interacting proteins	Function
Exophilin8/Slac2-c/MyRIP	Myosin Va, Actin, PKA	Translocation
Slp4/Granuphilin	Munc18-1, Syntaxin1a, Myosin Va	Docking
Noc2	Munc13-1	-
Exophilin7/Slp1/JFC1	-	Docking
Exophilin1/Rabphilin3A	Myosin Va	Translocation

Table 2 GDP-bound Rab27a effectors in pancreatic β -cells

GDP-bound Rab27a effectors	Interacting proteins	Function
Coronin 3/coronin 1c	F-actin	Actin bundling Endocytosis
IQGAP1	GTP-bound Cdc42 GTP-bound Rac1	Scaffold

myosin binding site. Although Exophilin8/Slac2-c/MyRIP functions as a linker protein between GTP-bound Rab27a and Myosin Va in melanocytes^[42,43], this effector may form a different complex in pancreatic β -cells. Indeed, some reports suggest that there are several conditions under which Exophilin8/Slac2-c/MyRIP does not interact with Myosin Va^[44]. Slp4/Granuphilin and Exophilin1/Rabphilin3A interact with Myosin Va in pancreatic β -cells^[45]. Moreover, they are linked to a different subset of insulin granules. Further studies are required to investigate the regulation of the transport of insulin granules to the plasma membrane.

Slp4/Granuphilin was identified as a molecule that associates with insulin granules in pancreatic β -cells^[46]. This molecule forms a complex with Syntaxin1a and Munc18-1 and regulates the docking step of insulin granules in the insulin secretion pathway^[47]. The number of insulin granules docked to the plasma membrane was decreased in pancreatic β -cells from Slp4/Granuphilin-KO mice^[48]. Interestingly, insulin secretion was promoted in these mice. These results suggest that Slp4/Granuphilin regulates the docking state and inhibits the fusion of the insulin granule membrane and the plasma membrane in unstimulated pancreatic β -cells (Figure 2). Exophilin7/Slp1/JFC1 also tethers insulin granules to the plasma membrane^[49]. There seem to be multiple docking states of insulin granules in pancreatic β -cells.

Noc2 is a potential Rab27a effector. Noc2 displays 78% similarity to the N-terminus of Exophilin1/Rabphilin3A^[50]. Ca^{2+} triggered insulin secretion from pancreatic islets of Noc2-KO mice was impaired, but was restored by treatment with the G-protein inhibitor pertussis toxin^[51]. Although Noc2 is involved in insulin secretion through G-protein Gi/o signaling, its role in pancreatic β cells has not been identified. A yeast two-hybrid experiment identified zyxin as a Noc2-interacting protein. Because zyxin has been reported to bind the actin-binding protein α -actinin in fibroblasts^[50], the interaction between Noc2 and zyxin may regulate insulin secretion by modulating actin dynamics in

pancreatic β -cells.

GDP-DEPENDENT EFFECTORS OF RAB27A

We have identified novel Rab27a-interacting proteins. Surprisingly, some of these proteins interacted with GDP-bound Rab27a (Table 2)^[52,53]. Since GDP-bound GTPases have been considered to be an inactive form, these proteins might be suspected to be regulators of Rab27a GTP/GDP cycles. Indeed, protrudin, which was identified as a GDP-bound small GTPase interacting protein, forms a complex with GDP-bound Rab11 *via* its GDI consensus sequence and regulates neurite formation^[54]. Therefore, protrudin is thought to be a GDI that regulates GTPase cycles. In contrast, the GDP-bound Rab27a interacting proteins that we identified do not contain any GDI consensus sequences. Moreover, these proteins interact with GDP-bound Rab27a and transduce downstream signals in a similar manner to classical GTP-dependent effectors of small GTPases. We consider that these proteins are GDP-dependent effectors of Rab27a^[40,41].

Coronin 3

We identified coronin 3 as a GDP-bound Rab27a interacting protein^[52]. Coronins have been purified from precipitated actin of *Dictyostelium discoideum* cells^[55]. To date, more than ten coronin subfamilies have been reported. Coronin 3 is a ubiquitously expressed coronin that shares a central domain with other coronin family members that contains five WD40 repeats, which are known to form β -propeller structures and to mediate protein-protein interactions (Figure 3). Initially, these repeats were thought to form a five-bladed β -propeller structure. However, recent studies of coronin structure demonstrated that the N-terminal region of coronin forms two additional blades (not identified from the sequence). Therefore, coronin 3 is now considered to possess a seven-bladed β -propeller structure^[56,57]. It was noted that this propeller structure of coronin 3 is a GDP-bound Rab27a binding site^[52]. Oligomerization of coronins and their interaction with F-actin are mediated by the C-terminal 30-40 amino acids, which form a coiled-coil structure. These interactions modulate actin assembly and participate in various cellular functions. Human coronin 3 also modulates F-actin through binding to Arp2/3^[57], a key protein in the formation of a branched actin filament network^[57-59]. These direct and indirect modulations of F-actin must play a crucial role in the cellular function of coronins, because they are conserved in eukaryote cells^[60]. Both F-actin-binding and -bundling activities of coronin 3 were promoted by its interaction with GDP-bound Rab27a^[61]. In contrast, GDP-bound Rab27a did not affect the interaction between coronin 3 and Arp2/3. GDP-bound Rab27a regulates F-actin bundling by modulating a direct effect of coronin 3 on F-actin (Figure 3).

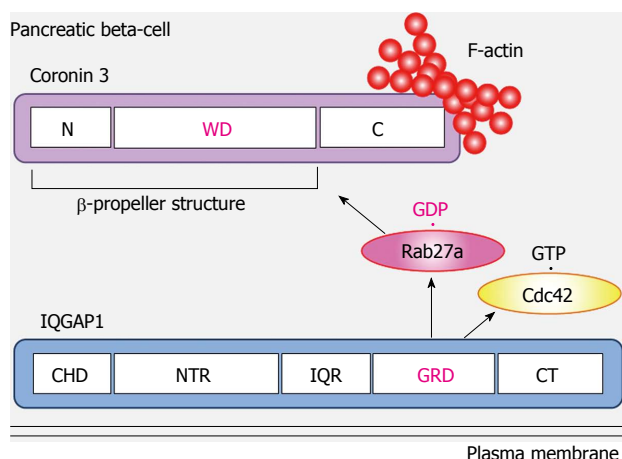


Figure 3 Interplay between Rab27a, coronin 3 and IQGAP1. GDP-bound Rab27a simultaneously binds coronin 3 and IQGAP1, resulting in the formation of a trimeric complex. WD: Five WD40 repeats; CHD: Calponin homology domain; NTR: N-terminal repeats; IQR: IQ repeats; GRD: RasGAP related domain; CT: Carboxy terminus of IQGAP1.

Coronins regulate membrane internalization in some types of cells through F-actin remodeling. In pancreatic β -cells, silencing of coronin 3 inhibited the internalization of both FM4-64 and phogrin^[52]. Internalization of these molecules was also inhibited when Rab27a-coronin 3 binding was inhibited by a dominant negative mutant of coronin 3. Moreover, the inhibition of F-actin binding to coronin 3 had the same effect^[61]. Thus, coronin 3 regulates the endocytosis of secretory membranes *via* the modulation of actin assembly in pancreatic β -cells.

Endocytosis is a complex process that involves cargo sorting, membrane invagination, vesicle scission and vesicle targeting (Figure 4). The uptake of an antibody against the extracellular domain of phogrin was inhibited and phogrin was located near the plasma membrane in MIN6 cells expressing a dominant negative mutant of coronin 3^[62]. Interestingly, phogrin staining near the plasma membrane remained when the cells were treated with acid wash, suggesting that the anti-phogrin antibody near the plasma membrane is separated from the plasma membrane. Thus the formation of GDP-bound Rab27a-coronin 3 complexes regulates the retrograde transport of internalized secretory membrane, at a stage after scission from the plasma membrane.

Insulin secretagogue glucose stimulation induced the intracellular redistribution of both Rab27a and coronin 3 from the cytosol to the plasma membrane^[62]. These redistributions were inhibited in Rab27a-silenced and Rab27a-Q78L-expressing MIN6 cells. Glucose-induced translocation of coronin 3 is due to its interaction with GDP-bound Rab27a. It has been reported that coronin 3 forms a closed conformation through an intramolecular interaction between its C- and N-termini^[59]. Therefore, the glucose-dependent binding of its N-terminus to GDP-Rab27a may shift coronin 3 to the open conformation, which enables this molecule to interact with F-actin^[41].

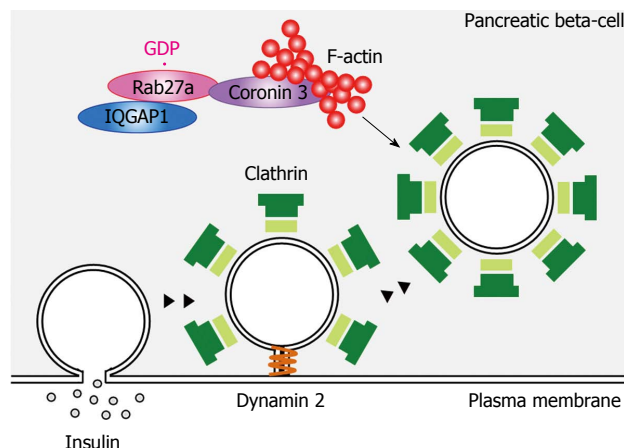


Figure 4 Schematic model of GDP-bound Rab27a function in pancreatic β -cells. Endocytosis is a complex process that involves cargo sorting, membrane invagination, vesicle scission and vesicle targeting. GDP-bound Rab27a modulates F-actin assembly and regulates the retrograde transport of the internalized secretory membrane, at the stage after scission from the plasma membrane. Scission is indicated by the dynamin step.

IQGAP1

We identified IQGAP1 as another GDP-bound Rab27a interacting protein^[53]. IQGAP1, an effector for Cdc42 and Rac1, is a member of the IQGAP family^[63]. IQGAP1 regulates cell-cell contacts and cell migration^[64-70]. In pancreatic β -cells, IQGAP1 interacts with vesicle-tethering exocysts under basal conditions. Moreover, this IQGAP-exocyst complex is dissociated by GTP-bound Cdc42^[71]. GTP-bound Cdc42 regulates insulin secretion by modulating F-actin bundling^[72,73]. Active Cdc42 also interacts with SNARE proteins such as VAMP2 and Syntaxin1a, and promotes the fusion step in insulin secretion^[74]. Since glucose converts Cdc42 from the GDP- to the GTP-bound form^[75], the IQGAP1-vesicle tethering exocyst complex is dissociated by glucose. It has been reported that vesicle-tethering to the plasma membrane is not a prerequisite for, but instead temporarily inhibits glucose-induced membrane fusion^[37]. This finding raises the possibility that the IQGAP1-exocyst complex may inhibit subsequent fusion events. IQGAP1 also binds A kinase anchoring protein 79 and functions as a scaffold protein^[76].

IQGAP1 binds GDP-bound Rab27a through its RasGAP related domain (Figure 3)^[53]. This domain lacks GAP activity and forms part of the GTP-bound Cdc42 and Rac1 interacting site^[63,70,77]. Moreover, IQGAP1 interacts with GDP-bound Rab27a when it forms a complex with GTP-bound Cdc42^[53]. IQGAP1 is distributed in the vicinity of the plasma membrane in both glucose-stimulated and -unstimulated cells. In contrast, glucose-induced redistribution of Rab27a and its binding protein coronin 3 was inhibited in both IQGAP1-silenced cells and in cells expressing the dominant negative mutant Cdc42-T17N. Since endocytosis of secretory membrane was also inhibited in these cells, these data indicate that activated Cdc42-bound IQGAP1, to which GDP-bound Rab27a binds,

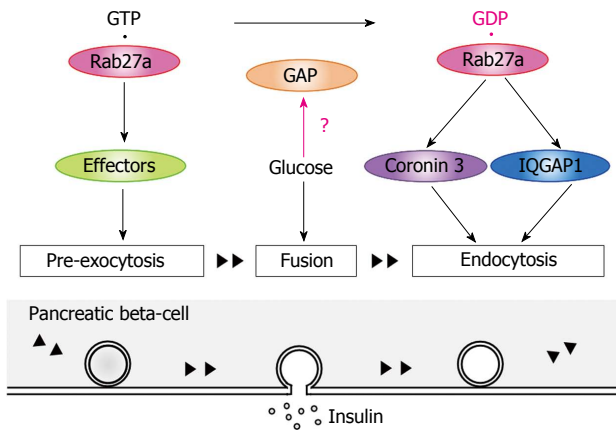


Figure 5 Rab27a GTP/GDP cycling synchronizes with the recycling of secretory membrane. In the basal state, GTP-bound Rab27a controls insulin secretion at pre-exocytotic stages *via* its GTP-specific effectors. Glucose stimulation causes insulin exocytosis. Glucose stimulation also converts Rab27a from its GTP- to its GDP-bound form. GDP-bound Rab27a interacts with IQGAP1, recruits coronin 3, and controls endocytosis of the secretory granule membrane. GAPs: GTPase-activating proteins.

recruits endocytic machinery including coronin 3 and regulates endocytosis of secretory membrane.

In summary, IQGAP1 functions at pre-exocytotic stages *via* interaction with the exocyst complex^[71] and this complex is dissociated by GTP-bound Cdc42. Our results indicate that IQGAP1 also plays a crucial role in the control of endocytosis *via* interaction with GTP-bound Cdc42 and GDP-bound Rab27a^[53]. Based on the combined data, we propose a model where IQGAP1 functions as a scaffold protein and is a key molecule for membrane recycling.

IQGAP1 also interacts with GTP-bound Rac1^[63,77]. Interestingly, IQGAP1 interacts with GDP-bound Rab27a when it forms a complex with GTP-bound Rac1^[53]. Moreover, endocytosis of secretory membrane was inhibited in MIN6 cells expressing the dominant negative Rac1-T17N mutant. These results suggest that Rac1 also recruits endocytic machinery and regulates endocytosis of secretory membrane. Cdc42 and Rac1 display some different characteristics in pancreatic β -cells. The most important difference is the timing of the glucose-induced conversion from the GDP- to the GTP-bound form. Glucose stimulation causes a shift from GDP-bound Cdc42 to GTP-bound Cdc42 within 3 min. In contrast, the glucose induced conversion of GDP- to GTP-bound Rac1 requires 20 min^[73,75]. These findings raises the possibility that Cdc42 regulates rapid endocytosis whereas Rac1 regulates subsequent, prolonged endocytosis, a pattern that may be associated with the biphasic release of insulin in response to glucose.

CONCLUSION

In the basal state, GTP-bound Rab27a controls insulin secretion at pre-exocytotic stages *via* its GTP-specific effectors (Figure 5). Glucose stimulation causes insulin exocytosis. Glucose stimulation also converts Rab27a

from its GTP- to its GDP-bound form, which interacts with IQGAP1, recruits coronin 3, and controls endocytosis of the secretory granule membrane. A long-term overexpression of a dominant negative coronin 3 mutant caused β -cell death (unpublished data), suggesting that the membrane recycling system controlled by Rab27a may be necessary for β -cell survival. Thus, we consider that Rab27a GTP/GDP cycling synchronizes with the recycling of secretory membrane to re-use the membrane and to keep the β -cell volume constant. It raises the possibility that a pharmacological agent that modulates the recycling system may become a new therapeutic option for the treatment of β -cell dysfunction in diabetes. Further studies are required to investigate whether Rab27a is involved in the pathogenesis of diabetes mellitus.

Typically, small GTPases are predominantly present in the GDP-bound form under unstimulated conditions and are converted to the GTP-bound form by stimulation. In contrast, glucose stimulation causes a shift of Rab27a in pancreatic β -cells from its GTP- to its GDP-bound form^[52]. The same conversion also occurs in thrombin stimulated platelets^[78]. These findings suggest that specific Rab27a-GAPs are activated by these stimulations. Two candidate Rab27a-GAPs, EPI64A and EPI64B, have been reported^[79]. In melanocytes, EPI64A has the higher GAP activity and functions as the main Rab27a-GAP. In pancreatic acinar cells, EPI64B regulates amylase secretion by modulating Rab27a GTP/GDP cycles^[80]. Further studies are needed to identify and characterize Rab27a-GAPs in pancreatic β -cells.

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Eating disorders in adolescents with type 1 diabetes: Challenges in diagnosis and treatment

Orit Pinhas-Hamiel, Uri Hamiel, Yael Levy-Shraga

Orit Pinhas-Hamiel, Uri Hamiel, Yael Levy-Shraga, Pediatric Endocrinology and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Ramat-Gan 52621, Israel

Orit Pinhas-Hamiel, Uri Hamiel, Yael Levy-Shraga, Sackler School of Medicine, Tel-Aviv University, Juvenile Diabetes Center, Maccabi Health Care Services, Ramat-Gan 52621, Israel

Author contributions: Pinhas-Hamiel O, Hamiel U and Levy-Shraga Y performed literature review, wrote the paper, and conceived the three level models and created the graphs.

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Correspondence to: Dr. Orit Pinhas-Hamiel, Pediatric Endocrine and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Ramat-Gan 52621, Israel. orithami@sheba.health.gov.il

Telephone: +972-3-5305015

Fax: +972-3-5305055

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Abstract

Eating disorders (ED) are characterized by a persistent disturbance of eating that impairs health or psychosocial functioning. They are associated with increased rates of medical complications and mortality. Insulin omission is a unique purging behavior available to individuals with type 1 diabetes mellitus (T1DM). The standard treatment regimen for T1DM requires a major focus on food and

eating patterns. Moreover, intensive insulin therapy is associated with increasing body weight. These factors, combined with the psychological burden of chronic disease management and depression, may contribute to ED. The comorbidity of ED in T1DM patients is associated with poorer glycemic control and consequently higher rates of diabetes complications. Early recognition and adequate treatment of ED in T1DM is essential.

Key words: Type 1 diabetes; Eating disorders; Insulin omission

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Core tip: Intentional insulin omission for the purpose of preventing weight gain is a unique behavior available to individuals with type 1 diabetes mellitus (T1DM). It is classified as either an inappropriate compensatory feature of bulimia nervosa or as a purging disorder component of other specified feeding or eating disorder (ED). Its prevalence increases with age, affecting up to 40% of young adult females with T1DM. The comorbid of ED in T1DM patients is associated with higher rates of short and long-term diabetes complications. A high index of suspicion is needed since ED behaviors are often well hidden and denied. Treatment involves a complex interplay of psychosocial, dietary and medical aspects and requires a multidisciplinary team.

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DEFINITIONS OF EATING DISORDERS

Eating disorders (ED) are characterized by a persistent

disturbance of eating that impairs health or psychosocial functioning^[1,2]. Based upon the fifth edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-V), which was released in May 2013, ED are divided into eight mutually exclusive categories^[3]. These categories are based upon observed symptoms and include anorexia nervosa (AN), bulimia nervosa (BN), binge eating disorder, avoidant/restrictive food intake disorder, pica, rumination disorder, other specified feeding or eating disorder (OSFED) and unspecified feeding or eating disorder. Some diagnoses include a dimensional component that enables clinicians to specify the severity of illness^[4-6].

In patients with type 1 diabetes mellitus (T1DM), intentional insulin omission or reduction for the purpose of preventing weight gain is recognized as either an inappropriate compensatory feature of bulimia nervosa or as a purging disorder, a component of OSFED. The latter is relevant when the recurrent purging behavior to influence weight or shape (*e.g.*, insulin omission) occurs in the absence of binge eating.

The two categories: "eating disorders not otherwise specified (EDNOS)" and "disturbed eating behavior (DEB)", compromised a wide spectrum of eating disorder pathologies, at a frequency or severity that does not merit a formal ED diagnosis. Though these categories are not included in DSM-V, they appear in the current review, due to their use by several studies involving individuals with diabetes.

ARE ADOLESCENTS WITH T1DM AT INCREASED RISK TO DEVELOP ED?

This question should be answered for the specific ED studied, according to the tools used for assessment and the prevalence rates in the general population.

Diagnostic tools of ED in persons with T1DM

The diagnosis of ED in individuals with T1DM is difficult, since eating behaviors are often well hidden and denied. The use of different questionnaires for assessing prevalence rates of ED makes comparisons across studies difficult. Furthermore, as Markowitz *et al.*^[7] pointed out, that general diagnostic questionnaires for detecting ED are not appropriate for individuals with T1DM for two main reasons. Firstly, these questionnaires do not identify eating disorder behaviors that are unique to T1DM, such as insulin omission^[7]. Secondly, such questionnaires may inflate the prevalence of eating problems in those with T1DM, because behaviors that are considered disturbed, such as particular concern about diet, reduced intake of certain food groups, and eating when not hungry, are integral to diabetes care^[8].

Prevalence rates of ED among persons with T1DM

Prevalence rates of ED among persons with T1DM vary according to the different ED categories and the populations studied. Some studies examined prevalence

rates of AN and BN only, while others examined the prevalence of insulin omission only, and some reported the prevalence of a combination of all ED together. Some studies reported the prevalence of full-threshold diagnoses of ED, whereas others also included subclinical ED. Reported prevalence rates differ also according to characteristics of the study populations, such as age range, and whether males were included or only females.

Prevalence of AN: A meta-analysis of controlled studies that defined ED in females according to DSM III-R or DSM IV criteria reported the prevalence of AN in T1DM subjects was not significantly different from that of controls^[9].

Prevalence of BN: This same meta-analysis showed a significantly higher prevalence of BN among the females with T1DM than those without diabetes^[9].

Overall prevalence of AN, BN and EDNOS: In a recently published meta-analysis^[10] of six studies of adolescents^[11-16], 7.0% of the 825 individuals with T1DM had ED, compared with 2.8% of the 2282 individuals without T1DM.

Prevalence of DEB: In a meta-analysis^[10] of five studies^[11,15-18], a higher proportion of adolescents with T1DM than without T1DM were classified as having DEB (39.3% vs 32.5%). The prevalence rate of DEB increased significantly with weight and age, from 7.2% in the underweight group to 32.7% in the obese group, and from 8.1% in the youngest age-group (11-13 years) to 38.1% in the oldest age-group (17-19 years)^[19].

Prevalence of insulin omission in T1DM: Insulin omission is a unique purging behavior available to individuals with T1DM. As presented in Table 1, insulin omission for weight loss is identified mainly in females; its prevalence increases with age, affecting up to 40% of young adult females with T1DM.

PREDISPOSING FACTORS FOR DEVELOPING ED

We suggest a three level model to describe the development of disturbed eating in adolescents with T1DM. This model comprises a hierarchically arranged continuum of potential effects on health problems (Figure 1). The first circle in the model, the premorbid status, includes a tendency to overweight, and factors relating to personality and family characteristics and dynamics. The second circle comprises factors arising at diagnosis of diabetes, such as the age of diabetes onset and satisfaction from weight loss. The third circle comprises factors associated with the chronic management of diabetes, such as recurrent hypoglycemic episodes,

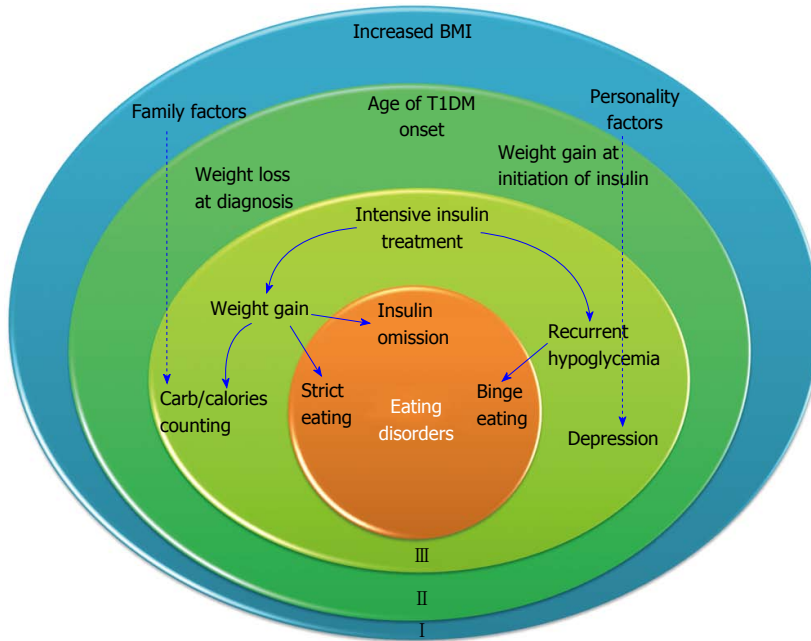


Figure 1 Three level models describing the development of eating disorders in adolescents with type 1 diabetes mellitus. The outer circle delineates the premorbid status. The middle circle delineates weight changes at diagnosis and with initiation of treatment. The inner circle includes factors associated with the management of T1DM that predispose to eating disorders. Dashed arrows imply that the specific factor extends on all levels. T1DM: Type 1 diabetes mellitus; BMI: Body mass index.

Table 1 Prevalence rates of insulin omission according to age range and gender

Prevalence of insulin omission (%)	Age range (yr)	Number and sex	Ref.
2	9-13	101 females	Colton <i>et al</i> ^[12]
26.2	11-19	390 females	Wisting <i>et al</i> ^[19]
4.5		380 males	
11	12-19	361 females	Jones <i>et al</i> ^[11]
14	12-19	356 females	Rodin <i>et al</i> ^[61]
10	12-21	70 females	Neumark-Sztainer <i>et al</i> ^[24]
1		73 males	
34	16-22	91 females	Rydall <i>et al</i> ^[46]
40	18-30	59 females	Stancin <i>et al</i> ^[74]
11	12-56	141 females	Philippi <i>et al</i> ^[75]
0		58 males	

strict insulin treatment and carbohydrate counting.

FIRST CIRCLE - PREMORBID FACTORS

Increased body weight and a tendency to gain weight

It has been suggested that the single question "Have you ever been overweight?" may be sufficient to screen for those with T1DM who are at a high risk for disordered eating attitudes/behaviors^[20]. Indeed, adolescent girls with T1DM who reported ever being overweight endorsed more disordered eating attitudes and behaviors^[21]. In addition, a higher body mass index percentile among teenagers and adults with T1DM, particularly among females, one to two years prior to the onset of ED, was found to be associated with disordered eating^[22]. Furthermore, youth at risk for disordered eating have been shown to have poorer

diet quality, including a higher intake of total fat and saturated fat^[23].

Low self-esteem and body dissatisfaction

Higher levels of weight dissatisfaction tended to be associated with unhealthy weight control/disordered eating among adolescent females^[24]. Low self-esteem predicts eating disturbances in the general population. Lower self-esteem was associated with disturbed eating behavior in girls with T1DM^[25]. Of note, low self-esteem and body dissatisfaction could also arise along the course of the disease. In a longitudinal study the development of DEB in girls with T1DM, was predicted by lower self-esteem related to physical appearance, and lower global self-esteem^[26].

Personality characteristics

Perfectionism has been associated with attitudinal aspects of eating disorders such as weight preoccupation. Borderline personality characteristics were related to insulin omission^[27]. Adolescent girls with T1DM and an ED showed deficits in self-regulation and narcissistic gain from illness^[28], and tended to blame themselves for the situation^[29].

Comparing adolescents with ED and adolescents with T1DM and ED, those with T1DM were less pathologically compromised, scored lower on several affective areas and had a lower prevalence of depression and anxiety^[30].

Family characteristics and dynamics

Several factors involving family characteristics and dynamics are associated with an increased risk for ED. Firstly, in many families of adolescent girls with T1DM,

interactions within the family center around food and weight. The prevalence of ED was found to be higher in families in which parents tended to make negative comments about eating or weight^[31]. Secondly, mothers who engaged in dieting and binge-eating themselves were more likely to have daughters with disturbed eating behavior. Maternal weight and shape concerns and impaired mother-daughter relationships significantly predicted eating disturbances in girls with T1DM, accounting for 57% of the variance^[32]. Finally, family dysfunctioning has been identified as an important risk factor for eating disorders among the general population. Specifically, among girls with T1DM, less support from, poorer communication with, and less trust in their relationships with their parents, were reported among those with eating disturbances than among those without^[33]. Families of girls with ED more often reported having infrequent family meals than did families without ED^[34].

SECOND CIRCLE - FACTORS ASSOCIATED WITH DISEASE ONSET

Age of diabetes onset

The age of onset of T1DM that is most closely related to the subsequent development of a severe eating disorder such as AN and BN was studied in 53 women with ED compared with 49 with T1DM who had no eating disorder - related problem^[35]. It was demonstrated that for diabetes onset age between 7 to 18 years, the density of the "eating disorder" group was higher, but for the younger and older onset ages the densities were lower. Thus the development of T1DM in preadolescence or adolescence seems to place girls at risk for the subsequent development of AN or BN. Similarly, frequent insulin reduction or omission was reported in type 1 diabetic patients with later disease onset (mean age at onset between 8 and about 17 years)^[36]. It was suggested that later age at diabetes onset, in particular during pubertal age with hormonal changes and gain in weight and fat mass may be associated with a greater risk for ED especially in female patients.

Weight issues

The cycle of weight loss at disease onset and subsequent weight gain with the initiation of insulin treatment is a risk factor for the development of ED in susceptible patients^[11].

THIRD CIRCLE - FACTORS ASSOCIATED WITH THE CHRONIC MANAGEMENT OF DIABETES

Intensive insulin treatment

Intensive insulin treatment conveys an increased risk of weight gain^[37]. A model of disordered eating and T1DM

proposed by Goebel-Fabbri *et al.*^[38,39] suggests that the increased weight gain causes negative feelings. Fear of further weight gain can lead to insulin restriction for caloric purging or weight regulation^[40].

Dietary restraint

Diabetes management imposes dietary restraint. This may lead to the yearning and craving of "forbidden foods", and result in bingeing, without administration of the appropriate insulin dose^[41].

Hypoglycemic episodes

The delicate balance between dietary regimen, insulin and exercise that is integral to T1DM management can result in recurrent hypoglycemic episodes. Hypoglycemia is accompanied by intense hunger and by eating sweetened foods and drinks that are normally forbidden. Patients may subsequently feel guilty about consuming these foods and restrict eating, which may result in yet another hypoglycemic episode. This vicious cycle of dietary restriction, over-eating and guilt is similar to that experienced by individuals with bulimia^[42].

Chronic disease and depression

The risk of significant depressive symptoms in individuals with T1DM has been assessed as about double that of the general population^[43]. Depression may increase the susceptibility for developing disturbed eating behavior. Indeed, among girls with T1DM, those diagnosed with depression scored higher on the Eating Disorder Examination than did those without depression; 75% and 45%, respectively^[44]. Furthermore, anxiety disorders and eating disorders co-occur in the general population, and 21% of children and adolescents with T1DM were reported to screen positively for anxiety^[45].

CLINICAL SIGNS OF ED AMONG INDIVIDUALS WITH T1DM

The diagnosis of subclinical or clinical eating disorders in individuals with T1DM is not easily established. Due to the frequent concealment and denial of eating disorder behaviors, a high index of suspicion is needed. In addition to the predisposing characteristics described above, a number of clinical signs should alert health care providers to the possibility of ED.

Poor glycemic control

Among females with T1DM, mean HbA1c levels were shown to be significantly higher in those with disturbed eating than among those with non-disturbed eating^[46]. HbA1c level of patients who confessed intentional under-dosing of insulin was $9.0\% \pm 1.6\%$ compared with $7.8\% \pm 1.2\%$ of compliant patients^[47]. Similarly, in a nationwide population-based study, conducted in Norway, a significantly higher HbA1c was documented in patients reporting insulin restriction ($9.0\% \pm 1.7\%$) than in nonrestrictors ($8.3\% \pm 1.2\%$)^[19]. In a meta-

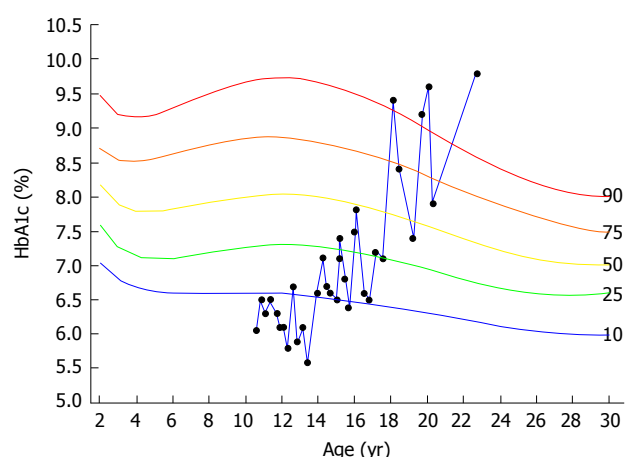


Figure 2 Typical example of the course of HbA1c levels in a patient, with intentional insulin omission for weight loss, plotted on the HbA1c percentile reference curves. HbA1c levels were around the 10th percentile from age 10 to 14 years. From age 18 the pattern changed and was characterized by sharp increases to levels above the 90th percentile, sharp decreases and multiple measurements above the 90th percentile.

analysis, eating problems, including both DEB and ED, were found to be associated with poorer glycemic control^[10]. Using a computer model of data mining, we recently showed that adolescent females with intentional insulin omission were discriminated by HbA1c > 9.2% and by more than 20% of HbA1c measurements above the 90th percentile^[48].

Recurrent episodes of diabetic ketoacidosis

Intentional insulin omission has long been recognized as a cause of recurrent episodes of recurrent episodes of diabetic ketoacidosis (DKA) in adolescents with T1DM^[49]. Thus, any recurrent episode of DKA in established diabetes should arouse suspicion^[50].

Recurrent hypoglycemic episodes

Recurrent hypoglycemic episodes may occur among individuals with T1DM and ED, mainly among those participating in binge eating and self-induced vomiting. Deliberately inducing hypoglycemia to justify eating sweets and high carbohydrate meals were described in individuals with T1DM. Eighteen percent of a cohort of males and females with T1DM, aged 10–22 years, reported intentional overdosing of insulin^[47]. The most common reason (49%) stated was the desire for uncontrolled binge eating, followed by self-destructive behavior in stressful situations.

Other characteristics and signs of ED

Other characteristic and clinical signs of ED are: frequently missed medical appointments, refusal to be weighed, preoccupation with appearance, a tendency to vegetarianism, and the calculating of caloric values and weighing of foods. Apparently, due to the availability of insulin omission as a weight loss strategy, females with diabetes are less likely to use other unhealthy weight control behaviors like vomiting, laxatives or diuretics,

and skipping meals or fasting, compared to their peers without diabetes^[51].

DIAGNOSIS

Several questionnaires were developed specifically for adolescents with T1DM. The revised Diabetes Eating Problem Survey (DEPS-R) is a 16-item diabetes-specific self-report measure of disordered eating that was designed specifically to identify patients with T1DM who are at risk for early intervention for disordered eating behaviors^[7]. The mSCOFF is a simple 5 item screening tool, with demonstrated reliability and validity, which can easily be used during a follow-up visit^[52]. Using data mining methods, a clinical prediction model that provides a decision support system for the detection of intentional insulin omission for weight loss in adolescent females with T1DM was developed^[48]. The model is based on identifying a pattern of HbA1c levels indicative of intentional insulin omission. After a period of apparently stable HbA1c levels, the onset of insulin omission is characterized by both high HbA1c levels and wide fluctuations between clinical visits. A typical example of the course of HbA1c levels in a patient who developed ED is depicted in Figure 2.

MORBIDITY AND MORTALITY

ED result in poor metabolic control and cause short and long-term complications.

Short term complications

Insulin omission is associated with recurrent events of DKA, and disturbed eating behavior is associated with recurrent episodes of severe hypoglycemia^[53]. Data from the diabetes patienten verlaufsdokumentation study, including 52215 T1DM males and females aged 8 to 30 years, revealed significantly higher rates of severe hypoglycemic episodes in adolescents with AN, BN or EDNOS, than with no ED: 12.1, 18.0, 12.9 and 5.7, respectively^[54]. Moreover, rates of hypoglycemia with coma, and DKA with hospitalization were higher, and the duration of hospital stay longer, among those with eating disorders^[54].

Long term complications

Long-term complications have been shown to be markedly increased among individuals with both T1DM and ED^[54]. Data from the DPV study revealed that both hypertension and dyslipidemia were more common in persons with T1DM and either EDNOS or BN than in those with T1DM without ED^[54].

Of T1DM patients who developed serious micro-vascular complications, 21% had a probable clinical ED, 47% had a history of DEB, and 48% had a history of insulin omission. The development of two or more serious complications was associated with the presence of a probable clinical eating disorder^[55].

Different ED are associated with varying prevalence

Table 2 Prevalence rates of retinopathy and nephropathy among type 1 diabetes mellitus patients with eating disorders

Complication	Average T1DM duration (yr)	T1DM patients with severe ED (%)	T1DM patients with moderate ED (%)	T1DM patients without ED (%)
Retinopathy ^[46]	11 ± 4	86	42	24
Nephropathy ^[46]	11 ± 4	43	20	18
Nephropathy ^[57]	28	25		10
Foot problems ^[57]	28	25		12

ED: Eating disorders; T1DM: Type 1 diabetes mellitus.

rates of morbidities. For example, data from the DPV study revealed a 2.5-fold higher risk for retinopathy in T1DM patients with BN than in those without ED. Patients with EDNOS also had a higher risk but without statistical significance, whereas those with AN had no increased risk for retinopathy^[54].

The duration of ED may also affect diabetes complications. The duration of severe insulin omission was the factor most closely associated with the development of retinopathy and nephropathy in T1DM females^[56].

As seen in Table 2, higher rates of retinopathy^[46] and nephropathy^[57] were documented in young T1DM patients with ED than in those without ED.

Mortality

Several studies documented increased relative risk of death among patients with ED. Mortality rates per 1000 person years were reported as 2.2 in girls with T1DM, 7.3 in girls with ED and 34.6 in girls with both T1DM and an ED^[58]. During an 11-year period, self-reported insulin restriction at baseline increased the relative risk of death by 3.2 times in women, mean age 45 years, mean diabetes duration 28 years; those with ED were younger when they died (aged 44 vs 58 years, $P < 0.01$)^[57]. In a 12-year follow-up of a cohort of 14 women with T1DM and ED (12 with AN), 5 died (36%)^[59]. The median age of the cohort was 37 years (range 25-46), with a median duration of diabetes of 26 years (14-33). The age of death of the 5 patients was 30 years (25-42).

PREVENTION

Promoting the understanding and awareness among healthcare providers of factors involved in the development of ED in young vulnerable individuals with T1DM may help prevent these disorders^[60]. These factors include the weight gain with the initiation of insulin treatment^[11], the dietary restraint necessitated by diabetes management, and recurrent hypoglycemic episodes. Diabetes treatment should therefore afford flexibility where possible, and non-depriving approaches to eating^[61]. As current clinical practice includes a focus on measuring body weight, health care professionals should be sensitive to preoccupation with body weight. Interventions aimed at increasing self-esteem and body acceptance, as well as family-based interventions aimed

to improve family management of obesity and diabetes, may also help to diminish the risk of developing ED in individuals with diabetes^[62].

Girls aged between 10 and 12 with a diagnosis of T1DM for at least 1 year, attended two group sessions, each of 4-h duration^[63]. The content of the sessions targeted perfectionism, media literacy, self-esteem. The program was found to have impact on self-efficacy relating to diabetes management. As lower T1DM regimen adherence is associated with lower self-efficacy in adolescents, it was concluded that a brief interactive program had a favorable impact on protective factors for disordered eating.

Among college-age women at highest risk for an eating disorder an internet-based intervention, has successfully reduced weight/shape concerns and prevented eating disorders^[64]. Specifically guided discussion group resulted in reduction in weight/shape concern. We did not find similar reports among adolescent girls with T1DM, however it is possible that online interventions may be successfully used to reduce eating disorder risk factors and preventing eating disorders in this group.

TREATMENT

Treatment of ED in patients with T1DM is challenging, especially since the clinical improvement achieved with insulin treatment is associated with weight gain. Compliance to the therapeutic regimen may therefore be poor.

Treatment should mirror the three circle model presented above, and address the factors predisposing to the development of ED (Figure 3).

Treating the first circle - premorbid variables

Increased body weight and a tendency to gain weight should be addressed by prudent dietary management: Since a tendency to gain weight was found to be associated with ED^[22], particular attention should be given to the constellation of excess weight and preoccupation with food.

Low self-esteem, body dissatisfaction and personality variables should be addressed by individual psychological treatment or group therapy: The concurrence of eating behavior disorder and mood disorder in patients with T1DM supports the importance of treating depression as a means of

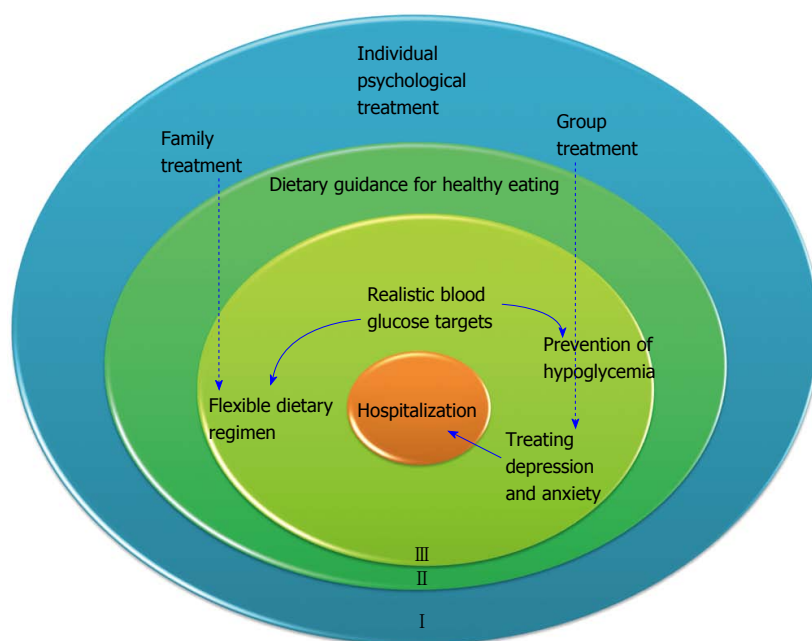


Figure 3 Three level models describing treatment modalities of eating disorders in adolescents with type 1 diabetes mellitus. The outer circle addresses treatment for premorbid variables. The middle circle delineates. The inner circle includes factors associated with the management of T1DM that predispose to eating disorders. T1DM: Type 1 diabetes mellitus.

treating ED. If depression and anxiety are suspected, a psychiatric consultation is needed. Psychiatrists have a distinct role in the diabetes care of children under their care, specifically in working through issues associated with the burden of the disease^[65]. The impact of antidepressant and anti-anxiety medications on recovery from eating disorders in adolescents with T1DM has not been studied systematically.

The effect of psychoeducation on adolescents with T1DM and ED is controversial. A psychoeducation approach with six weekly group sessions was associated with reductions in dieting, in body dissatisfaction and with preoccupation with thinness and eating; the reduction in disturbed eating behaviour was maintained for at least 6 mo^[66]. In another study, a six week intervention was as effective as a wait list control group^[67].

Group cognitive-behavioral therapy intervention has been shown to improve glycemic control, well-being and diabetes related stress among poorly controlled adult T1DM patients^[68], and among patients with T1DM and depression^[69]; however, it was not studied in patients with insulin omission.

As family dynamics may influence the development of ED^[34], family education is an important component of the treatment. However, we could not find data on the impact of family intervention as a treatment modality for adolescents with T1DM and ED.

Treating the second circle - factors associated with disease onset

Current T1DM management involves carbohydrate counting, which may lead to excessive preoccupation with food and diets. The cycle of weight loss at disease

onset, and subsequent weight gain with the initiation of insulin treatment, is a risk factor for the development of ED in susceptible patients^[11]. Dietary treatment should focus on healthy choices and low calorie alternatives with a high satiety index.

Treating the third circle - factors associated with the chronic management of diabetes

Intensive insulin treatment, dietary restraint, and hypoglycemic episodes should be addressed by the diabetes management team: As good metabolic control is associated with weight gain, changes in target blood glucose levels should be gradual. Setting higher than standard target blood glucose ranges (preprandial 120 to 150 mg/dL and postprandial < 200 mg/dL) may yield more benefit in the long run. In contrast, achieving excellent control may result in marked weight gain. Moreover, since low glucose target levels are associated with an increased risk of recurrent hypoglycemic episodes, which may result in additional increased calorie intake, setting higher target levels may be a better initial objective. In girls who were skipping insulin dosing at almost every meal, starting with adequate insulin injection at a single meal is sometimes helpful as a first step. Caregivers should be aware that patients often switch from one disturbed behavior to another, ie from insulin omission to restrictive eating or binge eating; thus, a prescribed diet should be flexible.

Treatment with an insulin pump was postulated as a means of enabling better physiological insulin delivery to adolescents with T1DM than achieved with multiple daily injections. Patients with insulin pumps require less insulin and thus gain less weight. Furthermore, the occurrence of hypoglycemic episodes is decreased, and

thus the need to consume extra calories. A recently published multicenter study demonstrated a decrease in disturbed eating behaviors, as assessed by the DEPS-R, in youth with T1DM, 6 mo after pump initiation^[70]. Moreover, among adolescent girls with T1DM and ED, the mean HbA1c level was significantly lower in those who were treated by insulin pumps than in those who were treated with multiple daily injections ($9.07\% \pm 1.33\%$ vs $10.40\% \pm 2.01\%$; $P = 0.04$)^[71].

Failure of outpatient treatment, the presence of a severe psychopathological state and poor glycemic control are key elements in the decision for hospitalization. Information is limited regarding the long-term outcomes of hospitalized adolescents with ED. Significantly lower levels of HbA1c and lower psychological test scores related to eating disorder psychopathology, depressiveness and anxiety were reported for 9 patients who were hospitalized, compared to 10 who were not^[72]. At 3-year follow-up, 78% of the hospitalized patients in that study no longer fulfilled any criteria for clinical or subclinical ED.

Thus, treatment of ED in individuals with T1DM involves a complex interplay of psychosocial, dietary and medical aspects and requires a multidisciplinary team. It is important to realize that adolescents with T1DM and ED tend to have lower motivation to change their eating habits^[73]. Furthermore, they are characterized by giving up easily when confronted with frustration and with low levels of accomplishment. These personality characteristics may explain the high dropout rate from therapy (79%) among patients with T1DM and ED^[73].

CONCLUSION

Deliberate insulin omission as a weight loss strategy is recognized as either an inappropriate compensatory feature of bulimia nervosa or as a purging disorder, a component of OSFED. About one-third of individuals with T1DM intentionally omit insulin. Diagnosis of eating disorders in individuals with T1DM is difficult, since eating disorder behaviors are often well hidden. Weight loss that is related to deteriorated glycemic control and recurrent DKA should raise suspicion. Early diagnosis is essential, as the combination of eating disorders and diabetes is associated with increased morbidity and mortality.

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Prospective Study

Perception of difficulty and glucose control: Effects on academic performance in youth with type I diabetes

Tiffany M Potts, Jacqueline L Nguyen, Kanika Ghai, Kathy Li, Lawrence Perlmutter

Tiffany M Potts, Jacqueline L Nguyen, Kathy Li, Lawrence Perlmutter, Department of Psychology, Rosalind Franklin University of Medicine and Science - College of Health Professions, North Chicago, IL 60064, United States

Kanika Ghai, Pediatric Endocrinology, Advocate Children's Hospital, Park Ridge, IL 60068, United States

Author contributions: Potts TM and Perlmutter L designed the research project; Ghai K provided the patient population and the testing space; Potts TM performed the research; Potts TM, Nguyen JL, Li K, and Perlmutter L analyzed the data; Potts TM, Nguyen JL, Li K and Perlmutter L wrote the paper.

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Correspondence to: Lawrence Perlmutter, PhD, Professor, Department of Psychology, Rosalind Franklin University of Medicine and Science - College of Health Professions, 3333 Green Bay Road, North Chicago, IL 60064, United States. lawrence.perlmutter@rosalindfranklin.edu

Telephone: +1-847-5788754
 Fax: +1-847-5788765

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Abstract

AIM: To investigate whether perceptions of task difficulty on neuropsychological tests predicted academic achievement after controlling for glucose levels and depression.

METHODS: Participants were type 1 diabetic adolescents, with a mean age = 12.5 years (23 females and 16 males), seen at a northwest suburban Chicago hospital. The sample population was free of comorbid clinical health conditions. Subjects completed a three-part neuropsychological battery including the Digit Symbol Task, Trail Making Test, and Controlled Oral Word Association test. Following each task, individuals rated task difficulty and then completed a depression inventory. Performance on these three tests is reflective of neuropsychological status in relation to glucose control. Blood glucose levels were measured immediately prior to and after completing the neuropsychological battery using a glucose meter. HbA1c levels were obtained from medical records. Academic performance was based on self-reported grades in Math, Science, and English. Data was analyzed using multiple regression models to evaluate the associations between academic performance, perception of task difficulty, and glucose control.

RESULTS: Perceptions of difficulty on a neuropsychological battery significantly predicted academic performance after accounting for glucose control and depression. Perceptions of difficulty on the neuropsychological tests were inversely correlated with academic performance ($r = -0.48$), while acute (blood glucose) and long-term glucose levels increased along with perceptions of task difficulty ($r = 0.47$). Additionally, higher depression scores were associated

with poorer academic performance ($r = -0.43$). With the first regression analysis, perception of difficulty on the neuropsychological tasks contributed to 8% of the variance in academic performance after controlling for peripheral blood glucose and depression. In the second regression analysis, perception of difficulty accounted for 11% of the variance after accounting for academic performance and depression. The final regression analysis indicated that perception of difficulty increased with peripheral blood glucose, contributing to 22% of the variance. Most importantly, after controlling for perceptions of task difficulty, academic performance no longer predicted glucose levels. Finally, subjects who found the cognitive battery difficult were likely to have poor academic grades.

CONCLUSION: Perceptions of difficulty on neurological tests exhibited a significant association with academic achievement, indicating that deficits in this skill may lead to academic disadvantage in diabetic patients.

Key words: Type 1 diabetes; Adolescents; Perception of difficulty; Academic performance; Glucose control

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Core tip: The objective of the current study was to investigate the association between perceptions of difficulty and academic performance in adolescents with type 1 diabetes. Perceptions of difficulty are reflected in executing cognitive activities as well as in the task of glucose regulation. Glucose control needs to be understood, not so much from a biological perspective but as an effortful process that is also reflected in academic challenges. The problem of anergia observed generally in patients with diabetes seems to broadly affect a variety of tasks and challenges. Thus, the novelty of the study is in showing that the regulation of glucose in diabetic patients is another example of the broad challenges confronted in the life of patients with diabetes.

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INTRODUCTION

Type 1 diabetes is one of the most common chronic diseases of childhood. In the United States, type 1 diabetes affects approximately 1 in 300 children and is usually diagnosed between the ages of 5 and 11 years^[1,2]. The course of this autoimmune version of diabetes is characterized by ongoing beta cell destruction

and the need for exogenous insulin^[3]. Since the central nervous system (CNS) is dependent on a supply of glucose for normal neural functioning, reduced glucose concentrations may result in temporary and/or enduring impairments in CNS function^[4]. Dysregulation of the CNS may have negative effects on neuropsychological functioning, such as impaired memory, attention, motor skills, and executive functioning^[5].

Adolescents with type 1 diabetes face many challenges that can impact metabolic control along with physiological and psychological complications. The need for adherence to metabolic control and compliance with regimen is effortful. Beyond the demands for medical compliance, adolescents are often burdened by depression^[6] as well as anergia^[7] and thus may be at academic disadvantage^[8].

Along with academic adversity, individuals with type 1 diabetes may be challenged by self-evaluative factors such as the perception of control, which affects behavioral and social functioning^[9,10]. The literature has described three prominent types of control: perceived control, perceived confidence (self efficacy), and perceived difficulty^[11]. Perceived difficulty, a subjective experience, can be defined as the extent to which individuals believe that performance on a task or behavior would range from easy to difficult^[11]. If adolescents believe they are substandard at a task because of its difficulty, they are likely to devalue the activity and decrease effort expenditure^[12].

Perceived difficulty has also been shown to relate to an individual's beliefs regarding how much effort is needed to succeed at a task and whether success is possible^[13]. The effort expended in performing a task is predicted to increase proportionally with the level of perceived task difficulty^[14]. Indeed, effort has been associated with academic performance. Studies^[15] have found that self-reported grades are positively correlated with effort. Moreover, adherence to regimen^[16] and metabolic control^[17] also reflect the expenditure of effort. Therefore, poor glucose control may be positively correlated with perceptions of task difficulty and inversely associated with academic performance^[18]. Indeed, self-control and willpower are both functions of available glucose levels^[19]. Thus, glucose control and effort are two highly related processes that need to be differentiated.

Perceptions of difficulty rely on executive functioning skills, such as problem solving and decision-making. These skills require an individual to make a decision regarding the degree of difficulty associated with the task and whether he or she will be successful in carrying out the task. Since problem solving and decision-making skills contribute to academic performance, these executive functioning skills may be impaired in diabetic individuals^[20]. A meta-analytic finding indicated that children with type 1 diabetes performed slightly, yet significantly, lower on attention/executive functioning tasks than non-diabetic individuals^[21]. This finding, in turn, was associated with poor glucose control and

hypoglycemic episodes as well^[21].

To open the question of effort, three brief but well validated neuropsychological tests were administered^[22] and participants rated the difficulty of each test. Secondly, academic performance and depression levels were evaluated and analyzed. Since long-term glucose control (HbA1c) is associated with cognition^[18], HbA1c levels from within the past three months were collected from medical records. Acute glucose levels immediately prior to and following neuropsychological testing were also collected and evaluated. These procedures enabled the distinction between the assessment of effort-associated processes supporting academic and neuropsychological processes vs those putatively supporting glucose control.

MATERIALS AND METHODS

Participants

This study was designed as a cross-sectional, prospective study. Participants were type 1 diabetic patients who attended a diabetes clinic in a northwest suburban Chicago hospital. Adolescents were approached to participate in the study during their regularly scheduled doctor's appointment. Adolescents were excluded from participation if they had co-morbid clinical health conditions, such as asthma, anemia, vascular disease, kidney disease, chronic tiredness, depression, psychiatric history, or obesity. Parents of the patients filled out consent forms that outlined the procedure and risks of participation. The subjects also signed an assent form. The study measures were carried out in a quiet, separate room for each participant individually. Pre-doctoral psychology students administered the assessments. Participants were provided modest monetary compensation for participation in the study. There were a total of 39 participants, consisting of 23 females and 16 males. The population was comprised of 71.8% Caucasian, 20.5% Hispanic, 5.1% Asian, and 2.6% African American. The mean age of the participants was 12.5 years (SD = 2.73). This study was approved by the Institutional Review Board at Rosalind Franklin University as well as the hospital's review board. There is no conflict of interest related to this study.

Materials

Glucose control: Blood glucose measurements were performed immediately prior to administering and again after completing the neuropsychological battery with glucose meters (One Touch, Blood Glucose Monitoring System). This procedure was used to obtain an average peripheral blood glucose measurement. A single HbA1c level was recorded from the medical records retrieved from the previous three months, prior to testing.

Neuropsychological battery: The neuropsychological battery included the Digit Symbol task (Wechsler Intelligence Scale for Children-Forth Edition), Trail Making test Parts A and B, and Controlled Oral Word

Association (COWA) in that order. This three-part neuropsychological battery was selected because the tests are brief, well researched, psychometrically sound, and are commonly used^[23]. Performance on these tests is reflective of neuropsychological status in diabetic patients in relation to glucose control^[22].

The Digit Symbol task of the Wechsler Intelligence Scale for Children-Fourth Edition (DS; WISC-IV)^[24] was used to assess psychomotor performance, attention, memory, and perceptual organization. It consists of Coding and Incidental Learning. In Coding, the individual was presented with several rows of a series of numbers (1 through 9). Each number was paired with a corresponding unique symbol. The individual was asked to fill in as many symbols as possible in boxes directly under the corresponding number and was allowed 120 s to complete this task. In Incidental Learning, individuals were asked to carry out two tasks: to recall as many digit-symbol pairs as possible (Pairing) and to recall as many symbols as possible without the corresponding numbers (Free Recall). Internal consistency of the Digit Symbol Coding task is 0.85.

The Trail Making Test^[23] was used to assess attention, visual-motor tracking, and speed of information processing. It consists of two parts: in Part A, individuals were asked to draw a line that accurately connected the correct sequence of numbers as quickly as possible. In Part B, individuals were asked to draw a line that accurately connects the correct sequence of numbers and letters in an alternating pattern as quickly as possible. Reliability for Part A is 0.78 and for Part B 0.67.

The COWA test^[25] assessed verbal functions. Individuals were asked to generate as many words as possible that began with the letter S in 60 s and then the letter F in 60 s as the examiner recorded these. Test-retest reliability is 0.88 and inter-scorer reliability was almost perfect.

Rating of task difficulty: After completing three tasks (the Digit Symbol task of the Wechsler Intelligence Scale for Children-Fourth Edition, Trail Making test Part A and B, and Controlled Oral Word Association test), participants were asked to rate the level of perceived difficulty of each task on a scale from 1-5, where 1 = very easy and 5 = very difficult. These evaluations were summed and averaged to develop a task difficulty measure.

Academic performance: Participants self reported academic grades in several courses during the current school year: Math, Science, and English. For the purposes of tabulation, grades were converted into a scale score from 1-5, where 1 = F, 2 = D, 3 = C, 4 = B, and 5 = A. Grades were then averaged for each participant.

Depression: To avoid possible reactive effects^[26], participants were lastly administered the Children's Depression Inventory (CDI)^[27] which is a 27 item self report inventory used to measure depressive symp-

Table 1 Characteristics of participants

	Mean	SD
Disease duration (mo)	48.05	41.08
BMI (kg/m ²)	21.18	3.85
Peripheral blood glucose (mm/dL)	190.99	89.27
HbA1c (%)	8.39	1.63
Digit symbol coding	55.38	14.7
Digit symbol paring	11.44	5.16
Digit symbol free recall	7.38	1.41
Trails A (s)	39.67	19.02
Trails B (s)	92.74	64.84
COWA	20.26	6.62
CDI	7.23	5.54
Perception of difficulty ¹	10.15	2.95
Grades ²	4.1	0.77

¹Range 1-20; ²Range low to high; 1-5. BMI: Body mass index; HbA1c: Hemoglobin A1c; COWA: Controlled Oral Word Association; CDI: Child depression inventory.

tomatology in youth aged 7-17. Respondents indicated which of three options best represented their mood in the context of everyday life: absence of symptom = 0; mild symptom = 1; and definite symptom = 2. The CDI provides a total score on five domains of depression. The reliability coefficient for the total inventory was 0.86^[27].

Data was collected and entered into Statistic Package for Social Science (SPSS) Version 13. Zero order correlation tables were examined to assess the degree of relationship between the variables. *P*-values of less than 0.05 were considered statistically significant. Multiple regression analyses were used to assess outcome variables.

RESULTS

Basic description of the sample population is presented in Table 1. The primary results (Table 2) indicated that perceptions of difficulty on the neuropsychological tests were inversely correlated with academic performance ($r = -0.48$), while acute (blood glucose) and long-term glucose levels (HbA1c) increased along with perceptions of task difficulty ($r = 0.47$). Finally, higher depression scores were associated with poorer academic performance ($r = -0.43$).

To evaluate the relationship of difficulty on neuropsychological tests and academic performance after controlling for peripheral glucose levels and depression, a multiple regression analysis was conducted (Table 3). The predictors in the regression analysis were entered in the following order: (1) peripheral blood glucose; (2) CDI; and (3) perception of difficulty with academic performance as the dependent variable. Results from the first predictor indicated that as peripheral blood glucose increased, academic performance decreased. The second predictor (CDI) indicated that increasing depression was associated with poorer academic performance after controlling for peripheral blood glucose. Lastly, the third predictor (perception of difficulty) showed

that higher levels of difficulty perceived on the neuropsychological test was inversely associated with academic performance after taking into account peripheral blood glucose and depression. The result of this analysis indicated that the perception of difficulty on the neuropsychological tasks contributed to 8% of the variance in academic performance after controlling for peripheral blood glucose and depression. Thus, stronger ratings of laboratory task difficulty were associated with poorer academic performance.

After controlling for academic performance and depression, a regression analysis was conducted with peripheral blood glucose as the dependent variable to test if perceptions of task difficulty were associated with glucose levels (Table 4). The predictors in the regression analysis were entered in the following order: (1) academic performance; (2) CDI; and (3) perceptions of difficulty on the neurological tests with peripheral blood glucose as the dependent variable. Results indicated that the first predictor (higher academic performance) was inversely related to peripheral blood glucose, contributing to 14% of the variance. The second predictor (CDI) showed that the levels of depression had insignificant effects on peripheral blood glucose. The third predictor (perception of difficulty) showed that the higher level of difficulty perceived on the test accounted for the higher peripheral blood glucose level. Perception of difficulty accounted for 11% of the variance after accounting for academic performance and depression. This indicated that higher perceptions of task difficulty were associated with higher blood glucose levels while depression seemed to play no significant role in this model. Individuals, who perceived the neuropsychological battery as challenging, may also experience difficulty controlling glucose levels.

The purpose of the final analysis (Table 5) was to determine whether academic performance would predict glucose levels after controlling for perceptions of task difficulty. The order of predictors was: (1) perception of difficulty; (2) academic performance; and (3) CDI with peripheral glucose levels as the dependent variable. The first predictor (perception of difficulty) showed that perception of difficulty increased with peripheral blood glucose, contributing to 22% of the variance. Most importantly, after controlling for perceptions of task difficulty, academic performance no longer predicted glucose levels. Additionally, it was found that the level of depression was not associated with peripheral glucose levels. Results from Table 5 indicated that peripheral blood glucose was positively correlated with the perception of task difficulty.

DISCUSSION

The general purpose underlying this research was to critically evaluate the various explanations for the problems that diabetic adolescents experience in the performance of cognitive tasks. In addition to elevations

Table 2 Correlations among the variables

	Academic performance	HbA1c	Peripheral blood glucose	CDI	Perceptions of difficulty
Academic performance ¹	-	-0.26	-0.37 ^a	-0.43 ^b	-0.48 ^b
HbA1c		-	0.42 ^b	0.03	0.34 ^a
Peripheral blood glucose ²			-	0.22	0.47 ^b
CDI ³				-	0.27

¹Low scores indicate poor grades; ²Low score-lower glucose levels; ³Higher scores more depression. ^a $P < 0.05$; ^b $P < 0.01$. HbA1c: Hemoglobin A1c; CDI: Child depression inventory.

Table 3 Linear multiple regression analysis for variables predicting academic performance¹

	β	R ² change
Peripheral blood glucose ²	-0.37	0.14 ^a
Child depression inventory ³	-0.36	0.12 ^a
Perceptions of difficulty on neurological tests	-0.33	0.08 ^a

¹Low scores indicate poor grades; ²Low score-lower glucose levels; ³Higher score more depression. ^a $P < 0.05$. β : Standardized coefficient beta.

Table 5 Linear multiple regression analysis for variables predicting peripheral blood glucose¹

	β	R ² change
Perceptions of difficulty on neurological test	0.47	0.22 ^b
Academic performance ²	-0.18	0.03
Child depression inventory ³	0.05	0.00

¹Low score-lower glucose levels; ²Low scores indicate poor grades; ³Higher scores more depression. ^b $P < 0.01$. β : Standardized coefficient beta.

in blood glucose, there are two alternatives, namely anergia^[7] and depression^[6]. Since these processes each exacerbate or contribute to the diminution of effort, it is possible that the actual mediator of compromised cognition can be found in reduced effort. Thus, the primary purpose of this introductory study was to assess the contribution of effort^[14] to both metabolic control and reduced cognition in these patients.

Through a series of multiple regression analyses, the results showed that after controlling for levels of effort, the role of metabolic control was compromised with respect to cognition. A key assumption in this process is that metabolic control is per se an effortful process. If effort is viewed as the primary process, metabolic control remains as an adjunctive player in cognition.

To examine this model, we turn to the results of the multiple regression analyses presented earlier. That is, adolescents who perceived the neuropsychological battery as more difficult were more likely to have lower grades in Math, Science, and English. Thus, perceiving the battery as difficult may indicate a broad weakness in executive functioning skills, such as problem solving and decision-making. Furthermore, increased levels of depression also negatively contributed to academic performance. As a result, academic performance was

Table 4 Linear multiple regression analysis for variables predicting peripheral blood glucose¹

	β	R ² change
Academic performance ²	-0.37	0.14 ^b
Child depression inventory ³	0.08	0.01
Perceptions of difficulty on neurological test	0.38	0.11 ^b

¹Low score-lower glucose levels; ²Low scores indicate poor grades; ³Higher scores more depression. ^b $P < 0.01$. β : Standardized coefficient beta.

poorer in adolescents with elevated peripheral blood glucose, which in itself may be a reflection of the difficulty or effort necessary for exercising metabolic control.

This study provided insight into how academic performance may be associated with self-ratings of difficulty on neuropsychological tasks after controlling for blood glucose levels and depression. Further, the study showed how peripheral blood glucose levels can be derived from perceptions of difficulty based on neuropsychological testing after controlling for academic performance and depression. Each of these observations will be discussed serially.

Previous findings^[28,29] have suggested a relationship between glucose control and academic performance. Greater difficulty in maintaining strong glucose control was associated with poorer academic performance. The list of predictors of academic performance included a neuropsychological battery that assessed psychomotor performance, attention, memory, speed of information processing, and verbal functioning.

Participants who perceived the neuropsychological battery as difficult were also more likely to exhibit poor grades (Table 4). However, this finding may not be surprising, since performance on the neuropsychological battery and academic performance share some conceptual space. Thus, a basic question in this study is whether the commonly observed relationship between glucose levels and cognitive functioning represents a basic physiological relationship between glucose and neuropsychological functioning? Alternatively, is the index of glucose control merely a reflection of the basic challenge between compliance with regimen, an effort-demanding task, and the challenge intrinsic to the cognitive task?

After controlling for appraisals of task difficulty, blood glucose levels seem to no longer account for cognitive

performance once task difficulty is factored into the equation (Table 5). Finally, by eliminating the influence of perceptions of difficulty in maintaining blood glucose control and complying with medical regimen, peripheral blood glucose and academic performance were no longer significantly related.

This may be the first study to illustrate how perceptions of task difficulty on neuropsychological tests are associated with poor academic performance in diabetic patients. While several studies have reported that individuals with diabetes exhibit relatively poor academic performance, the present study identifies how perceptual evaluative factors diminish academic performance.

Thus, careful disease management is needed to prevent or effectively treat potentially life-threatening complications such as hypoglycemia, diabetic ketoacidosis, and hyperglycemia as well as effort.

Limitations to the study include a relatively small sample size as well as the use of a single item to assess perceptions of task difficulty. However, this method of assessing perceptions of difficulty with one item was also utilized in a previous study^[11]. In their study, Rogers and colleagues assessed self-efficacy, perceived control and perceived difficulty with the use of a single item Likert scale question for each construct^[11]. Therefore, single item indicators may be regarded as similarly robust as multiple item indicators^[30,31]. Lastly, academic performance was assessed by participant self-report. Systematic biases may influence the validity of self-reported grades, but self-reported grades generally predict outcomes as effectively as actual or objectively culled grades^[32].

In conclusion, this study demonstrated that perceptions of task difficulty can predict academic difficulty in a sample of diabetic adolescents. Task difficulty was also associated with the relationship between glucose levels and academic performance. Hence, perceptions of difficulty can be viewed as a generalized executive function. When individuals perceive a task as more difficult, this may promote academic disadvantage along with increased challenges in controlling glucose. Overall, such information may be useful in training and counseling students in enhancing academic success and performance as well as in assistance with disease control.

COMMENTS

Background

Type 1 diabetes is one of the most common chronic diseases of childhood. In the United States, type 1 diabetes affects approximately 1 in 300 children and is usually diagnosed between the ages of 5 and 11 years.

Research frontiers

Adolescents with type 1 diabetes face many challenges that can impact metabolic control along with physiological and psychological complications. Cognitive functioning is an important aspect of life and can be impaired in individuals with diabetes. Academic performance in diabetic adolescents may also be compromised due to fluctuations in glucose.

Innovations and breakthroughs

Along with academic adversity, individuals with type 1 diabetes may be

challenged by self-evaluative factors such as the perception of control, which affects behavioral and social functioning. Perceived difficulty has also been shown to relate to an individual's beliefs regarding how much effort is needed to succeed at a task and whether success is possible. Perceptions of difficulty rely on executive functioning skills, such as problem solving and decision-making. Since problem solving and decision-making skills contribute to academic performance, these executive functioning skills may be impaired in diabetic individuals. The current study sought to examine the role of effort on glucose and academic performance.

Applications

This may be the first study to illustrate how perceptions of task difficulty on neuropsychological tests are associated with poor academic performance in diabetic patients. While several studies have reported that individuals with diabetes exhibit relatively poor academic performance, the present study identifies how perceptual evaluative factors diminish academic performance.

Terminology

Diabetes mellitus is a major health problem in the United States affecting approximately 29.1 million people. There are two major classifications of diabetes: insulin dependent diabetes (type 1) and non-insulin dependent diabetes (type 2). Diabetes is a group of metabolic diseases that either cause defects in insulin action, insulin secretion, or a combination of both, resulting in hyperglycemia. Perceived difficulty, a subjective experience, can be defined as the extent to which individuals believe that performance on a task or behavior would range from easy to difficult.

Peer-review

The study addresses a relevant scientific question and is well-conducted. The results are significant indicating that deficits in perception of difficulty may lead to academic disadvantage in diabetic patients. The manuscript is interesting and may provide important information for the reader of the journal.

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Effect of aerobic and anaerobic exercises on glycemic control in type 1 diabetic youths

Andrea Lukács, László Barkai

Andrea Lukács, László Barkai, Faculty of Health Care, University of Miskolc, 3515 Miskolc-Egyetemváros, Hungary
 László Barkai, Velkey László Center for Child Health, 3501 Miskolc, Hungary

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Correspondence to: László Barkai, MD, PhD, DSc, Faculty of Health Care, University of Miskolc, 3515 Miskolc-Egyetemváros, Hungary. barkai.l@t-online.hu

Telephone: +36-46-565111

Fax: +36-46-366961

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Abstract

AIM: To evaluate the long-term effect of aerobic and/or anaerobic exercise on glycemic control in youths with type 1 diabetes.

METHODS: Literature review was performed in spring and summer 2014 using PubMed/MEDLINE, Google Scholar, Scopus, and ScienceDirect with the following terms: aerobic, anaerobic, high-intensity, resistance, exercise/training, combined with glycemic/metabolic control, glycated haemoglobin A1c (HbA1c) and type 1

diabetes. Only peer-reviewed articles in English were included published in the last 15 years. It was selected from 1999 to 2014. Glycemic control was measured with HbA1c. Studies with an intervention lasting at least 12 wk were included if the HbA1c was measured before and after the intervention.

RESULTS: A total of nine articles were found, and they were published between the years of 2002-2011. The sample size was 401 diabetic youths (166 males and 235 females) with an age range of 10-19 years except one study, in which the age range was 13-30 years. Study participants were from Australia, Tunisia, Lithuania, Taiwan, Turkey, Brazilia, Belgium, Egypt and France. Four studies were aerobic-based, four were combined aerobic and anaerobic programs, and one compared aerobic exercise to anaerobic one. Available studies had insufficient evidence that any type of exercise or combined training would clearly improve the glycemic control in type 1 diabetic youth. Only three (two aerobic-based and one combined) studies could provide a significant positive change in glycemic control.

CONCLUSION: The regular physical exercise has several other valuable physiological and health benefits that justify the inclusion of exercise in pediatric diabetes treatment and care.

Key words: Type 1 diabetes mellitus; Glycemic control; Exercise; Aerobic; Anaerobic

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Core tip: Diabetic patients should be aware that exercise interferes with the glucose homeostasis. Anaerobic exercise can increase glycemia, whereas the aerobic exercise may cause a decrease during the exercise and post-exercise. By evaluating the long-term effect of exercise on glycemic control in type 1 diabetic youths

according to the major metabolic pathway involved in energy utilization (aerobic or anaerobic), we found insufficient evidence in the latest literature that any type of exercise or combined aerobic and anaerobic training would clearly improve the glycemic control. The regular physical exercise has several other valuable benefits that justify the inclusion of exercise in pediatric diabetes treatment and care.

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INTRODUCTION

Physiological, social and emotional benefits of regular exercise and physically active lifestyle are well documented mostly in a healthy population^[1-4], but they are also important for diabetic patients regardless of the type of diabetes^[5-8]. For type 1, successful diabetes management is based on individualized insulin therapy, adjusted diet and regular exercise^[9].

Exercise used as prevention and therapy in diabetes is not a new concept in the literature. Even the ancient Ayurvedic physician, Susruta Shamita (born around 600 B.C.), noted the reduction in the sweetness of urine from diabetic patients with exercise, and included moderate exercise within his treatment regimens^[10]. In 1919, Allen proved that exercise reduced blood glucose level and improved acutely the tolerance to a carbohydrate load^[11]. After the discovery of insulin in 1921^[12], Lawrence demonstrated that exercise has an effect on insulin requirements and glucose uptake^[13]. Joslin believed in "troika" (group of three) in the treatment of diabetes, symbolizing insulin, diet and exercise correlation^[14]. Regular exercise helps to improve overall health and fitness, and reduces risk factors for vascular complications. Diabetic youths with regular exercise have improved blood lipid profile, and increased insulin sensitivity, primarily in the skeletal muscles, which leads to a reduced need for insulin^[15-17]. The American Diabetes Association recommends all levels of physical exercise including leisure activities, recreational sport and competitive sport for youths with type 1 diabetes mellitus (T1DM) if they are in good blood glucose control and have no long-term complications^[18]. Until the sympathetic nervous and endocrine systems control the blood glucose level at the physiological levels during and after physical exercise in healthy subjects^[19], the regulation is external in diabetic patients considering many internal influences. Youths with T1DM should be aware that exercise interferes with the glucose homeostasis, although there are individual differences in blood glucose response due to type, duration and intensity of the exercise, the pre-exercise level of counterregulatory hormones, and blood glucose

concentrations^[20,21]. Several studies examined the role of physical activity and exercise in the treatment of T1DM and considered it an important component. The evidence for improvement in glycemic control is equivocal. Some studies suggest a positive effect^[22-27], whereas others fail to show this effect^[28-34]. Scientific questions remain concerning what is the exact effect of different types of exercise on glycemic control in youths with T1DM.

In this systematic literature review, we evaluated the latest studies examining the long-term effect of aerobic and/or anaerobic exercise on glycemic control in youths with T1DM. We also made distinguishing between concepts of physical activity, physical fitness, and aerobic-anaerobic exercise. Finally, we considered clinical applications and future directions.

Conceptualisation

Physical activity: Physical activity encompasses body movement produced by skeletal muscles which requires energy consumption^[35]. Physical activity can range from sports to any other lifestyle activities including school and out-of-school activities, weekend activities where youths play, are active and expend energy. There is evidence that behavioural patterns of physical activity in childhood are maintained throughout adulthood^[36,37]. This term is often used interchangeably with regular exercise and physical fitness in studies, although they are different concepts^[38].

Exercise: Exercise has been defined as any form of body movement that results in an increase in metabolic demand with the intention of developing one or more components of physical fitness. It is generally planned, structured and systematic^[35]. Regular exercise improves physical fitness. Exercise is characterised by five components: type [dynamic (isotonic) or static (isometric), and both of them can be performed aerobically or anaerobically on the basis of energy utilization]^[39], intensity (degree of effort that individual puts into exercise), duration (the length of each training session), repetition (number of times individual performs a complete movement of a given exercise) and frequency (number of exercise sessions per week)^[40].

Fitness: Fitness refers to the "possession of adequate levels of strength, endurance, and mobility to provide for successful participation in occupational effort, recreational pursuits, familial obligation, and that is consistent with a functional phenotypic expression of the human genotype"^[41]. Physical fitness determines cardiovascular, respiratory, and musculoskeletal systems in order to perform physically demanding activities such as exercise, sport, or work. Fit youth has normal body fat, good immune system and, in general, is in a physiologic state of well-being.

Aerobic-anaerobic exercise: Whether an activity is aerobic or anaerobic depends primarily on its intensity and duration. Most physical exercises are characterised

by both static and dynamic contractions as well as aerobic and anaerobic metabolism. Thus, exercise is classified by their dominant features.

Aerobic exercise includes any type of exercise, typically those performed at moderate levels of intensity for extended periods of time that maintains an increased heart rate. Activities such as cycling, swimming, jogging, rowing, cross-country skiing, and aerobic dancing require oxygen to produce ATP. Regular aerobic exercise improves maximal oxygen consumption and overall endurance performances. Anaerobic exercise is used to promote strength, power, and speed. Generally, anaerobic exercise has a short duration and high intensity activity. Unlike aerobic exercise, it does not depend on exogenous oxygen. Activities such as heavy weightlifting, all types of sprint (running, cycling, or swimming) or any hard exercises require anaerobic metabolism. During exercise various forms of energy sources are utilized markedly depending on the intensity and duration of exercise, but the activity is classified typically based on the predominantly used system. The anaerobic energy system is used for resistance training and increasing speed^[42]. During high intensity (anaerobic) exercise almost the entire metabolic fuel source is glucose, whereas during low intensity (aerobic) exercise fat utilization increases and glucose oxidation decrease^[43]. Both types of exercise increase the mechanical efficiency of the heart (cardiac adaptation), changes in morphology and functionality of the left ventricle^[44].

Measuring the intensity of exercise using maximal heart rate:

Maximal heart rate (HR_{max}) is one of the most commonly used values in clinical settings and physiology. HR increases nearly linearly with the increase in the intensity of exercise; and this is the simplest physiological response to measure. As maximal exercise intensity is approached, HR begins to plateau even as the exercise workload continues to increase. This HR_{max} is a reliable value and it remains constant for a longer period^[45]. To estimate HR_{max} for children, formula $208 - 0.7 \times \text{age in years}$ is applied^[46,47]. There are several more and less accurate types of estimation existing for determining the exercise intensity^[48]. Aerobic training zone is around between 60%-70% of the HR_{max} . Between 70%-85% of HR_{max} , the youths are in the mixed zone, and above 85% of HR_{max} the anaerobic metabolism will come to the fore.

Effects of different types of exercise on blood glucose level in type 1 diabetes patients

Different types of exercise produce different effects on blood glucose level. If the carbohydrate intake and the insulin dosage are not in line with the exercise the patient does, it will result in metabolic disturbances^[49]. The most common problems are the evaluation of pre-exercise level of blood glucose, evaluation of the intensity and duration of the expected exercise, and to

take into consideration the time of day when the patient exercises, because of the body's different physiological insulin need^[49]. Exercise functions like insulin, so the balance between the insulin therapy and diet could be facilitated if the daily schedule for exercise and the exercise parameters are consistent^[50], although this goal is almost impossible to obtain in the real life for youths. Findings from exercise training studies support the concept that moderate intensity aerobic workload increases the risk of hypoglycemia during the exercise and several hours after the exercise. High-intensity training with anaerobic utilization may increase the blood glucose level due to the release of adrenaline and noradrenalin in the blood, which then stimulates the liver to release glucose faster than normal. The exercise-induced rise in glucose level is followed by hypoglycemia after hours of finishing the exercise as counterregulatory hormone levels decrease^[21,51-53]. Both aerobic and anaerobic exercise training regimens improve glucose uptake and insulin sensitivity^[21,54]. School-aged children engage a combination of moderate- and high-intensity sessions in their everyday sport activities. Their exercise is often spontaneous and unplanned. Thus, a different plan is needed for each type of exercise to optimize blood glucose level, but problems with the management are well recognised. Patients differ in tolerance to exercise and insulin requirements and it is impossible to give precise guidelines suitable for everyone with T1DM, therefore experiences with regular blood glucose testing are inevitable. Continuous glucose monitoring may assist the active diabetic participants to follow the changes in blood glucose level and give information about the individual response to the exercise^[21,54]. There are several guidelines to discuss safe sport participation in children and adolescents with T1DM^[5,55-59]. These guidelines can be adjusted to own measurements and awareness of the usual responses to a particular type of exercise.

MATERIALS AND METHODS

Study eligibility

A systematic review was performed in spring and summer 2014 using PubMed/Medline, Google Scholar, Scopus, and ScienceDirect with the following terms: aerobic, anaerobic, high-intensity, resistance, exercise/training, combined with glycemic/metabolic control and type 1 diabetes. Only peer-reviewed articles in English were included published in the last 15 years with at least five subjects per group. Studies were selected from 1999 to 2014 that measured the long-term effect of aerobic and/or anaerobic exercise on glycemic control in type 1 diabetic children, adolescents and young adults without diabetes complications. Glycemic control was measured with glycated haemoglobin A1c (HbA1c). As the HbA1c provides an average of blood glucose control over a 12-wk period, we included studies with an intervention lasting at least 12 wk if the HbA1c was

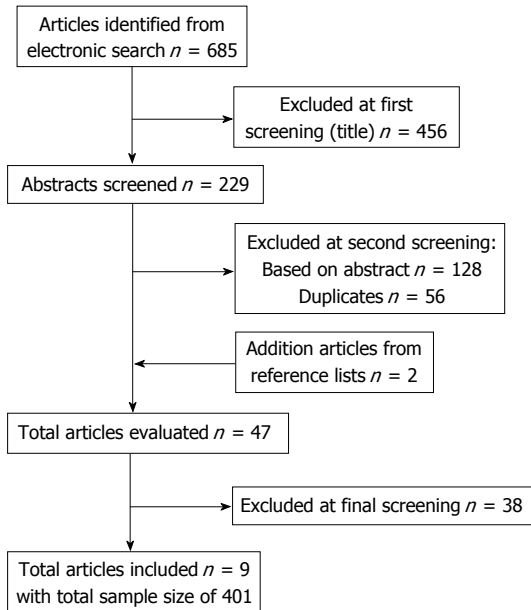


Figure 1 Flow diagram illustrating selection of the included studies.

measured before and after the intervention. The flow diagram of study selection is shown in Figure 1.

Statistical analysis

This manuscript does not describe basic or clinical research using biostatistics for raw data, therefore biostatistics statement is not relevant.

RESULTS

A total of nine articles was evaluated from nine different countries including Australia^[30], Tunisia^[60], Lithuania^[61], Taiwan^[62], Turkey^[63], Brazilia^[64], Belgium^[65], Egypt^[66] and France^[67], and they were published between the years of 2002-2011. The list of articles is presented in Table 1. Across included studies the total sample size was 401 (166 males and 235 females) with an age range of 10-19 years except one study, in which the age range was 13-30 years^[64]. Four studies were aerobic-based^[60-63], four were combined aerobic and anaerobic programs^[30,65-67], and one compared aerobic exercise to anaerobic one^[64]. The Turkish study^[63] conducted a Pilates program that is considered low-impact aerobic workout^[68]. All studies had diabetic control group except one^[61].

Effects of different types of exercise on glycemic control in youths with T1DM

Studies investigating aerobic exercise: Studies measuring the effect of aerobic exercise on glycemic control presented variable results^[60-63]. Only one study recognised a significant improvement in HbA1c after 12-wk exercise (girls swimming twice a week for 45 min a week)^[61]. In a study investigating the impact of the frequency of the supervised aerobic training on

glycemic control, exercise three times a week (one hour) for three months had no significant impact on glycemic control, but exercise four times a week (one hour) for 6 mo resulted in a significant improvement^[60]. Neither guided or self-directed home-based aerobic exercise program nor supervised Pilates exercise had a significant effect on glycosylated haemoglobin after a 12-wk session^[62,63].

Study investigating anaerobic exercise: We found only one study that measured the efficacy of resistance (anaerobic) and aerobic exercise in parallel. Both groups were trained three times a week (40 min) for 12 wk under supervision by a physical trainer and an endocrinologist. Interestingly, the HbA1c increased in the aerobic group significantly after 12 wk, whereas a non-significant reduction was observed in the resistance training group^[64].

Studies investigating combined exercise program:

Four randomised controlled trials examined the effect of combined (aerobic and anaerobic) exercise on glycemic control^[30,65-67]. Supervised combined aerobic and resistance training twice a week (70 min) for 20 wk resulted in no significant change in glycemic control^[65]. In a 12-wk supervised training followed by a 12-wk unsupervised training, HbA1c level remained unchanged regardless of the baseline HbA1c level^[30]. There were two trials that explored the effect of supervised combined exercise on glycemic control for a longer duration (6 mo)^[66,67]. The Egyptian study found significant improvements in HbA1c level in both training groups exercising three times a week and once a week. However, patients exercising three times a week produced significantly greater improvements^[66]. In the Heyman's study, the participants completed a two-hour supervised training and a one-hour unsupervised training a week including aerobic and strength exercises. The author could not prove a significant effect on glycated hemoglobin after a 6-mo session^[67].

DISCUSSION

The primary aim of diabetes treatment and care is to achieve as stable glycemic control as possible in order to prevent or delay the long-term complications^[69]. Regular exercise is recommended for diabetic youths for several psychological and health benefits^[7,70]. It is evidence-based that regular exercise has a preventive and curative role in type 2 diabetes, but its physiological role in type 1 diabetes is not fully explored. Exercise physiology in diabetes is described thoroughly by Robertson *et al*^[7] and Riddell *et al*^[53,59]. Another issue that remains unresolved is related to the type of exercise: aerobic or anaerobic workout is most beneficial for glycemic control. We evaluated the studies from the previous 15 years that investigated the long-term effect of exercise on glycemic control in youths with T1DM according

Table 1 Study characteristics, objective and result regarding glycemic control

Source	RCT	Objective	Sample size/gender	Age (y/o) gender	Intervention	Duration, frequency	Significant positive change in GC
Aouadi <i>et al</i> ^[61] , (2011) Tunisian	No	Effect of aerobic training on glycemic control and lipid profile	EG1: 11 (twice) EG2: 11 (4 times) DC: 11 no exerc	12-14 33 M	Supervised aerobic exercise	3 and 6 mo, twice <i>vs</i> four times a week (60 min)	Yes, but only 6 mo with four times a week duration
Sideraviciute <i>et al</i> ^[61] , (2006) Lithuanian	No	Effect of long-term physical activity in water on glucose control	EG: 19 (HC: 21)	14-19 19 F	Supervised aerobic exercise	14 wk, twice a week (45 min)	Yes
Wong <i>et al</i> ^[62] , (2010) Taiwan	No	Effect of home-based exercise programme on HbA1c and peak oxygen uptake	EG: 12 video-, 5 self directed DC: 11	7-17 8 M, 20 F	Unsupervised aerobic exercise	12 wk, three times a week (30 min)	No
Tunar <i>et al</i> ^[63] , (2012) Turkish	Yes	Effect of pilates training on metabolic control and physical performance	EG: 17 DC: 14	12-17 15 M, 16 F	Supervised aerobic exercise	12 wk, three times a week (40 min)	No
Ramalhõ <i>et al</i> ^[64] , (2006) Brazilian	Yes	Effect of aerobic <i>vs</i> resistance training on metabolic control	EG: 7 DC: 6	13-30 3 M, 10 F	Both aerobic and anaerobic exercise were supervised	12 wk, three times a week (40 min)	Sig. increase in aerobic group, not sig. decrease in anaerobic group
D'hooge <i>et al</i> ^[65] , (2011) Belgian	Yes	Effect of combined exercise training on metabolic control, physical fitness and quality of life	EG: 8 DC: 8	10-17 7 M, 9 F	Supervised combined exercise	20 wk, twice a week (70 min)	No
Roberts <i>et al</i> ^[66] , (2002) Australian	Yes	Whether the change of the glycemic control after intervention is dependent on the initiation of quality of glycemic control	EG1: 12 HbA1c > 9% EG2: 12 HbA1c < 9% DC1: 12 HbA1c > 9% DC2: 12 HbA1c < 9%	Circa 11-17 24 M, 24 F	12 wk supervised 12 wk unsuper-vised combined exercise	24 wk, three times a week (45 min)	No
Salem <i>et al</i> ^[66] , (2010) Egyptian	Yes	Effect of exercise on glycemic control, plasma lipids, blood pressure, severity and frequency of hypoglycemia, anthropometric measurements and insulin dose	EG1: 75 (once) EG2: 73 (3 times) DC: 48	12-18 76 M, 121 F	Supervised combined exercise	6 mo, once and three times a week (70 min)	Yes, in both exercise groups
Heyman <i>et al</i> ^[67] , (2007) French	Yes	Effect of exercise on quality of life, physical fitness, body composition, lipid and apolipoprotein profiles, and adiponectin and leptin levels	EG: 9 DC: 7	13-18 16 F	Supervised combined exercise	6 mo, 2 h supervised, and 1 h unsupervised	No

HbA1c: Haemoglobin A1c; RCT: Randomized controlled trial; EG: Exercise group; DC: Diabetic control; HC: Healthy control; M: Males; F: Females; y/o: Years old; GC: Glycemic control; sig.: Significant.

to the major metabolic pathway involved in energy utilization (predominantly aerobic or predominantly anaerobic). Results of investigated studies are open to debate. Most studies found no significant improvement in glycemic control regarding the aerobic^[62,63] and combined exercise intervention^[30,65,67]. The only study investigating the anaerobic effect on glycemic control presented a positive tendency, although there was no statistically significant effect^[64]. One of the possible reasons for these varied results could be the small sample sizes. The glucose profile varies greatly in patients with T1DM before, during and after exercise, and can be very different in patients with similar HbA1c. All except one study^[66] had less than 20 participants in a group in our studies. The other reason could be the short period. It seems that at least 6 mo of exercise intervention might lead to significant results^[60,66]. Kennedy *et al*^[71] also noticed in their systematic review and meta-analysis that longer duration of intervention shows a trend for HbA1c reduction. We had a total of 401 samples in our studies and the Egyptian study^[66] alone offered 187 participants. Weighting its results, it might be supposed that combined long-term exercise intervention is more beneficial for the glycemic control, than aerobic or anaerobic alone. Irvine *et al*^[72] in their systematic review with 372 patients with T2DM have reported that (anaerobic) progressive resistance exercise is not significantly better than aerobic exercise in improving glycosylated haemoglobin. There was a lack of evidence to suggest that one type of exercise was better than another^[72]. Yang *et al*^[73] in their systematic review with 595 patients with

T2DM also enunciated that either form of exercise appears to have comparable effects on glycemic control. A meta-analysis by Tonoli *et al.*^[74] with type 1 diabetic participants explored a tendency for improvement in glycaemic control due to aerobic or combined training, but they could not confirm this statistically. Individual studies on aerobic training had no significant evidence, but “in total” demonstrated a reduction in HbA1c, although they carefully interpret their results because of insufficient data on these topics^[74]. Neither Kennedy’s meta-analysis in children and adults^[71] nor Kavookjian’s systematic review in the adult population^[75] revealed evidence for statistically significant glycemic benefits of exercise. Kennedy *et al.*^[71] suggest that HbA1c may not be a sensitive indicator of glycaemic control, and that improvement in glycaemic variability may not be reflected in this measure. Tight metabolic control is very important for diabetic individuals, and the regular physical exercise is part of the diabetes management. Available studies provide insufficient evidence that any type of exercise or combined training would clearly improve the glycemic control in type 1 diabetic young patients. Long-term exercise-induced glycemic benefits are based on the continuous effect of each successive bout of exercise. Glycemic variability can be detected by continuous glucose monitoring that could help individuals to learn their own response to different type of physical exercise; and it might be a more appropriate marker to explore the exercise-induced beneficial effect than HbA1c^[71]. Youths with T1DM are recommended to be engaged in a sport they like, and do it regularly as much as possible at the same period of the day. Having the appropriate exercise physiology knowledge, they can accumulate own experiences regarding exercise in addition to insulin requirement and dietary program that may result in stable glucose levels. The insulin-diet-exercise adjustment must be personalized and discussed with the patient’s endocrinologist. The regular exercise is associated with numerous health benefits and chronically ill youths enjoy the same benefits as the healthy counterparts^[1,70].

There are miscellaneous results regarding the long-term effect of various forms of exercise on glycemic control. Meta-analyses also suffered from the insufficient randomised controlled trials with greater sample sizes to examine the short- and long-term effect of glycemic control on different exercise modalities in youths with T1DM. Patients and health professionals may want more information they currently received, but there are concerns that the results would be overestimated. Different forms of exercise generate different skills in youths. They need a comprehensive form of exercise to help their healthy many-sided physical development. Diabetic patients should be aware that glycemic control is influenced by multiple factors (initial blood glucose level, insulin absorption, time of the day), and shows individual responses to exercise. It is advisable that young patients choose some kind of sports or physical exercise they like and be engaged with it for a longer

time. Clinical treatment and management should be adjusted to the exercise form and the diabetic patients should acquire how to change nutrition and insulin dose according to their daily physical exercise. Patients who can monitor themselves intensively around periods of activity can learn how to keep glucose levels in an acceptable range.

Although current studies have insufficient evidence of the beneficial effect of any type of exercise on glycemic control, any type of regular physical exercise has several other valuable physiological and health benefits that justify the inclusion of exercise in pediatric diabetes treatment and care.

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COMMENTS

Background

Regular exercise is recommended for diabetic youth for several psychological and health benefits, but its physiological role in type 1 diabetes is not fully explored. So far, there are only few studies evaluating the long-term effect of different types of exercise on glycemic control.

Research frontiers

In this systematic literature review, the authors evaluated the latest studies examining the long-term effect of aerobic and/or anaerobic exercise on glycemic control in youths with type 1 diabetes. They also made distinguishing between concepts of physical activity, physical fitness, and aerobic-anaerobic exercise.

Innovations and breakthroughs

Most studies found no significant improvement in glycemic control regarding the aerobic and combined exercise intervention. The only one study evaluating anaerobic exercise observed a non-significant reduction in glycemic control. It might be supposed that longer duration (at least 6 mo) and more frequent (more than twice a week) exercise has a positive effect on glycemic control, but it is not proved yet.

Applications

Although current studies have insufficient evidence of the beneficial effect of any type of exercise on glycemic control, any type of regular physical exercise has several other valuable physiological and health benefits that justify the inclusion of exercise in pediatric diabetes treatment and care.

Terminology

Aerobic exercise includes any type of exercise, typically those performed at moderate levels of intensity for extended periods of time that maintains an increased heart rate. Anaerobic exercise is used to promote strength, power, and speed. Generally, it has a short duration and high intensity activity. Unlike aerobic exercise, it does not depend on exogenous oxygen.

Peer-review

The manuscript covers an important topic in type 1 diabetes management. The contribution that the manuscript provides is an overview of the dearth of scientific evidence to support exercise intervention in this population.

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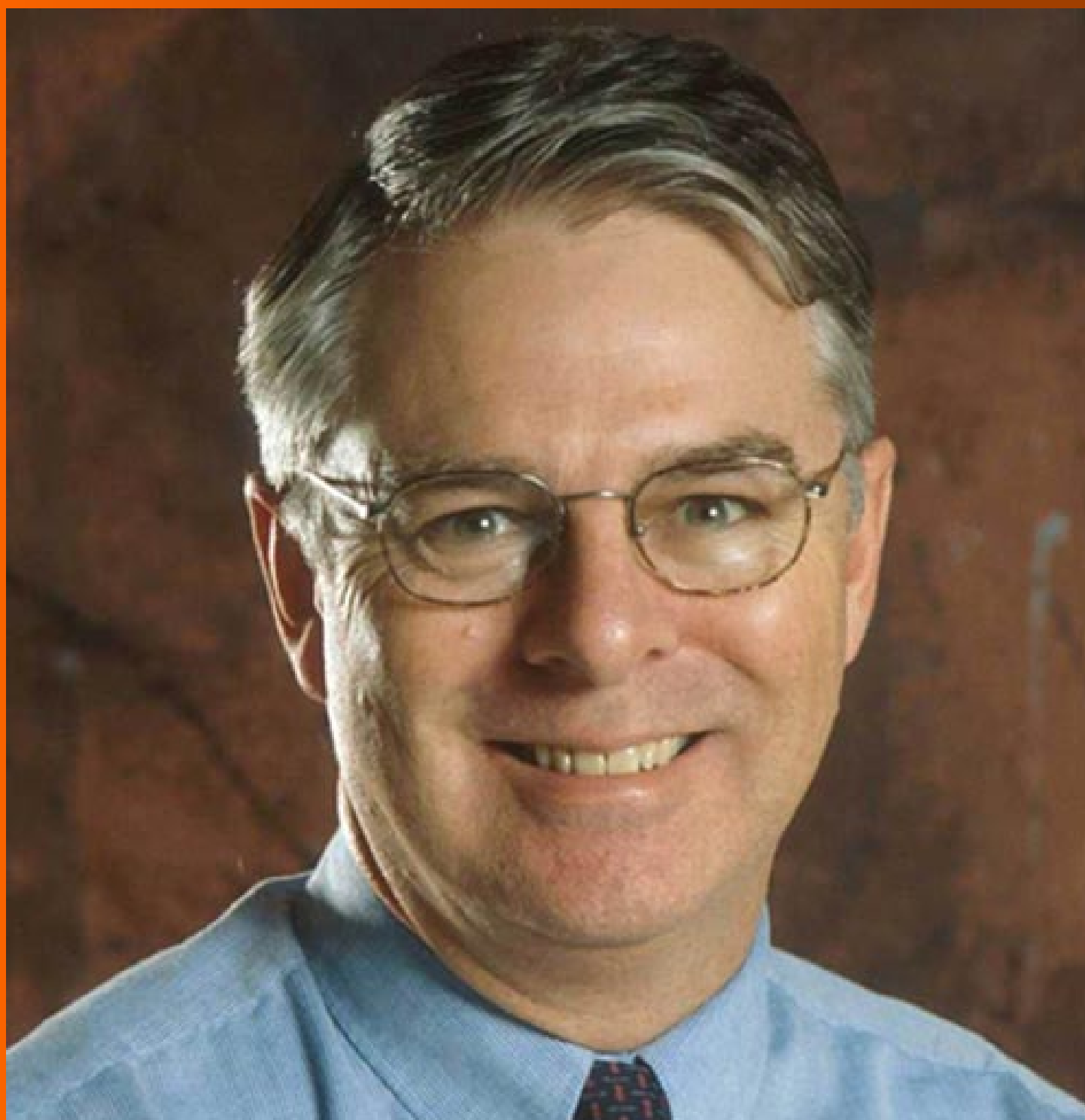
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Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

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Type 2 diabetes among Asian Americans: Prevalence and prevention

Tam H Nguyen, Thuc-Nhi Nguyen, Taylor Fischer, Won Ha, Thanh V Tran

Tam H Nguyen, Taylor Fischer, William F. Connell School of Nursing, Boston College, Chestnut Hill, MA 02467, United States

Thuc-Nhi Nguyen, Thanh V Tran, Graduate School of Social Work, Boston College, Chestnut Hill, MA 02467, United States

Won Ha, Graduate School of Social Work Library, Boston College, Chestnut Hill, MA 02467, United States

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Correspondence to: Tam H Nguyen, PhD, MSN/MPH, RN, Assistant Professor, William F. Connell School of Nursing, Boston College, 140 Commonwealth Ave, Cushing Hall 336C, Chestnut Hill, MA 02467, United States. tam.nguyen@bc.edu
 Telephone: +1-617-5523669

Fax: +1-617-5523666

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Asian American ethnic groups; with Filipino, Pacific Islander, Japanese, and South Asian groups consistently described as having the highest prevalence of T2DM. Disentangling and strengthening prevalence data is vital for on-going prevention efforts. The strongest evidence currently available to guide the prevention of T2DM in the United States comes from a large multicenter randomized clinical control trial called the Diabetes Prevention Program, which targets individual lifestyle behavior changes. It has been translated and adopted for some Asian American groups, and shows promise. However stronger study designs and attention to several key methodological considerations will improve the science. Increased attention has also been directed toward population level downstream prevention efforts. Building an infrastructure that includes both individual and population approaches is needed to prevent T2DM among Asian American populations, and is essential for reducing health disparities.

Key words: Type 2 diabetes mellitus; Asian American; Prevalence; Prevention; Health disparity

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Core tip: Current estimates suggest that type 2 diabetes affects approximately 9% of Asian Americans overall. However, when examining disaggregated data across different ethnic groups Filipino, Pacific Islander, Japanese, and South Asian groups consistently have the highest prevalence of type 2 diabetes mellitus. This highlights how aggregating Asian Americans into one category can potentially mask the disease burden in high risk groups, while inflating the burden in low risk groups. Prevention efforts therefore need be culturally tailored to meet the unique needs of the various Asian American ethnic groups. In addition, prevention efforts should address both individual and population level strategies.

Abstract

Type 2 diabetes mellitus (T2DM) is a growing problem among Asian Americans. Based on the Centers for Disease Control, the age-adjusted prevalence of T2DM for Asian Americans is 9%, placing them at "moderate risk". However differential patterns of disease burden emerge when examining disaggregated data across

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a growing epidemic in the United States. In 2012, over 21 million people were diagnosed with the disease as compared to 1.6 million in 1958^[1,2]. Moreover, the current cost associated with DM care in the United States is estimated at \$113 billion, and is expected to escalate to \$336 billion by 2034^[3]. The high prevalence of T2DM has been well documented for Native Americans, non-Hispanic Blacks, and Hispanic Americans^[4]. However, increasing attention has been drawn to the problem of T2DM among Asian Americans^[5,6], one of the fastest growing racial/ethnic minority groups in the United States^[7]. Disentangling and strengthening prevalence data will provide more support for activities and resources to address this important health problem among Asian Americans. In addition, understanding best practices and building an infrastructure to prevent T2DM among Asian American populations is essential for reducing health disparities, and more broadly for curbing the growing epidemic of T2DM in the United States.

PREVALENCE OF T2DM

For many years, health services for Asian American populations have been hampered by the model minority myth; the notion that Asian Americans are self-sufficient, well-educated, and have lower burdens of disease^[8,9]. In large part, the myth was perpetuated by the lack of reliable data that often lumped Asian Americans into one large category, when in fact they represent a heterogeneous group. This issue is particularly relevant when examining the prevalence of T2DM among Asian Americans. For example, the 2014 Centers for Disease Control (CDC) report estimated that the age-adjusted prevalence of T2DM for Asian Americans as a whole was 9%. This rate is lower than that of Native Americans (15.9%), non-Hispanic Blacks (13.2%), and Hispanic Americans (12.8%), but higher than that of non-Hispanic Whites (7.6%); placing Asian Americans at "moderate risk" for T2DM^[2]. When examining disaggregated data across various Asian American ethnic groups though, differential patterns of disease burden emerge.

For example, a study by Choi *et al.*^[10] using population based data from the 2009 California Health Information Survey (CHIS) found that Native Americans, non-Hispanic Blacks, and Hispanic Americans had higher overall age-adjusted prevalence of T2DM than

Asian Americans (as a whole); supporting findings from the CDC report. However, a more complex story is revealed when the data for Asian Americans are disaggregated across six different ethnic groups. Based on disaggregated data, Filipinos had the highest age-adjusted prevalence (15.8% men, 9.4% women), followed by Japanese (11.8% men, 7.6% women), Korean (6.7% men, 5.1% women), South Asian (6.3% men, 2.7% women), Chinese (5.0% men, 3.6% women), and Vietnamese (2.5% men, 2.1% women). These results demonstrated that among some Asian American groups the age-adjusted prevalence of T2DM was even higher than in non-Hispanic Black (8.8% men, 13.3% women), and Hispanics American (6.7% men, 10.7% women) groups. In particular, Filipino (15.8%) and Japanese (11.8%) American men were found to have among the highest rates of T2DM. Other population based prevalence studies have reported similar findings, with Filipino, Pacific Islander, Japanese, and South Asian groups consistently described as having the highest prevalence of T2DM across all Asian American ethnic subgroups^[11-14]. These examples highlight how aggregating Asian Americans into one category can potentially mask the disease burden in high risk groups, while inflating the burden in low risk groups.

While disaggregated data has clear advantages, much of the data available on the prevalence of T2DM among Asian Americans is at the aggregate level. When exploring aggregate data, there are some important patterns that can be used to underscore the urgency of addressing T2DM in Asian American populations. Specifically, when examining trends over time, several methodologically rigorous studies suggest that the prevalence of T2DM is increasing faster among Asian Americans than non-Hispanic Whites, non-Hispanic Blacks, and Hispanic Americans^[15-17]. Moreover, in a study that used fasting plasma glucose test to estimate the prevalence of T2DM, Asian Americans had higher levels of "pre-diabetes" than non-Hispanic Whites, non-Hispanic Blacks, and Hispanic Americans^[18]; foreshadowing further potential increases in the burden of T2DM among Asian Americans over the next few decades. Additionally, across DM prevalence studies that measure Body Mass Index (BMI), the relationship between T2DM and BMI appears to be different among Asians Americans than non-Hispanic Whites, with Asian Americans reporting T2DM at significantly lower BMI levels^[12,13,16,19]. These data provide strong evidence of disparities in T2DM among and across various Asian American ethnic groups, and underscores the urgency of addressing T2DM in Asian American populations.

To strengthen future prevalence data, providing disaggregated rates across ethnic subgroups is important. In addition, to simulate demographic data collected at the state and national level, it is helpful to report prevalence rates based on aggregated categories of "Asian" and "Asian Pacific Islander"

groups. When the goal of measuring prevalence is to compare rates across groups and time, it is also important to make sure that: (1) data are collected using a random population-base sampling strategy rather than with convenient samples; (2) adjustments are made to account for age given that T2DM significantly increases with age; and (3) rates are reported with their associated 95% confidence interval, which highlight the fact that prevalence rates are estimates of the population burden and allow readers to assess the precision of the estimate. These strategies will ensure that high quality data are collected and reflective of the realities and needs of the diverse Asian American population. Continued work in this area is essential to garnering ongoing support and resources to address the growing problem of T2DM among Asian Americans.

PREVENTION OF T2DM

The strongest evidence currently available to guide the prevention of T2DM in the United States comes from a large multicenter randomized clinical control trial ($n = 3234$) called the Diabetes Prevention Program^[20]. The program targets individuals with "pre-diabetes," and includes a 16-session "lifestyle" curriculum covering diet, exercise, and behavior change. Each session is taught on a one-to-one basis by a case manager trained in motivational interviewing techniques. In addition, six follow-up sessions are provided on a monthly basis to reinforce behavior changes. The main goals of this intervention include a 5%-7% weight reduction and ≥ 150 min of moderate physical activity per week. Study results demonstrated that this intervention reduced the development of diabetes by 58%^[20], and that the protective benefits persisted over 10 years^[21].

While the aims of the Diabetes Prevention Program are straight forward, the resource burden and lack of cultural relevance associated with the program have resulted in calls to test new models of program delivery, as well as to translate the program for use in minority populations^[22]. Several studies have demonstrated success in adopting the program in a variety of different minority populations; however they have largely been done with non-Hispanic Black and Hispanic American groups^[23,24]. Efforts to translate these findings with Asian American populations are limited, with most published studies designed at the pilot or quasi-experimental level, and focused on select Asian American ethnic subgroups including Chinese, Korean, Filipino, Pacific Islanders, and South Asians^[14,25-28]. Continued work in this area is critical because the diets and cultural norms among Asian Americans are vastly different from the general population, and vastly differ across Asian American ethnic groups^[29].

This heterogeneity likely explains most of the variance in prevalence rates described earlier, and

highlights the importance of culturally tailoring prevention interventions for a given ethnic subgroup. In 2008, the National Institutes of Health held a workshop to discuss strategic options to further investigate cardio-metabolic diseases among Asian American populations in the United States^[30]. Among their recommendations, the need to further understand dietary and physical fitness habits of Asian American subgroups was highlighted as a critical component to successfully tailoring interventions. Specifically, they suggests that more research is needed to understand the social context of eating, shopping, cooking, household dynamics on food choices (particularly related to influences on traditionally high carbohydrate diets and unhealthy eating patterns), as well as feasibility, perceptions, barriers (*i.e.*, social role strain), outliers, and motivators to exercising (perhaps by contrasting Asians who exercise and those who do not). To examine these topics, in-depth qualitative and mixed-methods studies will be vital. Of special note- given that the Asian American population represents individuals from over 60 countries with varying languages, cultures, and immigration status, a working group associated with the National Heart Lung and Blood Institute suggests clustering Asians into manageable groups that have similar risk profiles as a way to save cost and ensure broad generalization^[31]. These groups include: East Asians, South Asians, Southeast Asians, and Hawaiian/Pacific Islanders.

In addition to exploring these topics, future work aimed at adopting and/or translating the Diabetes Prevention Program should also consider some of the following opportunities and challenges. First, given that Asian Americans tend to have T2DM at lower BMI levels, expanding the inclusion criteria to include individual with BMI values between 23-25 kg/m² may be warranted. Related to this, it is advisable to also measure waist circumference, a potentially stronger predictor of DM risk given that Asian Americans tend to gain weight around the abdomen (*i.e.*, central adiposity). Second, many of the existing studies highlighted earlier use community health workers (CHWs) as the "interventionist". Future studies should describe in more detail how standardized training was provided for CHWs; this will enhance intervention fidelity, and provide stronger evidence for the value and use of CHWs. Other "burning" questions that will help move the field forward include evaluating whether or not outcomes differ when the interventionist is a "Certified Diabetes Educator", a designation that requires advanced training and increased cost. Addressing these questions will further promote the benefits of interventions like the Diabetes Prevention Program, and make them more generalizable to Asian American populations.

While there are clear benefits to interventions that target individual lifestyle changes, our current efforts underestimate how hard it is to change behavior not just once or twice, but every day of our

lives. As such, efforts directed toward population level downstream prevention efforts have gained increased attention. Primordial prevention refers to activities (*i.e.*, interventions and policies) that are put in place to prevent the development of risk factors (*i.e.*, obesity, inactivity, poor diet, and chronic stress) in the first place^[32]. Creating optimal defaults is the prevailing strategies for addressing primordial prevention. This involves creating environments that enables healthy choices and behaviors for all people across all age groups. This approach necessarily requires a population perspective that engages members of the community not traditionally involved with healthcare (*e.g.*, Boards of Education, Parks and Recreation, Department of Housing, Transportation, and Social Services). Engaging with communities and working with various stakeholders to change the environment (*i.e.*, improve social determinants of health) is especially essential in Asian American communities where coordinated advocacy work may not be as strong.

Whether prevention approaches are targeted at the individual or population level, some final thoughts that are important to consider include upfront planning to (1) estimate the cost and quality of these interventions, and (2) build in continuous longitudinal evaluation. This information will become increasingly relevant as the United States healthcare system enters an era of accountability. Together with improved reporting of prevalence data, these cumulative efforts will ensure that a strong infrastructure is built to prevent T2DM among and across Asian American populations.

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Adipose tissue fibrosis

Christa Buechler, Sabrina Krautbauer, Kristina Eisinger

Christa Buechler, Sabrina Krautbauer, Kristina Eisinger, Department of Internal Medicine I, University Hospital of Regensburg, 93042 Regensburg, Germany

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Correspondence to: Christa Buechler, PhD, Department of Internal Medicine I, University Hospital of Regensburg, Franz-Josef-Strauß-Allee 11, 93042 Regensburg,

Germany. christa.buechler@klinik.uni-regensburg.de

Telephone: +49-941-9447009

Fax: +49-941-9447019

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hypoxia contribute to immune cell immigration and activation which further aggravates adipose tissue fibrosis. There is substantial evidence that adipose tissue fibrosis is linked to metabolic dysfunction, both in rodent models and in the clinical setting. Peroxisome proliferator activated receptor gamma agonists and adiponectin both reduce adipose tissue fibrosis, inflammation and insulin resistance. Current knowledge suggests that antifibrotic drugs, increasing adipose tissue oxygen supply or HIF-1 antagonists will improve adipose tissue function and thereby ameliorate metabolic diseases.

Key words: Collagen; Hypoxia; Insulin resistance; Immune cells; Adipocyte

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Core tip: The close association of adipose tissue fibrosis and metabolic complications in obesity has been corroborated in rodent and human studies. Adipose tissue hypoxia initiates fibrosis which is further aggravated by inflammation. In adipose tissue preadipocytes, adipocytes and resident macrophages produce collagen showing that the fibrotic process differs from the extensively studied scar formation in the liver. Strategies to resolve fibrosis in fat tissues thereby promoting healthy adipose tissue growth are suggested to improve metabolic situation in obese patients.

Abstract

The increasing prevalence of obesity causes a major interest in white adipose tissue biology. Adipose tissue cells are surrounded by extracellular matrix proteins whose composition and remodeling is of crucial importance for cell function. The expansion of adipose tissue in obesity is linked to an inappropriate supply with oxygen and hypoxia development. Subsequent activation of hypoxia inducible factor 1 (HIF-1) inhibits preadipocyte differentiation and initiates adipose tissue fibrosis. Thereby adipose tissue growth is limited and excess triglycerides are stored in ectopic tissues. Stressed adipocytes and

Buechler C, Krautbauer S, Eisinger K. Adipose tissue fibrosis. *World J Diabetes* 2015; 6(4): 548-553 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i4/548.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i4.548>

INTRODUCTION

Obesity related diseases including type 2 diabetes and non-alcoholic fatty liver disease have become a major health problem. Inappropriate insulin production, insulin

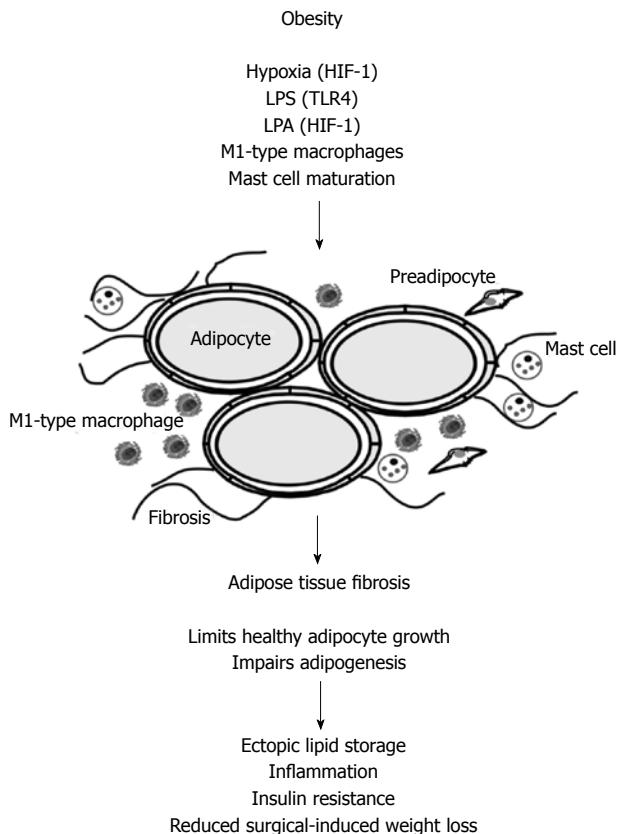


Figure 1 In obese adipose tissue hypoxia, lipopolysaccharide, lysophosphatidic acid, M1-type macrophages and matured mast cells contribute to adipose tissue fibrogenesis. Hypoxia and LPA mediated effects involve activation of HIF-1 and LPS activates TLR4. Fibrosis impairs adipogenesis and healthy adipocyte growth. Lipids are therefore stored in peripheral tissues like the liver. This is associated with impaired insulin sensitivity and inflammation. Adipose tissue fibrosis negatively affects surgery-induced weight loss. LPS: Lipopolysaccharide; LPA: Lysophosphatidic acid; HIF-1: Hypoxia inducible factor 1.

resistance and dyslipidemia are commonly associated with obesity. It is widely accepted that adipose tissue dysfunction is the major underlying reason for metabolic diseases in obesity^[1-3].

White adipose tissue is a highly dynamic organ which rapidly responds to nutrient excess and shortage. In obesity adipose tissue expands by hypertrophy and hyperplasia^[2,4]. In epididymal fat of rodents fed a high fat diet adipogenesis is detected after four weeks feeding while subcutaneous fat expands by hypertrophy for up to twelve weeks^[5]. Distinct adipose tissue depots also differ in gene expression, adipokine release and function^[4,6]. Accumulation of visceral adipose tissue is an independent risk factor for metabolic diseases while gain of subcutaneous fat may even be protective^[2,4]. The mechanisms regulating fat pad weight and distribution of body fat are, however, not well understood.

Macrophages are localized in adipose tissues and their number is strongly increased in obesity^[2,4]. Macrophages are classified as M1 and M2 types which is a very simplified approach in view of the high diversity of these cells^[7]. M1 cells express proinflammatory factors and M2 cells anti-inflammatory proteins. Adipose tissue resident macrophages in the lean state

are polarized to the M2 type and in the obese state to the M1 type (Figure 1). Various studies demonstrate a close association between adipose tissue resident macrophages and insulin resistance^[8].

Adipocyte inflammatory pathways are, however, essential for adipose tissue growth. Fat tissue expression of: (1) a dominant-negative tumor necrosis factor (TNF); (2) RID α/β , an adenoviral protein complex that inhibits proinflammatory signaling pathways like toll-like receptor 4 (TLR4)-, TNF- and IL-1 beta-mediated signaling; and (3) a mutated human I κ B α which inhibits NF κ B pathway, impairs adipogenesis and intestinal barrier function and favors ectopic lipid storage, systemic inflammation and insulin resistance. Therefore, adipocyte inflammation may be an adaption to an increased fat storage demand^[9].

Not all of the obese suffer from metabolic diseases. Obese people protected from metabolic complications display reduced adipocyte stress, lower inflammation and less accumulation of central fat than obese insulin-resistant individuals. Serum adiponectin is similar to levels in normal-weight controls. Adipose tissue growth of these individuals does not provoke adipocyte dysfunction, inflammation and fibrosis^[10]. Evaluation of the mechanisms underlying healthy and unhealthy obesity will help to identify the pathways associated with metabolic disturbances.

Research during the last 20 years revealed that rapid adipose tissue expansion is linked to adipocyte dysfunction^[2,3,11]. Adipose tissue inflammation, adipocyte death, low adiponectin, systemic inflammation, increased lipolysis and more recently adipose tissue fibrosis have been identified in obesity and are clearly associated with metabolic disturbances^[2,3,11].

ADIPOSE TISSUE EXTRACELLULAR MATRIX

Extracellular matrix proteins in adipose tissues regulate mechanical properties, adipogenesis and lipid droplet growth^[12,13]. Disruption of collagens impairs triglyceride storage during adipocyte differentiation and collagen 5 (COL5) and COL6 are essential for proper adipogenesis^[13]. High flexibility of the extracellular matrix guarantees healthy adipose tissue expansion. Inappropriately increased and rigid extracellular matrix hinders adipose tissue growth and promotes local and systemic pathologies associated with obesity^[3,14].

Fibrosis has been extensively studied in the liver. Liver injury activates "quiescent" hepatic stellate cells and these cells start to proliferate, synthesize connective tissue growth factor (CTGF) and extracellular matrix proteins. Transforming growth factor beta (TGF- β) is the main profibrotic factor in liver fibrosis and upregulates CTGF. CTGF stimulates binding of TGF- β to its receptor and thereby enhances TGF- β activity. CTGF is induced by TGF- β indicating an autocrine or paracrine loop that mutually enhances synthesis of both proteins^[1]. TGF- β also upregulates

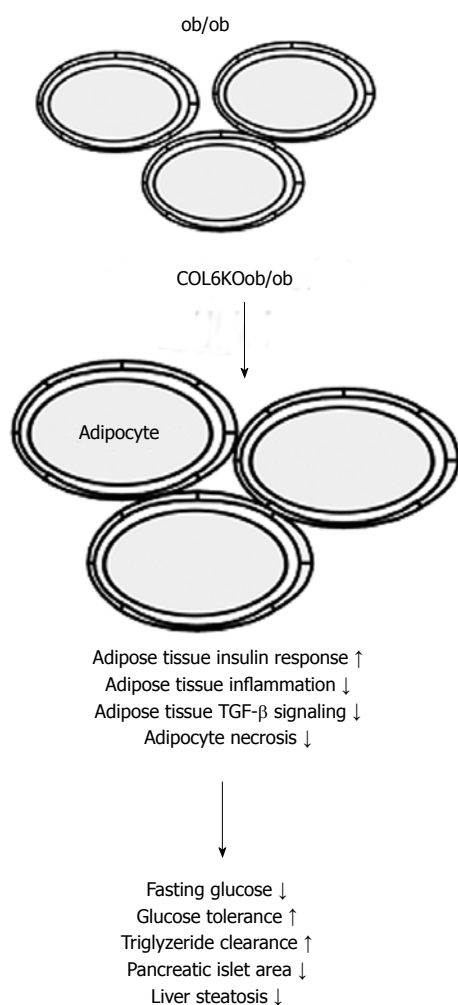


Figure 2 Mice with leptin deficiency (*ob/ob* mice) have large adipocytes and their size further increases when collagen 6 (*COL6KOob/ob*) is knocked-out in these animals. Weakening of the extracellular matrix is associated with improved adipose tissue insulin response, reduced inflammation and TGF- β signaling, and diminished adipocyte necrosis. Subsequently metabolic situation is improved.

CTGF in adipocytes which has been shown to inhibit adipogenesis^[15]. TGF- β correlates with adiposity in humans and rodents. Blockage of TGF- β signaling protects from obesity, insulin resistance and fatty liver. Beneficial effects are partly explained by browning of white adipose tissue^[16]. In fat tissue, preadipocytes, adipocytes and macrophages produce collagens demonstrating differences in adipose tissue and liver fibrosis where alpha-smooth muscle actin and collagens are mainly synthesized by activated hepatic stellate cells^[1,2,17,18].

HYPOXIA IN ADIPOSE TISSUE FIBROSIS

Adipose tissue grows by hyperplasia and hypertrophy which leads to a hypoxic state. Oxygen levels are markedly reduced in white fat of obese rodents and are also lower in white adipose tissues of humans. In obese adipose tissue capillary density is reduced and more large vessels are detected^[19].

Hypoxia-inducible factor 1 (HIF-1) is activated

when oxygen is low. Hypoxia is supposed to induce tissue fibrosis, and collagen, type I, alpha 1 (COL1A1), COL3A1 and the enzyme lysyl oxidase with a central role in collagen cross-linking are increased when mice are exposed to low oxygen. The HIF-1 α inhibitor PX-478 and expression of dominant negative HIF-1 α block high fat diet induced HIF-1 α activation, lower body weight gain and antagonize the development of metabolic diseases. Adipose tissue fibrosis and inflammation are improved^[20]. Therefore, hypoxia mediated activation of HIF-1 seems to be critically involved in limiting healthy adipose tissue growth (Figure 1).

Hypoxia and HIF-1 activation is also believed to significantly contribute to fibrogenic progression of chronic liver diseases and HIF-mediated processes independent of hypoxia have also been described to be involved herein^[21].

Hypoxia stimulates cytokine and chemokine release from adipose tissue resident macrophages and inhibits preadipocyte differentiation by lowering peroxisome proliferator-activated receptor gamma (PPAR γ) in these cells. Therefore, hypoxia is suggested to link adipose tissue growth, inflammation and adipose tissue fibrosis. Improving adipose tissue angiogenesis lowers hypoxia, HIF-1 α , TGF- β pathway and fibrogenesis^[22].

COL6 IN ADIPOSE TISSUE FIBROSIS

Inflammation and adipocyte death are reduced by the absence of COL6 which is a highly abundant extracellular matrix component of rodent fat tissues. The increased flexibility of the extracellular matrix enables healthy adipocyte growth, lowers local and systemic inflammation and improves metabolic disease (Figure 2). PPAR γ agonist and adiponectin reduce adipose tissue collagens in mice demonstrating that improvement of adipose tissue extracellular matrix composition is one of their beneficial features^[23].

The carboxy-terminal domain cleaved from COL-6A3 promotes adipose tissue fibrosis, angiogenesis and inflammation. Increased production of this so called endotrophin in obesity contributes to metabolic disturbances. Blockage of endotrophin by a neutralizing antibody protects from adverse metabolic effects of high fat feeding^[24]. Higher BMI is a risk factor for cancer and cancer-related deaths. Endotrophin enhances tumor growth and metastasis and thus may be one of the factors connecting obesity and malignant diseases^[25,26].

IMMUNE CELLS IN ADIPOSE TISSUE FIBROSIS

Increased cell death of adipocytes in obesity may be partly caused by the rigid extracellular matrix. Crown-like structures representing macrophages surrounding dead adipocytes are characteristic for obese adipose tissue and correlate with the extent of interstitial

fibrosis. Macrophage-inducible C-type lectin is expressed in these macrophages and enhances the formation of crown-like structures, activates myofibroblasts and expression of profibrotic genes^[27]. Macrophage depletion and blockage of TLR4 signaling improve while infusion of lipopolysaccharide aggravates adipose tissue fibrosis further confirming a central role of immune cells in fat tissue dysfunction^[28].

Mast cells are well known mediators of allergic reactions and it is known for a long time that their number is increased in obese adipose tissue^[29]. Mast cells release inflammatory mediators and promote immune cell recruitment. Obese animals lose body weight after mast cell inactivation^[30]. Progression of obesity is associated with mast cell maturation which induces COL5 shown to inhibit adipogenesis^[31] (Figure 1). COL5 has also been found to be important for adipocyte maturation demonstrating that different experimental designs reveal discordant results^[13]. Mast cells in human fat are activated and are mainly localized in fibrotic regions. Mast cell number is positively associated with fibrosis and macrophage accumulation^[32].

LYSOPHOSPHATIDIC ACID AND FIBROSIS

Autotaxin is a secreted lysophospholipase D and hydrolyzes lysophosphatidylcholine to produce lysophosphatidic acid. Autotaxin is increased in obesity and liver fibrosis^[33]. Lysophosphatidic acid inhibits adipogenesis and mice with an adipocyte-specific knockout of the lysophosphatidic acid receptor 1 (LPAR1) or treated with the receptor antagonist Ki16425 gain more weight and accumulate more adipose tissue. Despite being more obese animals show improved glucose tolerance^[34]. Treatment of db/db mice with the LPAR antagonist Ki16425 reduces COL1 and COL4 mRNAs and collagen protein in inguinal and perigonadal adipose tissues. Human adipose tissue explants release autotaxin spontaneously and its levels increase over time. Lysophosphatidic acid in supernatants increases in parallel along with elevated expression of COL1 and COL3, TGF- β and α smooth muscle actin and higher level of collagen protein. *In vitro* fibrosis is blocked by the LPAR antagonist and interestingly by the HIF-1 α inhibitor YC-1 while it is further increased by oleoyl-lysophosphatidic acid^[35] (Figure 1). Upregulation of HIF-1 α by lysophosphatidic acid has been shown in colon cancer cells^[36]. Current data suggest that HIF-1 is involved in fibrotic processes even in the absence of hypoxia.

COLLAGEN EXPRESSION IN HUMAN OBESITY

In humans COL6A3 is mainly expressed by stromal vascular cells and is higher in subcutaneous than omental fat depot. In both adipose tissues its expression

is reduced in obesity and increases upon weight loss in subcutaneous fat. Leptin dose dependently decreases COL6A3 demonstrating a role of this adipokine in adipose tissue extracellular matrix organization^[37]. Animal studies have proven that leptin directly promotes liver fibrogenesis. Leptin induces COL1, TGF- β and CTGF in hepatic stellate cells and this effect is mediated via enhancing TGF- β release from Kupffer cells^[38]. Whether leptin exerts opposing effects in the liver and adipose tissue or whether its activity may be affected by adipose tissue macrophages needs further studies.

In humans with a BMI between 35 and 55 expression of COL3A1, COL5A2 and COL6A3 is lower in omental and subcutaneous adipose tissue of those suffering from the metabolic syndrome compared to the healthy obese^[39].

In contrast, positive correlations of COL6A3 expression in abdominal subcutaneous fat with body mass index (BMI) and fat mass have been described in a further study while an association with type 2 diabetes has not been identified. Elevated COL6A3 mRNA levels are found in patients with greater visceral fat mass and higher inflammation. Eight weeks overfeeding increases and pioglitazone reduces COL6A3 expression. Further, in abdominal subcutaneous adipose tissue COL5 is higher expressed in the obese than the lean^[40].

In subcutaneous abdominal adipose tissue of patients with a wide range of BMI (19-40 kg/m²) there is a strong positive correlation of COL6 and CD68 mRNA expression. COL6 and CD68 expression are associated with BMI and inversely with insulin sensitivity. Fibrotic areas are increased in the obese fat tissue and are associated with macrophage number and negatively correlate with insulin sensitivity. Alternatively activated macrophages localize to fibrotic regions and express TGF- β ^[18]. COL5 is increased and elastin is reduced in obesity^[19]. Collagens quantified by picrosirius red staining are found increased in subcutaneous and visceral adipose tissues of obese vs lean patients^[32].

Picrosirius red has also been used to quantify subcutaneous and omental fat fibrosis in a further investigation. Positive associations with liver fibrosis and systemic IL-6 but not lipid and glucose parameters of the patients have been identified. The subcutaneous white adipose tissue stiffness measured by shear-wave velocity using a prototype vibration-controlled transient elastography method positively correlates with fasting glycemia and insulin, HbA1C and fat-free mass, and negatively with body fat and HDL cholesterol. Diabetes status is also significantly associated with increased shear-wave velocity. These data suggest that subcutaneous white adipose tissue stiffness is not solely defined by collagen content. Cross-linking of collagens and other extracellular matrix proteins such as elastin, laminin, and fibronectin most likely contribute to tissue rigidity^[41].

In summary most data in humans find increased adipose tissue fibrosis in obesity. There is, however, no simple explanation for the discrepant results on

the expression of single collagen species which are found reduced and induced in human obesity. Whether this is somehow related to differential composition of the extracellular matrix of the patients analyzed or to regional variations in adipose tissues needs further analysis. Techniques to directly measure adipose tissue stiffness may be more appropriate than determining mRNA expression of individual genes for the analysis of adipose tissue fibrosis.

ADIPOSE TISSUE FIBROSIS AND WEIGHT LOSS

Mild to modest liver fibrosis is reversible^[42] and previous studies have shown that adipose tissue collagen levels are reduced by adiponectin and treatment with PPARgamma agonists in mice^[23]. In patients, transcriptional and histological analysis of subcutaneous adipose tissue revealed persistence of fibrosis two years after bariatric surgery. As expected, adipocyte hypertrophy and inflammatory infiltration are improved^[43]. Adipose tissue fibrosis is even found negatively associated with surgery-induced weight loss^[44]. These findings have been confirmed in a second cohort and the association between collagen expression in white adipose tissue and gastric bypass induced weight loss persists even when age, diabetes and IL-6 have been considered^[41]. It has also been shown that diet and surgery-induced weight loss increase COL6A3 expression in subcutaneous adipose tissue in accordance with low COL6A3 expression in the fat tissues of obese patients described in this study^[37].

CONCLUSION

Adipose tissue fibrosis limits healthy growth of adipose tissue and is associated with metabolic complications in obesity. Hypoxia and subsequent activation of HIF-1 initiate profibrotic mechanisms in fat tissues. Fibrosis in obese fat tissues is not resolved upon weight loss and is even negatively associated with surgical-induced body weight reduction. More detailed analysis of the composition of extracellular matrix, biologic function of the individual constituents and non-invasive techniques to determine adipose tissue fibrosis will give further insights into the complex association of extracellular matrix proteins and metabolic health.

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Toxic stress, inflammation and symptomatology of chronic complications in diabetes

Charles A Downs, Melissa Spezia Faulkner

Charles A Downs, Melissa Spezia Faulkner, Nell Hodgson Woodruff School of Nursing, Emory University, Atlanta, GA 30322, United States

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Correspondence to: Melissa Spezia Faulkner, PhD, RN, FAAN, Nell Hodgson Woodruff School of Nursing, 1520 Clifton Road, Suite 244, Atlanta, GA 30322, United States. melissa.faulkner@emory.edu

Telephone: +1-404-7129693

Fax: +1-404-7274645

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Abstract

Diabetes affects at least 382 million people worldwide and the incidence is expected to reach 592 million by 2035. The incidence of diabetes in youth is skyrocketing as evidenced by a 21% increase in type 1 diabetes and a 30.5% increase in type 2 diabetes in the United States between 2001 and 2009. The effects of toxic stress, the culmination of biological and environmental interactions, on the development of diabetes complications is gaining attention. Stress impacts the hypothalamus-pituitary-adrenal axis and contributes to inflammation, a key

biological contributor to the pathogenesis of diabetes and its associated complications. This review provides an overview of common diabetic complications such as neuropathy, cognitive decline, depression, nephropathy and cardiovascular disease. The review also provides a discussion of the role of inflammation and stress in the development and progression of chronic complications of diabetes, associated symptomatology and importance of early identification of symptoms of depression, fatigue, exercise intolerance and pain.

Key words: Toxic stress; Type 1 diabetes; Inflammation; Type 2 diabetes; Chronic complications; Symptomatology

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Core tip: The incidence of diabetes and associated complications are increasing. Toxic stress and inflammation may be contributors to the development and progression of diabetes complications. Current evidence supports early identification of symptoms of toxic stress for preventative strategies of associated risks for diabetes complications as well as assessment of the exacerbation of symptoms related to neuropathy, cardiovascular disease and nephropathy.

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INTRODUCTION

The notion that exposure to chronic stressors predisposes individuals to developing diabetes or succumbing to worsening diabetes complications has gained

attention in recent years^[1-4]. The global epidemic of both type 1 and type 2 diabetes^[5-7] is occurring in an era of worldwide threats to personal, organizational and societal security due to psychosocial and economic burdens. According to the International Diabetes Federation, diabetes affects at least 382 million people worldwide, and that number is expected to reach 592 million by the year 2035^[8]. Although it is well-known that type 2 diabetes comprises the largest proportion of affected individuals, the number of individuals with type 1 diabetes around the world is increasing as well. Worldwide estimates for type 1 diabetes are unknown, but are estimated to be up to 3 million in the United States^[9]. A recent report on the prevalence of type 1 diabetes in youth in the United States indicated a 21 percent increase between 2001 and 2009. At the same time, rates of type 2 diabetes in youth rose 30.5%^[10].

In the midst of this public health crisis, there is tremendous need to embrace the impact of "toxic stress" from biological and environmental interactions on the development of chronic complications in persons living with diabetes. Toxic stress can result from strong, frequent, or prolonged activation of the body's stress response systems, particularly in the absence of protective mechanisms through daily coping strategies and healthy interpersonal relationships^[11]. The impact of toxic stress is apparent in current society and is garnering a paradigm shift regarding a more comprehensive understanding of health and disease across the lifespan^[11,12]. Toxic stress can be viewed as the catalyst of a physiological memory that confers lifelong risk for disease, especially due to inflammatory processes, well beyond its time of origin^[13]. How individuals, institutions, and governments respond to these stressors can have an enormous effect on the collective health of a nation. Health care clinicians serve on the front line of care delivery for identifying the most vulnerable individuals for the ravages of diabetes complications through an understanding of underlying etiologies associated with toxic stress and recognition of resultant symptomatology.

With the growing numbers of individuals diagnosed with diabetes, particularly in younger cohorts, the disease burden is ever apparent, as is the importance of minimizing the role of toxic stress on associated diabetes complications. According to Shonkoff^[14], the future consequences of significant adversity and chronic stress in early childhood extend beyond socioemotional and cognitive development. They also have significant implications for the pathogenesis of adult disease^[15], including biological manifestations of alterations in immune function^[16] and measurable increases in inflammatory markers^[17,18] that are known to be associated with poor health outcomes such as cardiovascular disease^[19-21], liver cancer^[22], asthma^[23], chronic obstructive pulmonary disease^[24], autoimmune diseases^[25], poor dental health^[26], and depression^[27-29]. Although there is no absolute evidence that chronic stress has a direct effect on the development of

diabetes in adults or children, stress can influence the onset of type 2 diabetes secondary to obesity and metabolic syndrome^[2].

With regard to the effects of stress on the neuroendocrine system, the hypothalamus-pituitary-adrenal (HPA) axis exerts considerable importance^[30]. Upon experiencing a stressor, the hypothalamus secretes corticotropin-releasing factor, which causes the release of adrenocorticotropin (*i.e.*, ACTH). This in turn stimulates the adrenal cortex, which leads to the secretion of glucocorticoid hormones, in particular cortisol. Under normal circumstances, cortisol is secreted according to a circadian rhythm, with cortisol levels highest in the morning and lowest in the evening. However, exposures to stress stimulate the HPA axis to release additional amounts of cortisol to maintain homeostasis and reduce the effects of stress. Cortisol influences a wide range of processes, including the breakdown of carbohydrates, lipids, and proteins to provide the body with energy. Cortisol has an immunosuppressive effect and therefore plays a role in the regulation of immune and inflammatory processes.

The relationship between inflammation and the HPA axis is a complex one since pro-inflammatory cytokines also stimulate the HPA axis and contribute to stress-induced elevation in cortisol^[31]. Cortisol in turn, normally plays a fundamental role in limiting the further production of pro-inflammatory cytokines *via* the important cytokine-glucocorticoid feedback cycle. This occurs through cortisol binding to glucocorticoid receptors in the white blood cells (WBCs), which once activated, leads the activated receptor [*e.g.*, Nuclear factor- κ B (NF- κ B)], to block intracellular cytokine signaling pathways, ultimately stopping the further production of pro-inflammatory cytokines^[32] and promotion of anti-inflammatory cytokines^[33]. NF- κ B consists of a family of transcription factors that play critical roles in inflammatory processes, immune regulation, cell proliferation, differentiation, and survival^[34].

With toxic stress, chronic exposure of the WBCs to high cortisol leads to down regulation of the glucocorticoid receptors, resulting in their resistance to cortisol. This stops the cytokine-glucocorticoid feedback cycle, leading to dysregulated cytokine production and chronically elevated cortisol; two states known to worsen disease outcomes. Thus, toxic stress has been associated with inflammation due to glucocorticoid receptor resistance, a mechanism of dysfunctional inflammation regulation that allows proinflammatory mediators to be uncontrolled, adding to stress-related morbidity^[35].

ROLE OF INFLAMMATION IN THE PHYSIOLOGY OF DIABETIC COMPLICATIONS

Chronic inflammation contributes to diabetes and its

complications. Features of chronic inflammation include an up-regulation of proinflammatory cytokines, such as TNF- α , interleukin (IL)-1, IL-6, IL-8, monocyte chemo attractant protein-1, and C-reactive protein that are produced by activated immune cells, resident macrophages and adipocytes^[36]. Production of these proinflammatory cytokines functions to amplify the immune response. It is recognized that a chronic, low-grade inflammatory response that occurs with an activated immune system is involved in the pathogenesis of obesity-related insulin resistance and type 2 diabetes^[37].

Markers of systemic inflammation correlate with risk for the development of diabetes related macrovascular complications^[38]. For example, in obesity-related type 2 diabetes, adipose tissue, liver, muscle and pancreatic tissues are sites of inflammation. There is an infiltration of macrophages and other immune cells coupled with a shift in cell population from anti-inflammatory to a pro-inflammatory profile. The shift in the inflammatory profile promotes insulin dysfunction leading to hyperglycemia^[39].

One complication of hyperglycemia is the formation and accumulation of advanced glycation endproducts (AGEs), ubiquitous irreversible end products of protein glycation which are formed from Amadori protein products^[40]. AGEs crosslink proteins to form stable complexes that are resistant to enzymatic degradation. In addition to hyperglycemia, oxidative stress appears to increase AGE formation. AGEs ligate with their receptor, RAGE, to amplify and perpetuate the inflammatory response through nuclear factor $\kappa\beta$ (NF- $\kappa\beta$), cAMP regulated element binding protein (CREB), and activator protein-1 (AP-1) signaling pathways. RAGE is a promiscuous receptor and has multiple ligands including lipopolysaccharide, S100/calcium binding proteins, High Mobility Group Box Protein 1 (HMGB1) and Amyloid- β peptide (A β), as well as many others^[40,41]. Data from multiple studies demonstrate that AGEs and their receptor, RAGE, are important contributors to the development of diabetes related complications^[40,42].

Oxidative stress, an alteration in redox regulation and control, occurs in response to excessive reactive species production that overwhelms antioxidant defenses^[43]. Reactive species may modify glucose, free fatty acids, oxysterols or lipids through oxidation-reduction reactions. For example, oxidize glucose is involved in the formation of AGEs. AGEs ligate with their receptor RAGE to promote an inflammatory response; modification of lipids has been shown to affect mitochondrial metabolic pathways leading to mitochondrial damage^[44,45]. Inflammation and mitochondrial damage result in oxidative stress thereby producing an autocrine feedback pathway to perpetuate inflammation and oxidative stress^[46]. This pathway has been described in the macrovasculature as well as in peripheral neurons and is recognized as a contributor to the complications of diabetes^[47,48].

Vascular dysfunction characterized by an activated

endothelium that is primed to facilitate immune cell migration into tissue also occurs in diabetes. Indeed vascular dysfunction is a key contributor of neuropathy, impaired cognition, nephropathy and cardiovascular diseases (e.g., atherosclerosis, cardiomyopathy, etc.) that underlie complications of diabetes.

DIABETIC NEUROPATHY

Peripheral neuropathy (PN) affects up to 50% of people with diabetes and the diffuse peripheral neuropathies (distal sensori-motor polyneuropathy and autonomic neuropathy) are major risk factors for foot ulceration and amputation^[49]. The etiology of PN is complex; however, studies show that altered blood flow, hyperglycemia and alterations in metabolites (oxidative/nitrative stress, advanced glycation end products and a pro-inflammatory response) are involved.

In animal models of diabetes, evidence of reduced blood flow to the nerve is seen within the first few days of the induction of diabetes with a chemical agent such as streptozosin (STZ). These changes often precede changes in nerve conduction velocity^[50-52]. However, the loss of blood flow results in neuronal hypoxia sufficient to compromise nerve function and initiate neurodegeneration^[53]. This effect has also been described in autonomic ganglia, dorsal root ganglia and in the hippocampus^[54-56].

Hypoxia also induces the expression of numerous pro-angiogenic and pro-inflammatory genes in macrophages^[57]. Alterations in the microvasculature effect associated peripheral nerves^[58]. Indeed capillary occlusion induces ischemia to the nerve producing ischemic nerve fiber damage and perineural capillary luminal occlusion (due to endothelial cell hypertrophy and hyperplasia)^[59]. In rats, hypoxic conditions reduced nerve velocity conduction, and within the context of hyperglycemic hypoxia, blockade of potassium channels leads to intra-axonal acidification by anaerobic glycolysis. This suggests that hypoxia induced neuronal changes may play a role in the development of neuropathy^[60,61]. However, reversal of hypoxia in the ischemic limbs of individuals with diabetes does not improve nerve function^[62].

Hyperglycemia appears to contribute to the pathogenesis of diabetic neuropathy. Within the first month of inducing diabetes in rats, hyperglycemia resulted in slowing of sensory^[63-65] and motor^[66,67] nerve conduction velocity coupled with hyperalgesia^[68,69] and allodynia^[70]. Over time prolonged hyperglycemia produces axonopathy, demyelination and nerve degeneration in diabetic animals^[71,72].

Metabolic alterations are thought to play a central role in the development of neuropathy in diabetes. Elevation in polyol pathway activity, oxidative stress, the formation of advanced glycation end products and a persistent pro-inflammatory response through activation of the NF- $\kappa\beta$ and p38 mitogen activated protein kinase signaling have been consistently shown

to contribute to diabetic neuropathy^[73-75].

There is considerable evidence that pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 are involved in the pathogenesis of diabetic neuropathy. TNF- α is a potent proinflammatory cytokine that appears to play a role in the pathogenesis of diabetic neuropathy and have a central role in central and peripheral sensitization of neuropathic pain^[76]. Pharmacologically inhibiting TNF- α in mice ameliorates the electrophysiological and biochemical effects of the cytokine^[77].

IL-1 β is an important cytokine that induces the production of a wide variety of cytokines through NF- κ B activation. Studies show an increase in the mRNA expression of TNF- α and IL-1 β in the spinal cords of STZ-diabetic rats^[78]. Activated astrocytes in the spine increase IL-1 β expression, which may induce *N*-methyl-*D*-aspartic acid receptor phosphorylation in spinal dorsal horn neurons to enhance pain transmission^[79]. Hyperglycemia induces the production of IL-1 β through the NOD-leucine-rich repeats-and pyrin domain containing inflammasome^[80]. In the spinal dorsal horns of db/db mice, increased IL-1 β , TNF- α and IL-6 levels are inhibited by anti-high-mobility group box protein-1, a known RAGE ligand^[81].

IL-6 is a member of the neurotrophic cytokine family that participates in neuronal development and has neurotrophic activity. IL-6 is a sensitive marker of diabetic neuropathy and predicts progression and severity of type 1 diabetes^[82]. Increased levels of IL-6, IL-1 and TNF- α correlated with the progression of nerve degeneration in diabetic neuropathy^[83]. It is believed that these proinflammatory cytokines affect glial cells and neurons to set the pathological process of diabetic neuropathy in motion. However, the role of these cytokines in diabetic peripheral neuropathic pain is unclear^[84]. It is clear that inflammation is a complex scenario. To that end other signaling molecules such as interferon- γ , IL-10, C-reactive protein, adhesion molecules, chemokines and adipokines may also play a role in the inflammatory process associated with diabetic neuropathy and neuropathic pain.

NEUROPATHIC PAIN

Pain is the body's perception of actual or potential damage to the nerve or tissue by noxious stimuli. Large A-delta myelinated fibers and small C unmyelinated fibers are sensory afferent nerves that are mainly responsible for carrying nociceptive sensation from the skin, joints, and viscera. Tissue damage results in the release of inflammatory mediators such as prostaglandins, bradykinins, and histamines at the site of injury, which triggers the depolarization of nociceptors, thereby generating an action potential. The action potential transmits the nociceptive sensation, *via* the dorsal root ganglion (DRG) to the dorsal horn of the spinal cord. The release of glutamate and substance *P* results in the relay of nociceptive sensations to the

spinothalamic tract, thalamus, and subsequently, the cortex where pain is interpreted and perceived.

Nociceptive pain is the normal response to noxious stimuli and nociceptive pain usually subsides upon removal of the stimulus (e.g., healing of injured tissue). Neuropathic pain occurs in the absence of noxious stimuli and represents a pathological change affecting the somatosensory system. Neuropathic pain is characterized by the activation of abnormal pathways of pain at the peripheral nerve and posterior nerve roots. Neuropathic pain is a critical feature in diabetic neuropathy.

The development of painful diabetic neuropathy is complex and not completely understood. However, evidence suggests that glycemic shifts, inflammation and oxidative stress are important contributors. Hyperglycemia affects glial cells leading to demyelination and impaired neurotrophism that culminates in impaired regeneration and decreases nerve conduction velocity; ultimately this results in pain. Hyperglycemia also activated the microvascular endothelium causing endothelial hypertrophy affecting downstream endoneurial circulation to promote hypoxia and ischemia of the nerve. Hyperglycemia and hypoxia affects neurons by promoting axonopathy and neuronal degeneration. Hyperglycemia may also contribute to painful diabetic neuropathy through the polyol pathway^[85], advanced glycation end-products^[86], hexosamin flux^[87], mitogen-activate protein kinases^[73], altered activity of the Na⁺/K⁺-ATPase^[88], poly-ADP ribose polymerase (PARP) over activation^[89], and cyclooxygenase-2 activation^[90]. Nerve cells are prone to hyperglycemic injury as the neuronal glucose uptake is based on glucose concentration.

The expression of voltage-gated sodium and calcium channels and voltage-independent potassium channels in the DRG has a significant role in the generation of nociceptive sensation and peripheral sensitization. Indeed voltage gated sodium channels are active following nerve injury and demonstrate continued generation of ectopic impulses; similar findings have been observed from some voltage-gated calcium channels suggesting that voltage-gated calcium channels play a role in neuropathic pain. Calcium entry through voltage-gated calcium channels causes the release of substance *P* and glutamate, which results in the modulation of pain at the dorsal horn. The transient receptor potential vanilloid 1 (TRPV1) channel has been found to be associated with neuropathic pain as well. Methylglyoxal, a reactive intracellular by-product of glycolysis and hyperglycemia, depolarizes the sensory neuron by activating the TRPV1 channel^[91] in the DRG and also induces posttranslational modification of the voltage-gated sodium channel Nav1.8^[92]. In addition, these changes increase electrical excitability and facilitate firing of nociceptive neurons.

Neuroplasticity is the brain's response to changes within the body or the external environment. In response to chronic neuropathic pain, neuroplasticity

is associated with somatosensory cortex remodeling, reorganization, and hyperexcitability in the absence of external stimuli. Provoked pain and spontaneous stimuli may reverse the remodeling and reorganization at the somatosensory cortex^[93]. In a study of patients with chronic neuropathic pain and nonneuropathic pain Gustin *et al*^[93] found using functional and anatomical resonance imaging cortical reorganization and changes in somatosensory activity in patients with neuropathic pain.

IMPAIRED COGNITION AND DEPRESSION

Diabetes can lead to a number of secondary complications, and the most common brain complications include cognitive decline and depression. The incidence of cognitive decline, measured by behavioral testing may be as high as 40% in people with diabetes^[94]. Subjective feelings of cognitive decline have also been reported from persons with diabetes^[95], which illustrates the impact of diabetes on the individuals perception of how well their brain functions. Indeed multiple studies have reported that diabetic patients have a 2-5 fold increased risk for Alzheimer's Disease compared to non-diabetic subjects^[96,97]. Furthermore, alterations in cognitive functioning in type 1 diabetic children (less than 5 years old) has been reported^[98,99], as well as evidence of changes in white matter structure^[100].

The mechanisms responsible for the development of high rates of cognitive decline in diabetics are not well understood, although evidence suggests that neuroplasticity may play an important role. The dentate gyrus of the hippocampus and the subventricular zone are two important areas in neurogenesis^[101], the process of proliferation of progenitor cells or their differentiation into astrocytes, oligodendrocytes or neurons and survival and incorporation of the newborn cells into target regions. Hippocampal neurogenesis is diminished by exposure to environmental stress, HPA axis hyperactivity and increased inflammation^[102,103]. Changes in neurogenesis alter a number of key functions of the hippocampus, such as learning and memory, affective expression and regulation of the HPA axis^[104,105].

Wide variations in glucose levels and oxidative stress may also play an important role in the development of cognitive decline in diabetics. In animal models, studies show that repeated bouts of hypoglycemia inhibits hippocampal neurogenesis, presumably through oxidative injury to hippocampal CA1 dendrites^[106]. Hyperglycemia also promotes oxidative stress and neurodegeneration^[107]. Prolonged hyperglycemia promotes the development of AGEs which bind to their receptor, RAGE, to promote and sustain an inflammatory response through NF- κ B, AP-1 and CREB signaling pathways. RAGE ligation also promotes increases expression through an autocrine feedback mechanism^[108]. RAGE is also responsible

for the transport of amyloid- β (A β) across the blood-brain barrier. A β contributes to the development of Alzheimer's Disease^[109,110] by participating in the formation and accumulation of amyloid plaques and fibrils that facilitate neurodegeneration and impair cognition^[107]. Also, A β and hyperglycemia have been shown to activate microglia to induce oxidative injury^[111].

The relationship between diabetes and depression is reciprocal as either is known to be a risk factor for the other^[112]. The importance of depression in diabetes is highlighted by studies consistently report a higher prevalence rate for depression among type 1 and type 2 diabetics compared to the general population^[113]. Comorbid depression and diabetes is associated with poor self-care, lack of exercise, and nonadherence to dietary or medication routines, leading to inadequate glycemic control.

The mechanisms responsible for the development of depression in diabetics is unclear, although there is likely overlap between physiological and non-physiological factors to account for the pathogenesis of their comorbidity. Non-physiological factors such as sedentary life style, lack of self-care, and diet, as well as the emotion burden of managing diabetes, contribute to the development and progression of diabetes. Insulin resistance is gaining attention as a potential link between diabetes and depression and cognitive decline^[114,115]. Neuroendocrine signaling, through hyperactivity of the HPA axis, is thought to cause or exacerbate depression in diabetics^[116]. Indeed antidepressant treatment has been shown to abrogate abnormal HPA responses while facilitating recovery from depression^[117].

Stress has been shown to decrease brain derived neurotrophic factor (BDNF) in the hippocampus. Stress also appears to decrease the expression of other types of neurotrophic and growth factors such as nerve growth factor and neurotrophin-3^[118], which could lead to the alteration in the structure and function of hippocampal neurons. Stress also decreases the expression of vascular endothelial cell growth factor, a growth factor that influences vascular permeability and the proliferation of endothelial cells, in the hippocampus^[119]. The significance is that antidepressant treatment increases expression of BDNF and other growth factors in individuals recovering from depression^[120-122].

There is also considerable evidence that inflammation plays an important role in the pathogenesis of depression and diabetes^[123]. Many studies describe an increase in peripheral cytokine of individuals with depression that is often comorbid with other chronic diseases such as coronary artery disease and chronic obstructive pulmonary disease^[124]. Interestingly, cytokines have been shown to be associated with suicidality and depression^[125]. Diabetes and inflammation have been associated with alterations of dopamine, serotonin, brain derived neurotrophic factor and insulin growth factor-1 which have been implicated

in depression^[126].

CARDIOVASCULAR DISEASE, NEPHROPATHY AND ASSOCIATED SYMPTOMS

Given the worldwide increase in the incidence of diabetes, the dual complications associated with cardiovascular disease and nephropathy heighten the importance of preventive therapies through early identification of biomarkers of inflammation and causative etiologies for stress responses regardless of age or type of diabetes^[127]. In 2010, high blood pressure was the leading risk factor for deaths due to cardiovascular diseases, chronic kidney disease, and diabetes in every region of the world, causing more than 40% of worldwide deaths from these diseases^[128]. The National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014^[129], indicated that from 2003-2006 after adjusting for population age differences, cardiovascular disease (CVD) death rates were approximately 1.7 times higher among adults (≥ 18 years) with diabetes than among adults without diabetes. Regardless of the type of diabetes, the risk of CVD is evident and likely begins at an earlier age for those diagnosed with type 1 diabetes. Endothelial dysfunction is an integral part of the pathogenesis underlying the increased cardiovascular complications seen in individuals with T1D but it is unclear how early it appears^[130].

Results from the Epidemiology of Diabetes Interventions and Complications study, a long term follow up study of the Diabetes Control and Complications Trial (DCCT), showed that adults with T1D had increased carotid intima medial thickness (CIMT) compared to a healthy non-diabetic population 6 years into the study. Individuals receiving intensive insulin treatment during the DCCT had much less progression in their CIMT compared to those who had received conventional treatment. However there was not a significant difference in their percent HbA_{1c} at that time, suggesting the effect of "metabolic memory"^[131]. These data suggest that glycemic control may have long lasting effects on cardiovascular morphology and function^[130]. Hence, there exists a caveat to minimize exposure to toxic stressors in early life and at the onset of T1D that may aggravate optimal glycemic targets.

Cardiovascular morbidity related to diabetes is associated with vascular changes due to inflammation, resulting in both macrovascular (*i.e.*, atherosclerosis)^[132] and microvascular (*i.e.*, cardiovascular autonomic neuropathy)^[133] alterations. In type 1 diabetes, several causative factors are implicated in these inflammatory vascular changes^[134]. The oxidative modification of LDL and associated immune responses^[135] may be one of these key factors, resulting in damage to the endothelium^[136], activation of macrophages, adherence

of monocytes^[137] and impairment of nitric oxide action with resulting vascular cell cytotoxicity^[138]. Although markers of inflammation have not been extensively studied in the development of CAD in T1D, the Eurodiab study group, using a standard score based on combined levels of C-reactive protein, IL-6, and TNF- α , reported a significant difference between those with and without CAD ($P < 0.001$) after adjusting for age, gender, HbA_{1c}, diabetes duration, and systolic blood pressure^[139]. Research has also indicated that in subjects with known coronary atherosclerosis, low-degree inflammatory activity (*i.e.*, C-reactive protein, fibrinogen, erythrocyte sedimentation rate and white blood cell count) is not only increased in patients with T1D and T2D diabetes, but also increased with increasing HbA_{1c} in non-diabetic individuals. This later finding indicates an early association between degree of glycaemia, inflammation and atherosclerosis prior to the development of diabetes^[140].

Cardiovascular autonomic neuropathy is a common form of autonomic neuropathy and one of the most overlooked of all serious complications of diabetes, resulting from microvascular damage to parasympathetic and sympathetic fibers and increased risks for cardiovascular arrhythmias, sudden death, and myocardial infarction in adults with diabetes^[141]. There are multiple etiologies of diabetic neuropathy, including hyperglycemic activation of the polyol pathway leading to accumulation of sorbitol causing direct neuronal damage and/or decreased nerve blood flow^[142], oxidative stress with increased free radical production leading to vascular endothelium damage and reduced nitric oxide bioavailability^[143,144], and the formation of advanced glycosylated end products with reduced blood flow, activation of inflammatory cytokines (*e.g.*, IL-6, TNF- α), nerve hypoxia and altered nerve function^[141].

Cardiovascular autonomic neuropathy has been linked to postural hypotension, exercise intolerance, enhanced intraoperative cardiovascular lability, increased incidence of asymptomatic (*i.e.*, painless) ischemia, myocardial infarction, and decreased likelihood of survival after myocardial infarction^[145]. The presence of palpitations and tachycardia at rest due to loss of parasympathetic modulation can be present early in the development of this complication prior the onset of other associated symptoms. Cardiovascular autonomic neuropathy occurs in approximately 17% of patients with T1D and 22% of those with T2D. An additional 9% of T1D and 12% of T2D have borderline dysfunction^[133]. Since the 1970s, the seminal work by Ewing *et al.*^[146] unveiled the predictive relationship between cardiovascular autonomic neuropathy and mortality in adults with T1D. The Hoorn Study also found increased mortality in adults with T2D who had decreased cardiovascular autonomic function^[147]. Within the pediatric literature, heart rate variability (a measure of cardiovascular autonomic function) was lower in adolescents with T1D compared with healthy control

subjects^[148,149] and lower in youth with T2D vs T1D^[150].

New pathways in the development of diabetic nephropathy also implicate inflammatory processes due to hyperglycemia, renin-angiotensin system and oxidative stress, involving infiltration of the kidneys with monocytes and lymphocytes that increase pro-inflammatory cytokine production, reactive oxygen species and tissue damage^[151,152]. This leukocyte activity amplifies the inflammatory response and promotes cell injury and organ tissue fibrosis. Improved future understanding of the inflammatory response in diabetic kidneys is expected to identify novel anti-inflammatory strategies for the potential treatment of diabetic nephropathy. Familial predisposition to disease, including risks for toxic stress, race and other environmental factors interact with hemodynamic changes producing advanced glycation end products, glucose reduction and sorbitol accumulation into the cell, and overproduction of reactive oxygen species^[151]. For individuals exposed to toxic stress that may further exacerbate dysglycemia, glycemic control is of upmost importance for preventing the onset and progression of nephropathy by influencing both hyperglycemia itself and hyperglycemia induced metabolic abnormalities. Evidence for this premise is supported by randomized controlled clinical trials in both type 1 and type 2 diabetes^[153,154].

CLINICAL IMPLICATIONS FOR SYMPTOM RECOGNITION

The complications of diabetes related to neuropathy, nephropathy and cardiovascular disease are the major contributors to morbidity and mortality in this population. Given the projected increase in the worldwide numbers of individuals to develop diabetes in the coming years, the potential additional burden of toxic stress on the development of disease related complications is of tremendous concern. Key symptoms that warrant clinician recognition during routine assessment in persons with diabetes include signs of cognitive decline, depression, fatigue (including disturbed sleep patterns), exercise intolerance and pain associated with peripheral neuropathy. Although the emphasis in diabetes management is achievement of glycemic targets, weight, lipid and blood pressure control, the environmental and physiological effects of daily stress may be "ticking away" at the emergence of subtle inflammatory changes leading to devastating complications. Therefore, diabetes care management should emphasize symptom palliation as well as cardiometabolic control^[155].

Chronic low-grade inflammation in metabolic disorders such as diabetes contributes to behavioral symptoms, including depression, cognitive impairment, fatigue, sleep disturbance and pain^[156]. The quality and quantity of sleep may play a key role in the inflammatory processes associated with diabetes and

related cardiovascular disease^[157]. Additionally, several biomarkers of inflammation, specifically IL-6 and CRP, have been found to be associated with fatigue, poor concentration and sleep quality in a healthy adult cohort^[158], which has implication for the stress-induced inflammatory effect on individuals prior to the development of diabetes. There is increasing evidence that hypercytokinemia and activated innate immunity affect the pathogenesis of T2D and related symptoms of fatigue, sleep disturbance and depression^[159].

CONCLUSION

Toxic stress exposes individuals at all ages to chronic, low-grade inflammation that is a risk for the development of diabetes and may increase the physiological alterations leading to neuropathy, nephropathy and cardiovascular disease that are so prevalent in diabetes. Evidence supports the importance of minimizing toxic stress to promote glycemic control and lessening immune and inflammatory responses in an attempt to prevent the emergence or worsening of diabetes complications. At a time when the evaluation of immune and inflammatory biomarkers is not standard clinical practice, routine examination strategies are essential for the assessment of stressful life experiences and the effects of these experiences that contribute to the symptoms related to neuropathy, nephropathy and cardiovascular disease and overall quality of life.

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Adapting chronic care models for diabetes care delivery in low-and-middle-income countries: A review

Grace Marie V Ku, Guy Kegels

Grace Marie V Ku, Guy Kegels, Department of Public Health, Institute of Tropical Medicine, B-2000 Antwerp, Belgium

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Correspondence to: Grace Marie V Ku, MD, MPH, PhD, Department of Public Health, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. gracemariakumd@yahoo.com
 Telephone: +63-91-53615683

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grassroots level, involving the leaders and members of the community, the patients, the local health system and the healthcare providers. A second analysis making use of certain organizational theories was done to explore on improving feasibility and acceptability of organizing care for chronic conditions. The analyses indicated that care for chronic conditions may be introduced, considering the needs of people with diabetes in particular and the community in general as recipients of care, and the issues and factors that may affect the healthcare workers and the health system as providers of this care. The context-adapted chronic care model-based service delivery model was constructed accordingly. Key features are: incorporation of chronic care in the health system's services; assimilation of chronic care delivery with the other responsibilities of the healthcare workers but with redistribution of certain tasks; and ensuring that the recipients of care experience the whole spectrum of basic chronic care that includes education and promotion in the general population, risk identification, screening, counseling including self-care development, and clinical management of the chronic condition and any co-morbidities, regardless of level of control of the condition. This way, low-to-middle income countries can introduce and improve care for chronic conditions without entailing much additional demand on their limited resources.

Key words: Chronic care models; Context adaptation; Diabetes mellitus type 2; Low-to-middle income countries; Service delivery model

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Abstract

A contextual review of models for chronic care was done to develop a context-adapted chronic care model-based service delivery model for chronic conditions including diabetes. The Philippines was used as the setting of a low-to-middle-income country. A context-based narrative review of existing models for chronic care was conducted. A situational analysis was done at the

Core tip: This paper introduces strategies that low-to-middle-income countries can employ to introduce feasible care and prevention for diabetes amidst problems of the double burden of disease and scarcity of resources, and presents a context-adapted service delivery model that integrates care for diabetes and similar chronic conditions in the current health services

and assimilates the delivery of diabetes care with other responsibilities of the health system so that people under the care of health services and the health system can experience the whole spectrum of diabetes prevention and care.

Ku GMV, Kegels G. Adapting chronic care models for diabetes care delivery in low-and-middle-income countries: A review. *World J Diabetes* 2015; 6(4): 566-575 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i4/566.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i4.566>

INTRODUCTION

Chronic conditions are said to be a global crisis that threatens human development, especially in low-to-middle income countries (LMIC)^[1,2]. A large number of people from LMICs live with chronic conditions. This includes around 291 million with diabetes^[3].

The macroeconomic effects of chronic conditions including diabetes in LMICs are substantial. These disproportionately affect the poor^[4] and the care for these conditions leads to continued and, as the condition worsens and complications develop, escalating expenditures while decreasing productivity. The costs of care and the disability or death of a household income earner may cause (further) poverty^[5]. For every 10% rise in mortality from chronic conditions, the yearly economic growth of a country is estimated to be reduced by 0.5 percentage points^[6]. Abegunde *et al*^[7] computed projections of foregone national income due to heart disease, stroke and diabetes in 23 LMICs and showed that these countries combined are at risk of losing US\$ 84 billion in economic output over the ten-year period 2006-2015.

Thus, LMICs should move towards strategies to deal with chronic conditions including the provision of good quality chronic care in order to address the evolution that is threatening their people. However, the acute disease-oriented health systems of LMICs may face a number of difficulties in adjusting health care delivery to accommodate the growing burden of chronic conditions in general and diabetes in particular. This could be attributed to various reasons including resource constraints, absence of programs directed towards chronic conditions, and difficulties in introducing and/or integrating care for chronic conditions.

An adequate approach to care for chronic conditions such as diabetes is very different from the acute disease-oriented approach practiced in most LMICs: in addition to the disease prevention and drug prescription activities usually done in acute disease care, chronic care also needs to focus on disability limitation and rehabilitation^[8]; should give attention to the psychosocial aspects of the patient^[9]; and should involve and enable the patient in caring for the

condition^[10]. Other features that make chronic care different from acute disease care include: (1) case finding for assessment of risk factors, detection of early disease, and identification of high risk status; and (2) long term follow-up with regular monitoring and promotion of adherence to pharmacological and psychological interventions^[11].

Analyses have demonstrated that in spite of increased funding in LMICs, progress towards agreed Millennium Development Goals, including the health-related ones, remains slow^[11]. This could be attributed to weak health systems, human resource constraints, and over-concentration of resources to specific programs. Introducing chronic disease care *in toto* as practiced in high income countries (HIC) or separately structured and resourced vertical programs to address specific chronic disease problems to LMIC health systems may prove detrimental if not fatal. A better approach could be to strengthen the first line and progressively integrate care for chronic conditions into primary care activities, taking into consideration the capabilities of the health system.

LMICs can take the initiative to undertake the first steps towards the provision of good quality chronic care. Adapting models for chronic care to fit the context of a country and selecting specific elements for implementation is likely to stand a better chance of improving chronic care. This way, specific problems such as resource constraints may be addressed; certain characteristics of the people, the health system and the country that could be capitalized on may be identified; and particular context-adapted strategies may be employed.

For this research, the investigators reviewed existing models for chronic care considering the results of a situational analysis of a low middle-income country, the Philippines, to come up with a model for diabetes care delivery that could be adapted in low-to-middle-income countries with similar characteristics.

BACKGROUND ON THE PHILIPPINES: HEALTH SYSTEMS, CHRONIC CONDITIONS AND DM TYPE 2

Public health care in the Philippines was devolved in 1992 and the responsibility of providing basic health care services for the people was handed down to the local government units, specifically municipalities and cities, through their respective local government health units (LGHU)^[12]. A decade before this health care devolution, the country implemented a primary health care policy which led to the creation of a large cadre of community-based health care workers locally called barangay health workers (BHW)^[13]. The barangay (village) is the smallest unit of government; a city or a municipality would be composed of a number of barangays. Organizationally, the BHW fall under the governance of the barangay and are selected to work

in their respective areas of residence; functionally, they are under the LGHU. A BHW is assigned approximately 10-20 families and is responsible for dissemination of health information and health promotion activities, and conducts other health-related undertakings to any member of the families being attended to.

The Philippines is among the 23 low-and-middle-income countries where 80% of the LMIC mortality due to chronic conditions is accounted for^[7]. It is likewise predicted to be among the 10 countries worldwide with the highest numbers of people with DM type 2 by 2030^[14]. For the past decade, eight of the 10 leading causes of mortality in the Philippines are chronic conditions and DM type 2 has been consistently among these^[15]. Furthermore, the complications and consequences of DM type 2 in the Philippines are on the rise and have become alarming. For renal complications alone, it is seen that 55% of Filipino diabetics will eventually develop kidney disease; in 2007 there was an increase of more than 2800 diabetic nephropathy patients requiring dialysis^[16]. Aside from these, the International Diabetes Federation estimated undetected type 2 diabetes (UDD) in the Philippines at 58.8% in 2011^[3]. It seems that the current screening strategies in routine conditions cannot adequately identify previously undetected cases of DM type 2 in the Philippines. The high rate of UDD, the rapidly increasing prevalence of DM type 2, and the poor control of disease progression and emergence of complications only show that current case management of diabetes mellitus in the Philippines is below optimum, and the burden will only escalate if no measures are employed to address these problems. On the macroeconomic level, the Philippines lost US\$60 million in 2006 from coronary heart disease, stroke, and diabetes alone^[7].

METHODS OF REVIEW AND CONTEXTUAL ANALYSIS

Narratives on models for chronic care and their elements, records of implementation and outcomes of implementation, if any, were analyzed and adapted to the context of the Philippines, an LMIC where the health system is still acute disease-oriented, there is limited organized care for chronic conditions, if at all, and healthcare expenditures are mostly out-of-pocket. The investigators focused on the two main models for chronic care, which have been used by HIC health systems as bases for the organization of chronic care. For the situational analyses, they considered a number of organizational theories: on how an organization may respond to pressure to change and what factors could influence an organization's response; and the factors that may facilitate or hinder adoption of innovations introduced to an organization. Key factors affecting the adaptation of chronic care models and the development of a context-adapted chronic care model-derived service delivery model were explored

making use of theories presented by Oliver^[17] in her analysis of organizational responses to pressures towards conformity and Greenhalgh *et al.*^[18]'s theories on diffusion of innovation, taking into consideration the background of the country and its health system and the profiles of the healthcare workers, the people with chronic conditions, and the community. The Philippine context was used and informal interviews with representatives of the community (the government and the people), the healthcare system/service and people with diabetes were conducted.

MODELS FOR CHRONIC CARE

In HICs, models and frameworks for chronic care and its delivery have been implemented, most of which were derived from Wagner's Chronic Care Model (CCM)^[19]. The CCM was conceptualized from a primary care perspective and advocates improvements in six essential elements: self-management support, clinical information systems, delivery system redesign, decision support, health care organization, and community resources^[20]. The basic idea of the CCM is quite sound: to optimize "productive interactions" between "informed, activated patients" on one hand, and "prepared, pro-active practice teams" on the other, resulting in "functional and clinical outcomes" (Figure 1). However, the CCM appears to be grounded in a preponderantly clinic-based perspective with a background of abundant resources and a highly technological environment such as can be found in HICs. Its focus seems to be on optimizing clinical interaction for more effect in dealing with chronic conditions.

To adapt the basic principles and elements of the CCM to something actionable in developing countries, the World Health Organization^[21] introduced the Innovative Care for Chronic Conditions framework (ICCCF) (Figure 2). The same essential chronic care elements specified in Wagner's model were retained. The guiding principles of the ICCCF are evidence-based decision making, population focus, prevention focus, quality focus, integration, and adaptability. It has the following essential elements for taking action: supporting a paradigm shift, managing the political environment, building integrated health care, aligning sectoral policies for health, using health care personnel more effectively, centering care on the patient and family, supporting patients in their communities, and emphasizing prevention. The ICCCF seeks to improve health care at the macro, meso, and micro levels. However, essential components for the policy environment (macro level) are needed (leadership and advocacy, integrated policies that span different disease types and prevention strategies, consistent financing, developing human resources, legislative frameworks and partnership working), requirements that many low-to-middle-income countries, especially those encountering scarcity in human resources for health,

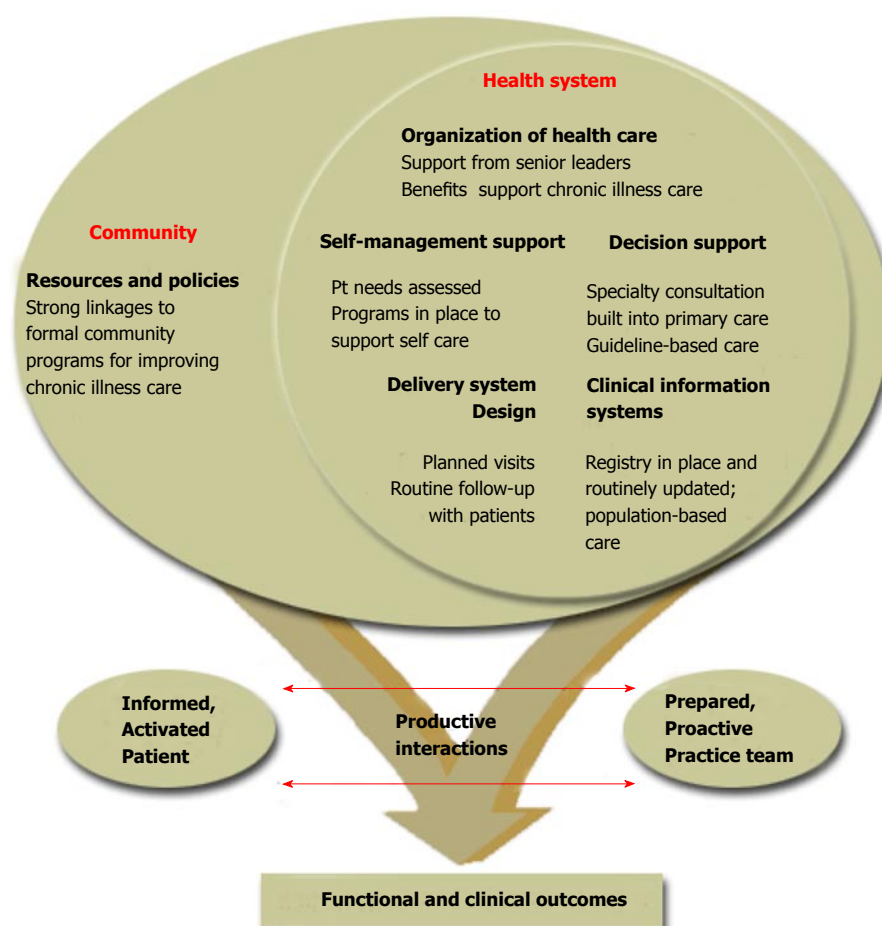


Figure 1 The chronic care model.

economic crisis, and instabilities in leadership, may be unable to fulfill.

In Canada, the Province of British Columbia formulated the Expanded Chronic Care Model (Figure 3) integrating population health promotion and prevention with the existing elements of the CCM to address the social, environmental and cultural factors that affect health^[22]. This way, the role of the community has become well delineated and added to the clinically focused initial CCM.

Some countries chose to implement selected elements of these models. In Scotland, key principles in chronic care have been established, namely: pathways of care focused on individuals with chronic conditions; partnership between health care professionals and people with chronic conditions; partnership between primary care, social care, and other agencies; integrated solutions that respond to the needs of people with chronic conditions; focus on providing care in primary care and community settings; and focus on self-care^[23].

Certain LMICs have made use of the CCM or the ICCCF to design systems of care for chronic conditions. The CCM-based Vera-Cruz Initiative for Diabetes Awareness in Mexico reports improved glycemia among its study participants 18 mo after implementation^[24] while Rwanda made use of ICCCF elements to strengthen its health system and design a system of care for

HIV/AIDS, with impressive results^[25,26]. However, such implementations of elements of the CCM/ICCCF in LMICs are exceptions rather than the rule. The health systems response in many LMICs is still characterized by a public health system focused on prevention programs; little consideration for the organization, coordination and regulation of health care services; routine medical practice without attention to the opportunities and resources for the specific aspects of chronic care; and large out-of-pocket expenses for patients^[27].

Studies conducted on the implementation of the CCM in HICs demonstrated significant correlations between specific elements of the CCM and better health outcomes^[28,29]. The number of elements of the CCM and the type and intensity of implementation may vary, depending on many contextual and organizational factors^[30].

THE ORGANIZATIONAL THEORIES USED

Oliver's typology of strategic responses to institutional processes^[17] lists five behaviors that organizations may enact in response to pressures toward conformity with the institutional environment: acquiescence and its alternative forms of habit, imitation, and compliance; compromise including pacifying tactics and bargaining; avoidance, concealment, buffering and

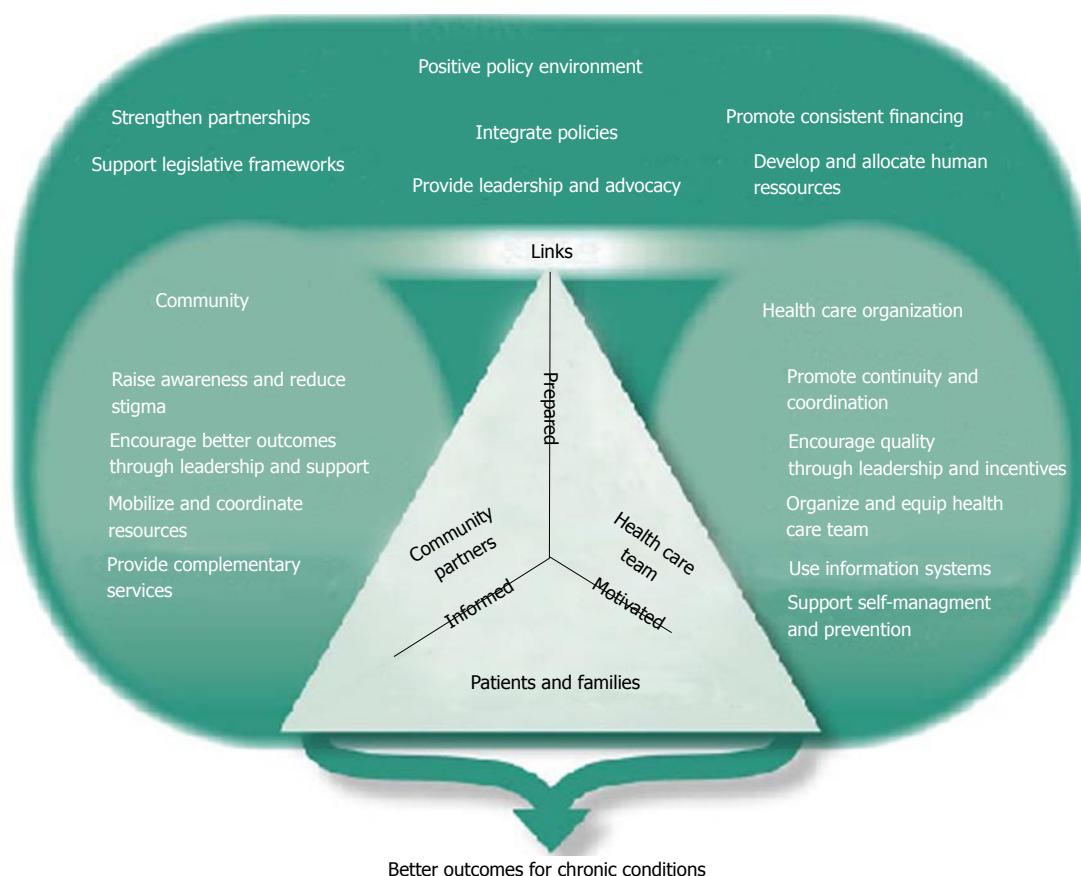


Figure 2 The innovative care for chronic conditions framework.

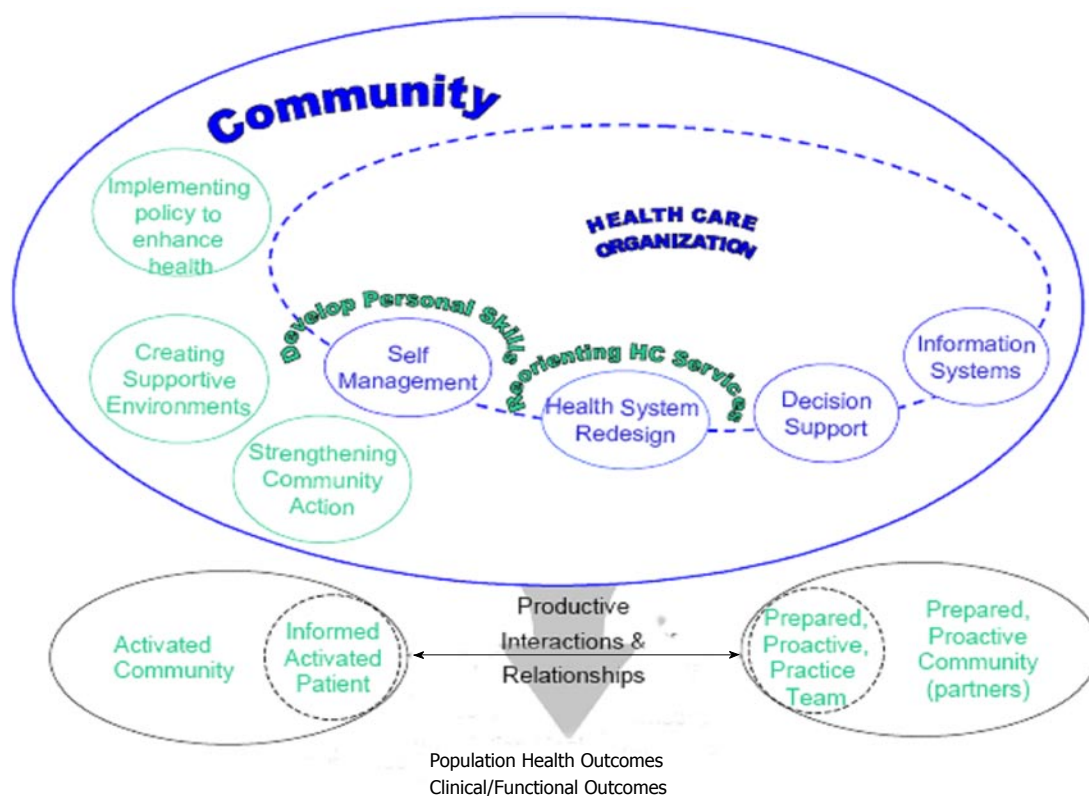


Figure 3 The expanded chronic care model.

escape; defiance, dismissal, challenge and attack; and manipulation including use of co-opting, influencing and controlling tactics. Oliver further states the following antecedents of strategic response: (1) the cause - why the organization is being pressured to conform to institutional norms or expectations; (2) the constituents - who are exerting institutional pressures on the organization; (3) the content - to what norms or requirements is the organization being pressured to conform; (4) the means of control - how or by what means are the institutional pressures being exerted; and (5) the context - what is the environmental context within which institutional pressures are being exerted. Ten predictive dimensions on which the response of the organization will depend are theorized from these antecedents. The investigators considered seven of these theoretical dimensions: social legitimacy; economic efficiency; external dependence on institutional constituents; consistency with organizational goals; constraints on decision-making imposed on the organization; voluntary diffusion of norms; and environmental interconnectedness. The other three dimensions namely multiplicity of constituents demands, legal coercion and environmental uncertainty, although necessary for implementation of top-down policies, were deemed to be less relevant for the present purpose, the perspective of which is rather how institutions would react to bottom-up innovations.

Greenhalgh *et al.*^[18] theorized that adoption of any intervention by an organization is variable - some interventions may be fully adopted, partially or not at all, while some may be eventually abandoned in time - and presented a unifying conceptual model for considering the different aspects of a complex situation and their many interactions. Certain attributes influence the (non)adoption of interventions based on the characteristics of the innovation, the individuals who will adopt the intervention and the system where the innovation will be assimilated. For this research, the investigators considered the following characteristics of an innovation in designing the context-adapted chronic care model-based service delivery model: relative advantage; compatibility; simplicity; trialability; observability; reinvention; risk; consideration of task issues; knowledge required; and augmentation/support. In constructing the model, the investigators envisioned a care model that could be applied in the context of an LMIC such as the Philippines, and that would engage the two main groups of stakeholders: those who are involved in the provision of care and prevention activities (the care providers, the health service/health system, and the policy makers); and the intended recipients of these activities (the person with the condition and the community members).

A 2-step situational analysis was conducted to help determine what specific interventions for chronic care could be feasibly applied to successfully organize care for chronic conditions, particularly type 2 diabetes mellitus (DM type 2), in the Philippines.

RESULTS OF THE CONTEXTUAL ANALYSIS

Results of analysis of key factors on the proposed organization of care for chronic conditions considering the current situation including awareness and level of knowledge on chronic conditions in general and on DM type 2 in particular, and skills for its care among the leaders and members of the community, the patients, the health system and the healthcare providers are listed in Table 1.

The community, the patients, the health system and the healthcare providers were taken into consideration as these would be the key potential players in implementing any of Wagner's six essential chronic care elements (self-management support, clinical information systems, delivery system redesign, decision support, health care organization, and community resources). The patient is central to all of these chronic care elements; the community, separately as an organized group and as a unit of government, would be responsible for the community resources; and the healthcare provider and the health service would be involved in at least five if not all of the elements.

This first analysis would indicate that there are no insurmountable barriers to the introduction of at least basic interventions for the care of chronic conditions.

The analysis of these factors was taken a step further by applying Oliver's typology and the theories of Greenhalgh *et al.*^[18] (Table 2), which explored aspects that could affect adaptation of chronic care models towards the development of a chronic care service delivery model.

Based on these, a feasible service delivery model for DM type 2 and similar chronic conditions was constructed, taking into consideration the existing healthcare organization and design, the current duties and responsibilities of individual cadres of healthcare workers, and the chronic care activities that need to be and can be provided.

THE CONTEXT-ADAPTED SERVICE DELIVERY MODEL FOR DM TYPE 2 AND SIMILAR CHRONIC CONDITIONS

Health care reorganization to concentrate primarily on chronic care is neither feasible nor desirable for developing countries still dealing with the problems of acute diseases concurrently with the rising prevalence of chronic conditions. Gradual accommodation of care to include chronic conditions is a better choice as LMICs continue their battle against malaria, pneumonia, diarrheal diseases and other acute illnesses. With this double burden of disease, the health care system should address the care for both chronic and acute conditions in terms of a more inclusive priority setting.

As mentioned previously, creating more vertical programs with specialized structures, dedicated

Table 1 Key contextual factors that is expected to affect chronic care model adaptations for the development of a service delivery model for chronic conditions including type 2 diabetes mellitus

Key factors	Analysis based on context
Community-related	
Policy	No specific policies on chronic care delivery exist at both national and local levels
Politics	Informal interviews with government officials suggested some awareness of chronic conditions such as DM type 2 and the needs that must be addressed for the care of chronic conditions in general and DM type 2 in particular in the political environment
Support	National support is limited mostly to prevention and one-day health promotion campaigns on specific chronic conditions Support from private organizations and civil societies is currently untapped
Awareness	Informal interviews with local government officials and community members suggested a low level of awareness of DM type 2, the care for DM type 2 and other associated factors, and the prevalence and burden of DM type 2 in the locality
Patient-related	
Support	Informal interview with healthcare staff and people with diabetes gave an impression of low level of support given to people with diabetes by the community and health services
Awareness	Informal interview with healthcare staff and people with diabetes gave an impression of low level of knowledge on the condition and care for the condition
Perceived need	Informal interview with people with diabetes revealed a moderate level of perceived need to improve care delivery for their condition
Perceived benefits	Informal interview with people with diabetes revealed a moderate level of perceived benefits of improving care delivery for their condition
Self-efficacy	Informal interview with healthcare staff and people with diabetes suggested a low level of self-efficacy in managing the condition
Provider-related	
Perceived need	Informal interview with healthcare staff revealed a high level of perceived need to improve primary care for chronic conditions
Perceived benefits	Informal interview with healthcare staff revealed a high level of perceived benefits of delivering good quality chronic care
Self-efficacy	Informal interview with healthcare staff suggested an impression of low level of self-efficacy in the provision of good quality chronic/diabetes care
Skill proficiency	Informal interview with healthcare staff suggested an impression of a need for skills and knowledge development regarding delivery of good quality chronic/diabetes care
Health service-related	
Leadership	The (local) government leaders and health officers are supportive of project implementation
Shared vision	The health system has a shared vision in improving the quality of care for chronic conditions
Organizational norms regarding change	The healthcare workers may be open to small, incremental changes as long as these do not lead to a drastic increase in demands on resources and workload
Administrative support	Administrative support for the project is limited

DM: Diabetes mellitus.

personnel and earmarked budget may prove more detrimental to an already-weak health system, and may inadvertently lead to inattention and cause neglect to other health issues that also need to be addressed. Primary care strengthening and capacity building of an existing health service may pave the way towards health care delivery to the people rather than prevention and care of a specific disease, moving health care towards a person-centered, comprehensive approach and veering away from being disease-centered.

LMICs can deliver prevention and care for chronic conditions such as DM type 2 by applying carefully thought-through implementation principles.

Although the care for acute and chronic diseases may seem contrasting, the people involved in health care delivery are basically not. The usual personnel complement of a health service can also be used for chronic care. Chronic care activities may range from simple, standardizable procedures that require low expertise to complex ones that require more expertise and more extensive training and education. These activities may be distributed to different types of health care personnel. A model for the delivery of chronic care services where the activities were stratified according

to the level of expertise of health care personnel in LMIC was conceptualized (Figure 4). In this model, healthcare personnel may range from volunteers/community-based health workers and expert patients, constituting the health care personnel with lesser formal expertise, to paramedical personnel (midwives, nurses) to physicians (general practitioners, specialists) constituting health care personnel with more formal expertise, although the highest tier may only involve up to the nurses in certain settings. Chronic care activities may involve health promotion and prevention in the general population, among a population at risk, and a subpopulation with high risk of developing certain chronic conditions; clinical management, counseling and health education of those with good control of their chronic conditions and with stable co-morbidities; and clinical management, counseling and health education of those with poor control of their chronic conditions and/or with unstable co-morbidities.

For DM type 2 (and similar chronic conditions, *i.e.*, hypertension, coronary artery disease, cerebrovascular accidents, some cancers, chronic obstructive pulmonary disease), health care workers with the least formal expertise may perform population-based health promotion and prevention activities, carry out pre-screening activities

Table 2 Contextual analysis of key factors affecting adaptation of chronic care models and subsequent selection of CACCM elements for implementation making use of Oliver's typology and characteristics enumerated by Greenhalgh *et al.*^[18]

CACCM- and project-related	
Oliver's dimensions	
Social legitimacy	Improving care for chronic conditions and protection and promotion of the health and wellbeing of the LGU population enhances the social fitness of the LGHU and the local government
Economic efficiency	The introduction of additional activities in any organization entails additional expenses. Cost-effective or cost-saving innovations would be preferred
External dependence on institutional constituents	The LGHU are dependent on the LGU for funding; the LGU officials who decide on the allocation of these resources are dependent on the populace for their seats in office
Consistency with organizational goals	The primary goal of the LGHU is to provide good quality healthcare to the people
Discretionary constraints imposed on the organization	The LGHU expects full autonomy especially in substantive decision-making such as resource-allocation, resource acquisition, organizational administration, <i>etc.</i>
Voluntary diffusion of norms	A moderate to high degree of voluntary diffusion with some degree of pressure from the LGU officials to diffuse said norms may be most effective in promoting adoption of the intervention
Environmental interconnectedness	A certain degree of predictability of the environment is seen: the general population, especially the people with diabetes and their families will most likely appreciate the intervention. Such appreciation may be reflected on goodwill towards the LGU officials and consequently to the LGHU (for example additional budget allocated to health)
Greenhalgh's characteristics	
Relative advantage	Implementing a diabetes-care project gives the advantage of improving the care for this condition and a number of its comorbidities, but without reduction of other health benefits
Compatibility	Compatibility of the intervention with current/pre-existing activities in the LGHU and with the current duties, responsibilities and workload of the LGHU staff is sought
Simplicity	Simplicity and ease of use of the intervention favors adoption of the intervention
Trialability	Flexibility in accomplishing a number of tasks, <i>i.e.</i> , giving leeway to the healthcare staff regarding performance of activities related to the intervention will increase acceptability of the intervention
Observability	Providing information to the intended adopters of the benefits of the intervention, <i>e.g.</i> , improvements of glycemia, favors adoption of the intervention
Reinvention	Flexibility of the intervention allowing adaptation and refinement to suit the context, the needs of the individual person with diabetes and the capabilities of the healthcare provider favors its adoption
Risk	Based on outcomes of previous studies conducted on implementation of chronic care models and provision of self-management education, it is certain that the benefits far outweigh the risks
Task issues	Workable and easy to use interventions favor adoption Relevance of the intervention to the work of the staff and tasks that may contribute to the relevance of the work of the individual health care worker is preferred However, the intervention may also be interpreted as an added workload to the LGHU staff
Knowledge required	Knowledge and skills required for full implementation of the intervention need to be supplied/supplemented
Augmentation/support	Provision of a training workshop prior to implementation increases the probability of adoption of the intervention

LGHU: Local government health units; LGU: Local government units.

to identify a subpopulation at risk for developing DM type 2, and may follow-up on people noted to be at high risk or already identified to have prediabetes. These activities are standardizable; operating procedures and work flow diagrams/decision trees may be constructed to instruct the health care worker, and checklists may be prepared to serve as guides. On the next tier of health care workers, activities that may be assigned include specific screening or confirmatory testing for dysglycemia (DM type 2 and prediabetes), identification of co-morbidities, and counseling, health education and clinical management of patients in good glycemic control and with stable co-morbidities. Although certain guidelines and diagrams still make these activities standardizable, a higher level of expertise is expected to clinically manage optimally controlled DM type 2 and co-morbidities and to recognize and know when to refer impending instabilities; special skills also need to be developed to initiate effective communication and counseling/health education. At the higher end of the spectrum, activities are focused on those with poor glycemic control and/or unstable co-morbidities and

complications, which would require clinical expertise and judgment for appropriate clinical management and counseling.

CONCLUSION

The context-adapted service delivery model for DM type 2 and similar chronic conditions may be far-removed from that in HICs, but it is designed to deliver prevention and care that encompasses the spectrum of diabetes from those at risk to those with poor glycemic control and/or unstable co-morbidities and includes counseling for self-management education and support. It is likewise designed for the general population to experience this service through general health (diabetes) education and healthy lifestyle promotion. The model incorporates care for diabetes into a current package health care activities making use of pre-existing human resources for health. It taps the potential of a workforce that may assume simple and standardizable diabetes prevention and care activities. In so doing, the additional burden on

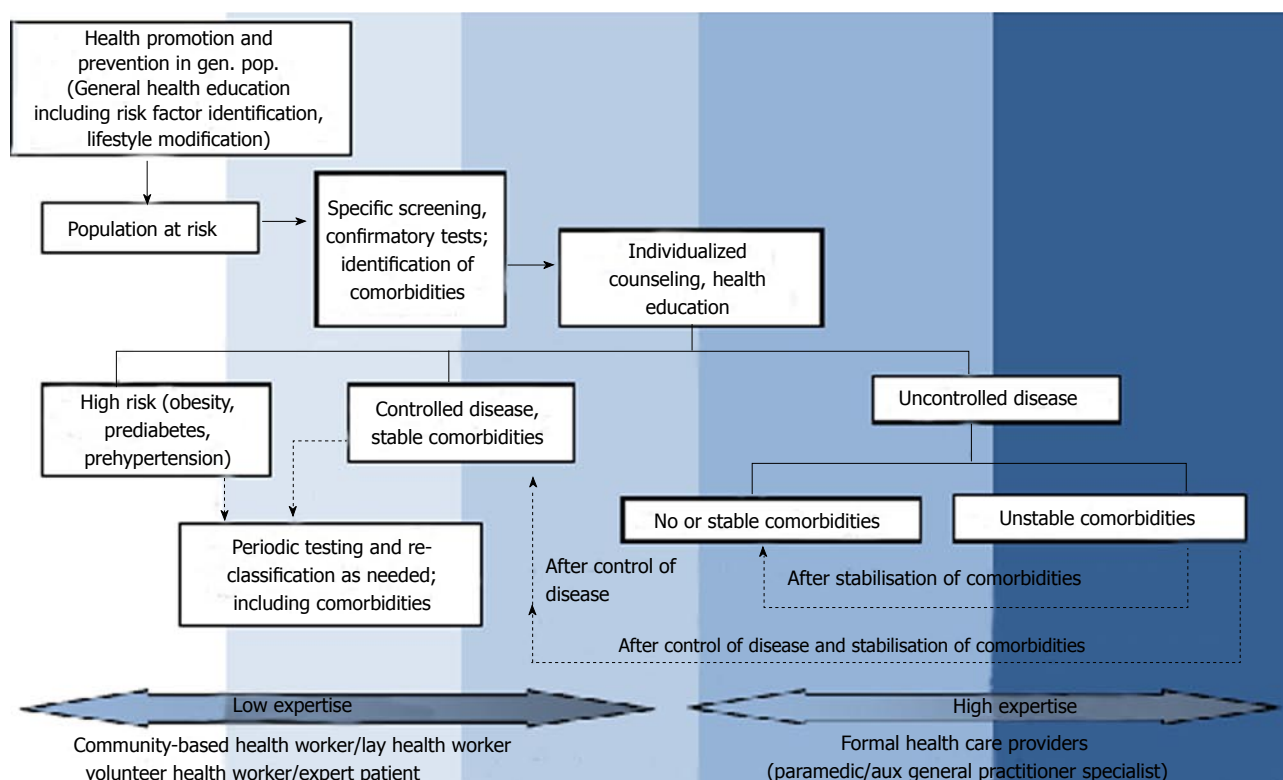


Figure 4 The context-adapted service delivery model for type 2 diabetes mellitus and similar chronic conditions.

professional healthcare workers who are now required to focus attention on both acute and chronic conditions is decreased. This service delivery model, adapted to the Philippine context, may be applicable to other LMICs having a similar situation as the Philippines. The model, however, requires additional support in terms of preparing all cadres for the delivery of diabetes care. These include sustained decision support, and materials such as the flowcharts, decision trees and checklists.

Taking inspiration from models of chronic care and carefully selecting essential elements according to effectiveness potential and local feasibility can result in basic but efficient care strategies^[31].

A low resource healthcare system with no specific attention at all for chronicity can be induced to include chronic/lifelong conditions among its priorities, even with minimal means. Well applied, however, such minimal means can make a lot of difference for increasing numbers of people.

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How the kidney hyperfiltrates in diabetes: From molecules to hemodynamics

Tsuneo Takenaka, Tsutomu Inoue, Yusuke Watanabe

Tsuneo Takenaka, Department of Medicine, International University of Health and Welfare, Tokyo 107-0052, Japan

Tsuneo Takenaka, Department of Medicine, International University of Health and Welfare, Clinical Research Center, Sanno Hospital, Tokyo 107-0052, Japan

Tsutomu Inoue, Yusuke Watanabe, Department of Nephrology, Saitama Medical University, Iruma Saitama 350-0495, Japan

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Correspondence to: Tsuneo Takenaka, MD, PhD, Professor, Department of Medicine, International University of Health and Welfare, Clinical Research Center, Sanno Hospital, 8-10-16 Akasaka Minato, Tokyo 107-0052, Japan. takenaka@iuhw.ac.jp
 Telephone: +81-3-34023301

Fax: +81-3-34043652

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Abstract

In this review, we focused on two molecules, connexin and sodium-glucose cotransporter, which can link to diabetic hyperfiltration. In diabetic kidney, the activation of renin-angiotensin system occurs simultaneously with glomerular hyperfiltration. The latter largely depends

on pathophysiological afferent arteriolar dilation in the presence of high angiotensin II. As a mechanistic basis for the above, tubular hypothesis has been proposed for type 1 diabetic patients as well as experimental models. Although tubular hypothesis has not been well evaluated in type 2 diabetes, clinical observations support that tubular hypothesis is true also in type 2 diabetes. Recent results on tubular hypothesis along with connexin abnormality in type 2 diabetes were revisited. In addition, the importance of sodium-glucose cotransporter in diabetic hyperfiltration is discussed. The link between salt paradox and the activation of renin-angiotensin system will be also reviewed.

Key words: Tubuloglomerular feedback; Salt paradox; Connexin; Glomerular hyperfiltration; Sodium-glucose co-transporter

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Core tip: A diminished tubuloglomerular feedback (TGF) in diabetes can explain both glomerular hyperfiltration and the activation of renin-angiotensin system. An enhanced absorption through sodium-glucose co-transporter in proximal tubule decreases the delivery to macula densa, reducing TGF signal generation in diabetes. Connexin phosphorylation and subsequent ubiquitination by oxidative stress in type 2 diabetes reduces its expression in juxtaglomerular apparatus, disabling TGF signal transduction. Clinical as well as experimental evidences support that this tubular hypothesis is working, and suggest that drugs targeting the above to normalize TGF, an intrinsic physiological system, would be effective to ameliorate diabetic nephropathy.

Takenaka T, Inoue T, Watanabe Y. How the kidney hyperfiltrates in diabetes: From molecules to hemodynamics. *World J Diabetes* 2015; 6(4): 576-582 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i4/576.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i4.576>

OPENING REMARKS

As reported recently^[1], many clinical trials have demonstrated that the inhibitors of renin-angiotensin system (RAS) are effective to prevent the development and progression of diabetic nephropathy (DMN). There is an emerging agreement that the activation of RAS give deleterious influences on DMN. Why is RAS activated even in the early course of diabetes? Many experimental hypotheses for an overproduction of angiotensinogen by hyperglycemia and pathological activation of pro-renin in diabetes have been proposed experimentally^[2,3]. They appear very true at least in some aspects of DMN. From the renal hemodynamic point of view, DMN is characterized as glomerular hypertension and hyperfiltration from its early stage. In non-diabetic chronic kidney disease, single nephron glomerular hypertension and hyperfiltration occur in remnant nephrons to suffice the function of lost glomeruli due to its underlying renal disease. Thus, glomerular hyperfiltration starts when renal injury has progressed to some extent. However, all nephrons in diabetes show glomerular hypertension and hyperfiltration before microalbuminuria is developed^[4]. The main character of DMN is abnormal afferent arteriolar dilation^[5]. Tubular hypothesis is proposed more than 2 decades ago, which explain both glomerular hyperfiltration and RAS activation^[6]. Is tubular hypothesis true for type 2 diabetes, which now provides medical as well as socio-economical problems over the world? This has not been well examined. Let us start from basic experiments.

TYPE 1 DIABETES

Tubular hypothesis

Tubular hypothesis is based on physiological responses to hyperglycemia and its mechanisms are following^[6]. In type 1 diabetes, insulin deficiency causes marked hyperglycemia, resulting in the ultrafiltrate with high glucose concentration in Bowman capsule. Although proximal tubules reuptake most amounts of filtered glucose, glucose exceeding the capacity of tubular reuptake excretes into urine (glycosuria). Since proximal tubule possesses sodium glucose co-transporter 1 (SGLT1) and SGLT2, glucose is up-taken together with sodium. Then, the reuptake of sodium chloride through SGLT is increased in hyperglycemic condition, which is considered as a cause of salt-sensitive hypertension in diabetes. Furthermore, the delivery of sodium and chloride to macula densa is decreased by the enhanced reuptake through SGLT by proximal tubules (Figure 1). A reduced delivery to macula densa dilates the afferent arteriole by removing constrictor signals from tubuloglomerular feedback (TGF), to induce glomerular hypertension and

hyperfiltration. Moreover, TGF signal from macula densa inhibits renin release. Again, a reduced delivery to macula densa during hyperglycemia (due to increased proximal tubular absorption through SGLT) removes TGF signals, to activate RAS which constricts efferent arterioles, worsening glomerular hypertension^[7].

TGF mechanisms (adenosine triphosphate + adenosine) and salt paradox

There are still debates how macula densa cell transduces TGF signal to afferent arterioles. Although two hypotheses have been raised for "second messenger" for TGF, our data support the notion that both ATP and adenosine are required for full expression of TGF responses^[8]. Macula densa cell releases adenosine triphosphate (ATP) into the interstitium when it reabsorbs sodium chloride delivered by tubular flow (Figure 2). On the one hand, ATP released from macula densa binds to ATP receptor located on extraglomerular mesangial cells to induce membrane depolarization and/or an increase in cytosolic calcium^[9]. These signals travel to neighboring mesangial cells through gap junctions, and finally the signals are transduced to afferent arteriolar myocytes through gap junction^[10]. Gap junction constitutes an important intercellular communication tool. Indeed, the inhibiting the function of connexin (Cx37 or Cx40), which compose of gap junction, elicits both suppression of TGF-dependent autoregulation and RAS activation. There is a possibility that ATP secreted from macula densa diffuses to afferent arteriolar myocytes and directly interacts with ATP receptors to induce afferent arteriolar constriction. On the other hand, ATP is degraded to adenosine by nucleotidase on extraglomerular mesangial cells, and subsequently adenosine binds to its specific receptor on afferent arteriolar myocytes to induce constriction^[11].

Many paracrine factors modulate TGF. Angiotensin and endothelin enhance TGF responsiveness, whereas nitric oxide and prostaglandin diminish it^[9]. Under physiological condition, salt load that enhances the renal production of nitric oxide and prostaglandin weakens TGF. However, experimental data indicate that salt intake enhances TGF in type 1 diabetes. Salt load reduces proximal tubular reabsorption, which is enhanced in diabetes. As a result, the delivery to macula densa is increased, thereby restoring TGF that constrict afferent arteriole, thereby ameliorating glomerular hypertension, hyperfiltration and albuminuria in type 1 diabetic model^[12]. This is called salt paradox. An inverse relationship between salt intake and glomerular filtration rate is seen in type 1 diabetic patients as well as the animal models. Collectively, both tubular hypothesis and salt paradox are truly working in human^[13].

Insulin deficiency and resistance

In contrast to type 1 diabetes, which is characterized by absolute insulin deficiency due to beta-cell damage, type 2 diabetes shows normal or excessive insulin

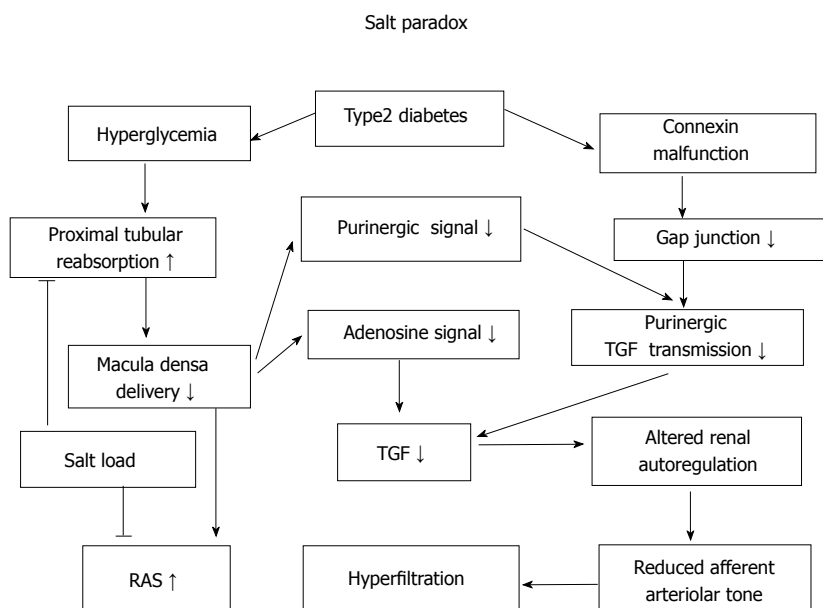


Figure 1 Working hypothesis for glomerular hyperfiltration in diabetes. On the one hand, hyperglycemia enhances sodium reabsorption in type 1 and type 2 diabetes, thereby decreasing the delivery to macula densa with resultant weakening of tubuloglomerular feedback (TGF). The latter impairs renal autoregulation that dilates afferent arterioles, and activates renin-angiotensin system (RAS). On the other hand, TGF signal by adenosine triphosphate (P2) is damaged in type 2 diabetes due to connexin phosphorylation and gap junction malfunction, worsening glomerular hyperfiltration. High salt intake inhibits proximal tubular reabsorption, thereby increasing the delivery of sodium chloride to macula densa. This ameliorates pathological afferent arteriolar dilation by the restoration of TGF through adenosine (A1) signal^[18].

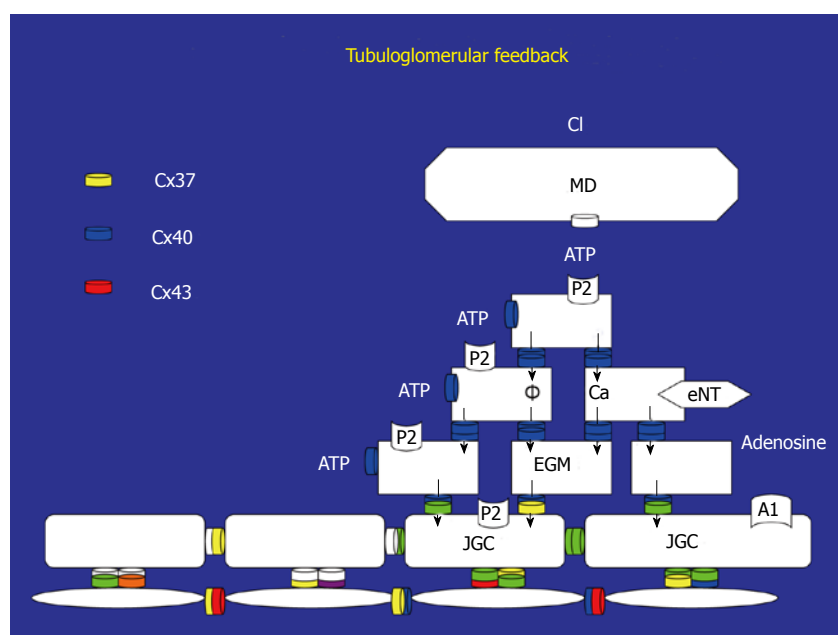


Figure 2 Sodium chloride is delivered to macula densa, macula densa releases adenosine triphosphate. Adenosine triphosphate (ATP) binds to P2 receptor on extraglomerular mesangial cell (EGM), and induces membrane depolarization and/or elevations of cytosolic calcium. These signals are transduced to juxtaglomerular cells (JGC) by intercellular communication through gap junctions consisted of connexins (Cx). In addition, ATP is degraded to adenosine by ecto-nucleotidase (eNT) on EGM. Adenosine binds to A1 receptor on JGC, increasing cytosolic calcium. Calcium waves generated in JGC transduce through gap junctions between afferent arteriolar myocytes to its upstream, eliciting ascending vasoconstriction (refs 8 and 10). MD: Macula densa; Cl: Chloride.

secretion, especially during its early clinical course. The patients with type 2 diabetes are usually obese and manifest insulin resistance, underlying hyperglycemia in type 2 diabetes. The mechanisms mediating insulin resistance are out of focus of this review, but involve oxidative stress that inhibit insulin signaling by facilitating serine phosphorylation of insulin receptor

substrate^[14].

HOW ABOUT TYPE 2 DIABETES?

Connexin

As mentioned above, gap junctions are required for the transmission of TGF signal. Juxtaglomerular apparatus

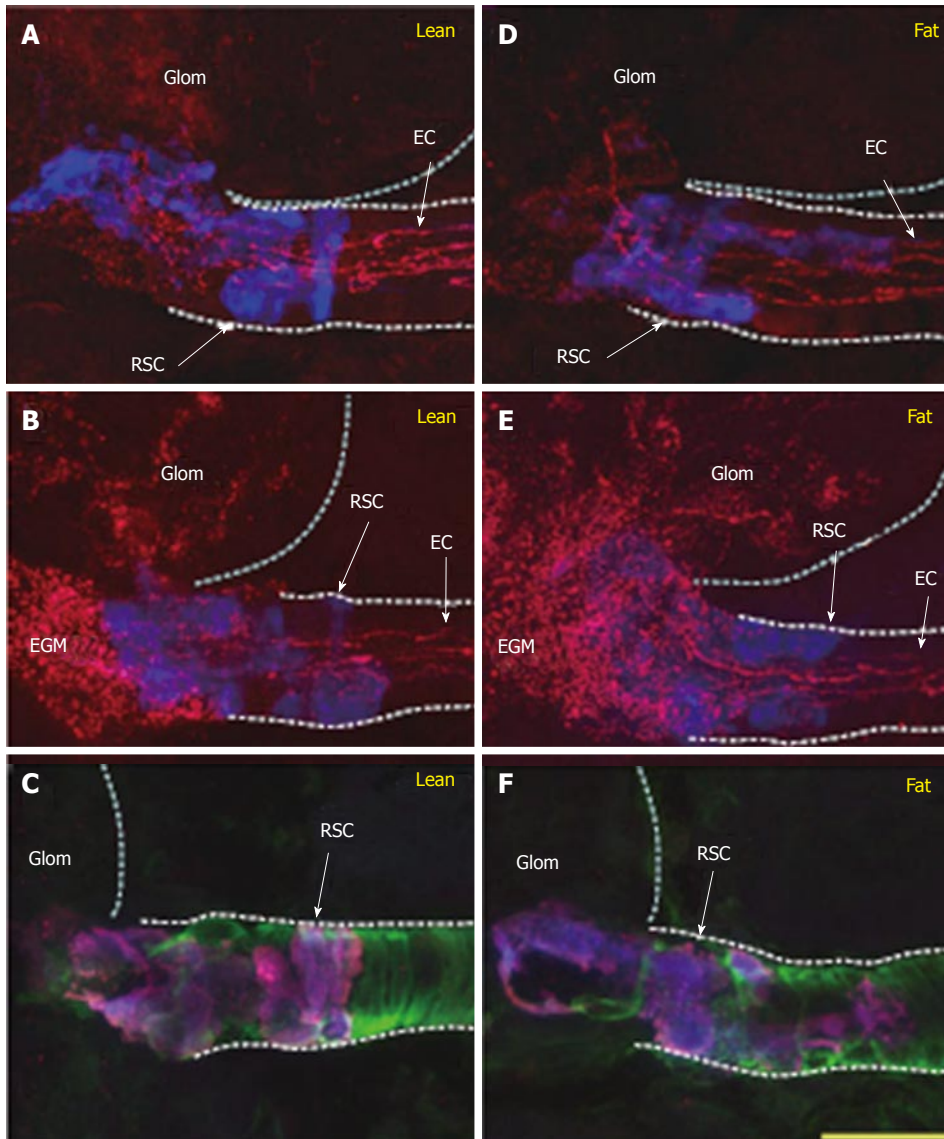


Figure 3 Expression of connexins (Cx37, 40, 43) in juxtaglomerular apparatus of type 2 diabetic (Zucker diabetic fatty) and control (Zucker lean) rats. A, B, D, E: Renin secreting cells (RSC, blue) and endothelial cells (EC) express Cx37 (A: Lean; D: Fat) and Cx40 (B: Lean; E: Fat). Cx40 is expressed on glomerular and extraglomerular mesangial cells (EGM) in control (B) and diabetes (E). Cx43 is expressed in cytosol of RSC in both groups (C, F). Quantification reveals that the expression of Cx37 in RSC is reduced in diabetic model^[15].

shows the expression of Cx37, Cx40 and Cx43. Type 2 diabetic model animals exhibit Cx abnormality^[15]. Six Cxs form one hemichannel on cell membrane^[16]. The binding of a hemichannel in a cell with the other one in an adjunct cell forms a gap junction. Gap junctions pass through small molecules such as inositol triphosphate and/or calcium, and transduce membrane depolarization, enabling intercellular communication. Post-transcriptional alterations of Cx induce conformational changes and prevent hemichannels to bind each other, especially when their extracellular loops are modified. Serine residue of Cx can be phosphorylated by protein kinase C and/or MAP. In type 2 diabetes, insulin resistance and associated oxidative stress activate these kinase activities. Phosphorylated Cx diminishes its ability to form gap junction, impairing intercellular signal transduction. Abnormal function

of gap junction/connexin is considered to be one of the causes of arrhythmia in diabetes. Indeed, the abundance of phosphorylated Cx43 is elevated in type 2 diabetic animal model. Furthermore, functional analyses demonstrated abnormal gap junction function in juxtaglomerular apparatus that inhibiting Cx37 or Cx40 failed to stimulate renin release. Phosphorylated Cx is prone to be ubiquitinated and broken down. Expression of Cx37 on renin-secreting cells is reduced in type 2 diabetic animals (Figure 3). In type 2 diabetes, functional derangements of Cx induce the removal of TGF signal, which dilates afferent arterioles and activates RAS (Figure 1). As discussed, functional impairments of Cx in DMN cause glomerular hyperfiltration through reductions of TGF signal transmission. The latter may allow direct transmission of systemic blood pressure to glomeruli, facilitating glomerular sclerosis together with

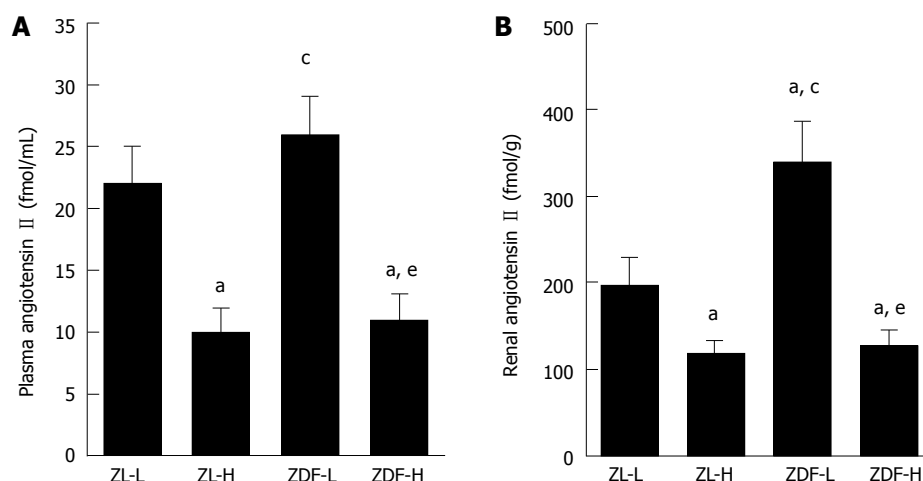


Figure 4 Plasma and kidney concentration of angiotensin II in type 2 diabetic model (Zucker diabetic fatty rat) and control rat (Zucker lean rat). ZL-L and ZL-H indicate ZL fed normal and high salt diet, respectively. Alike, ZDF-L, ZDF-H describe ZDF fed normal and high salt diet, respectively. ^a*P* < 0.05 vs ZL-L, ^c*P* < 0.05 vs ZL-H, ^e*P* < 0.05 vs ZDF-L^[18]. ZDF: Zucker diabetic fatty rat; ZL: Zucker lean rat.

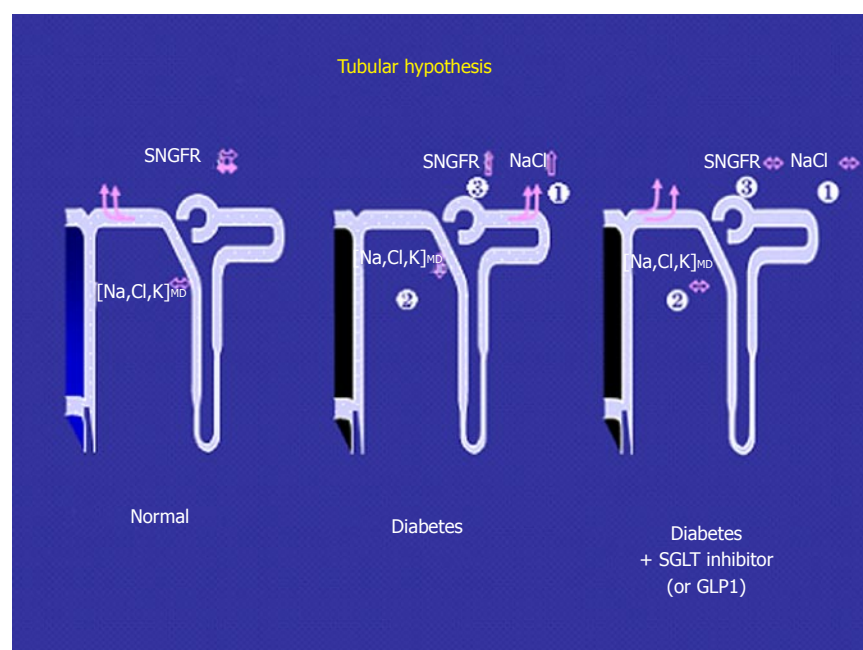


Figure 5 Compared to normal condition (left), ultrafiltrate in Bowman capsule contains significant amount of glucose in diabetes (middle). Because proximal tubules reabsorb more sodium with glucose through SGLT in diabetes (1), the delivery to macula densa is decreased (2). This weakens TGF signals to increase glomerular filtration rate (3), accounting for hyperfiltration in early stage of diabetes. Either GLP or SGLT inhibition (right) inhibits proximal tubular reabsorption (1), restoring sodium chloride delivery to macula densa even under hyperglycemic condition (2). This would have TGF work and normalize glomerular filtration rate (3), ameliorating glomerular hyperfiltration. SGLT: Sodium glucose co-transporter; GLP: Glucagon-like peptide; TGF: Tubuloglomerular feedback.

systemic hypertension induced by RAS activation^[4]. Of interest, there is a report that abnormal Cx is related to the prognosis of DMN in type 2 diabetic patients^[17].

Hyperglycemia and salt

Is tubular hypothesis true for type 2 diabetes? The answer appears to be YES. Our recent data indicate that enhanced proximal tubular reabsorption and glomerular hyperfiltration exist in type 2 diabetic animal model^[18]. In addition, renal RAS is activated in this model, as evident that renal angiotensin concentration is elevated. Furthermore, in this model, acute salt load induces the

suppression of proximal tubular reabsorption and the amelioration of glomerular hyperfiltration, together with the decrease in renal angiotensin concentration (Figure 4). The observations that salt load reduces albuminuria in this diabetic model suggest that glomerular hypertension is also controlled by high salt intake. These findings provide compelling evidence that salt paradox exists in type 2 diabetes. It is proved that adenosine is a mediator of salt paradox in type 1 diabetes. When salt consumption is increased in type 1 diabetes, the delivery of sodium chloride is increased, restoring TGF signals to produce adenosine that constricts afferent

arterioles and ameliorates glomerular hyperfiltration. Our experimental results demonstrated that under adenosine receptor blockade, the amelioration of glomerular hyperfiltration by salt load was not happened. Because TGF signal transmission pathway for ATP has been already diminished due to Cx abnormality in type 2 diabetes, residing adenosine pathway works for salt paradox in type 2 diabetes. Is salt paradox truly functioning in type 2 diabetic patients? No answer was given for this question until recently. However, there is a report that proximal tubular reabsorption positively relates to glomerular hyperfiltration in type 2 diabetic black patients^[19]. Furthermore, an inverse relation between salt intake and albuminuria is demonstrated in type 2 diabetic Japanese patients^[20]. Taken together, salt paradox is working in type 2 diabetic patients regardless of difference in race.

HOPE FOR NEW ANTI-DIABETIC DRUGS

We would not recommend for diabetes to take high salt diet to prevent the development and progression of DMN. Salt load could induce hypertension and facilitate the development of cardiovascular diseases. How can we prevent DMN? Although it should be important to strongly inhibit RAS, new anti-diabetic drugs have some hope. The inhibition of DDP-4 elevates GLP1, which binds to its specific receptor on proximal tubules to induce natriuresis through suppressing proximal reabsorption of sodium chloride (Figure 5). Emerging evidences indicate that DDP-4 inhibitors show blood pressure lowering effects^[21]. Thus, according to tubular hypothesis, GLP1 ameliorates RAS activation, glomerular hypertension and hyperfiltration in DMN independently of its blood glucose lowering actions (Figure 1). Indeed, it was recently reported that DDP-4 inhibitors exhibit antiproteinuric effects in DMN. Many SGLT inhibitors are becoming available for clinical use. SGLT inhibitors may share similar renal actions with DDP-4 inhibitors. According to tubular hypothesis, SGLT inhibitors suppress proximal tubular reabsorption, which is enhanced in diabetes, suggesting that SGLT inhibitors possess blood pressure lowering and renal protective actions beyond its blood glucose lowering effects^[22]. Indeed, experimentally SGLT inhibitor showed protective effects on diabetic nephropathy. However, the influences on RAS of SGLT and DDP-4 inhibitors have not been examined. Further studies are required to clarify this issue.

CONCLUDING REMARKS

Recently, the number of patients with DMN is progressively increased, showing medical-economic problem. Research on DMN is promising from many aspects, and numerous new findings have been obtained. This is a good news, and we hope that new results could be applied for clinical care as soon as possible. However, we are sorry that they do not intend

to integrate the findings, so that they are isolated to each other. We have tried to sum up several findings in this review, although it might be inadequate. Discussion was focused on the mechanisms underlying DMN common in diabetic patients as well as animal model. The treatment with RAS inhibitors on DMN is effective, but we have to admit that they are not enough. In near future, we wish that new therapy that eventually halts DMN will be developed along with complete understanding of underlying mechanisms for DMN.

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Effects of exercise on brain functions in diabetic animal models

Sun Shin Yi

Sun Shin Yi, Department of Biomedical Laboratory Science, College of Medical Sciences, Soonchunhyang University, Asan 336-745, South Korea

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Correspondence to: Sun Shin Yi, DVM, PhD, Assistant Professor, Department of Biomedical Laboratory Science, College of Medical Sciences, Soonchunhyang University, 646, Eupnae-ri, Sinchang-myeon, Asan 336-745, South Korea. admiral96@sch.ac.kr

Telephone: +82-41-5304873

Fax: +82-41-5308085

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using experimental animals are a suitable option to overcome this drawback, and animal studies have improved continuously according to the needs of the experimenters. Since brain health is the most significant factor in human life, it is very important to assess brain functions according to the different exercise conditions using experimental animal models. Generally, there are two types of DM; insulin-dependent type 1 DM and an insulin-independent type 2 DM (T2DM); however, the author will mostly discuss brain functions in T2DM animal models in this review. Additionally, many physiopathologic alterations are caused in the brain by DM such as increased adiposity, inflammation, hormonal dysregulation, uncontrolled hyperphagia, insulin and leptin resistance, and dysregulation of neurotransmitters and declined neurogenesis in the hippocampus and we describe how exercise corrects these alterations in animal models. The results of changes in the brain environment differ according to voluntary, involuntary running exercises and resistance exercise, and gender in the animal studies. These factors have been mentioned in this review, and this review will be a good reference for studying how exercise can be used with therapy for treating DM.

Key words: Diabetes mellitus; Involuntary and voluntary exercise; Resistance exercise; Brain function; Animal models

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Abstract

Human life span has dramatically increased over several decades, and the quality of life has been considered to be equally important. However, diabetes mellitus (DM) characterized by problems related to insulin secretion and recognition has become a serious health problem in recent years that threatens human health by causing decline in brain functions and finally leading to neurodegenerative diseases. Exercise is recognized as an effective therapy for DM without medication administration. Exercise studies

Core tip: Brain is a highly sensitive and vulnerable tissue easily influenced by diabetes mellitus (DM). Physical exercise has been known to be one of the best non-pharmacologic ways to prevent and treat DM. Animal exercise experiments are very useful for research on DM because experiments cannot be performed in humans. Exercise has various benefits that help to improve brain function by reducing chronic inflammatory responses, accumulation of adipose tissue, appetite, insulin resistance, and dysfunction of the negative

feedback mechanism. In this review, the author reports a battery of animal models of exercise, and presents the beneficial effects of exercise on the brain.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine disorders and is mainly divided into two types according to the activity of β -cells in the pancreas: type 1 DM (T1DM) is characterized by degeneration of β -cells, while the main cause of type 2 DM (T2DM) is a progressive decline in insulin sensitivity resulting in sustained hyperglycemia^[1-3]. Particularly, DM is known as the main factor that can cause various pathologic brain complications and can promote cognitive impairment and vascular dementia in humans^[4-8]. A number of studies have reported that DM can cause hormonal dysregulation, systemic vascular changes, dysregulation of the plasma glucose level, changes in blood chemistry, and other organ dysfunctions such as kidney and heart failure^[9-19]. Various medical treatments are available to regulate glucose dysregulation, correct hormonal changes and vascular conditions in DM patients; however, these medical treatments cannot always cure the metabolic disorder completely, and physicians also cannot predict the progression of the complications with uncontrolled patient's life styles^[20-24].

Particularly T2DM is significantly related to the incidence of obesity and its associated disorders^[25-27]. Obesity is defined as a surplus of body fat accumulation, with the excess of adipose tissue really being a well-established metabolic risk factor for the development of obesity-related comorbidities such as insulin resistance, T2DM, cardiovascular diseases, and some common cancers^[2,28-32]. The mechanisms linking excess adiposity and cancer are unclear, but the obesity-related low-grade chronic inflammation is widely accepted as a critical factor in the pathogenesis of various diseases such as T2DM, cardiovascular disorders, dementia, cancers, dietary control failure^[26,28,33-42]. Currently, particular attention has been placed on the pro-inflammatory microenvironment in the body associated with obesity, specifically underlining the involvement of obesity-associated hormones/growth factors in the cross-talk between macrophages, lymphocytes, adipocytes, and epithelial cells involved in the development of T2DM^[28,43]. In addition, accumulated peripheral white adipose tissue (WAT) is an endocrine tissue that secretes hundreds of cell-signaling molecules known as cytokines, chemokines, and adipokines^[29,32,33,44,45]. The endocrine

function of adipose tissue might be a key factor in the mechanisms linking adipose tissue to insulin resistance, leptin resistance, dietary control failure, T2DM-associated dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, neurodegenerative diseases, vascular diseases related to aging, cognitive impairment, and dementia^[27,35,45-50]. Hence, uncontrolled chronic obese condition can be a critical factor in the development of T2DM, and it also acts as an agent that affects normal brain functions.

Recently many studies have shown the positive effects of regular physical activity on improving complications caused by DM, and hence regular physical activity intervention is regarded as a promising adjuvant therapy^[7,37,51-62]. Exercise can affect various physical environments and has decisive effects on improving brain functions for a better quality of life^[7,59-61,63-72]. However, the precise mechanisms responsible for the positive effects of exercise on brain functions under obesity and T2DM conditions have not yet been well understood, and many studies have been performed using animal models of different diabetic stages regardless of the DM type and under various kinematic conditions to assess the related mechanisms for changing the microenvironment of the brain. Thus, experiments with animal exercise models mimicking the etiology and progression of human DM have been actively performed and developed to assess the preventive and therapeutic effects of exercise on brain functions^[1,73-81].

Therefore, we review recent evidences on the role of exercise in promoting brain functions mainly under T2DM conditions in animal models and provide practical applications for the management of T2DM.

PHYSIOPATHOLOGICAL CHANGES CAUSED BY DM

Most of the DM conditions gradually impair normal brain functions by causing excessive production of pro-inflammatory cytokines, insulin resistance, and reactive oxygen species due to certain causes such as prolonged obese condition or hormonal dysfunction^[15,27,28,31,32,35,82-88]. Excessive and/or compulsive overeating disturbs the normal blood composition, deteriorates cardiovascular circulation, induces insulin resistance, and increases the visceral fat^[29,45,82,89-91]. Particularly, the infiltrated inflammatory immune cells such as macrophages and lymphocytes in adipose tissues secrete a variety of cytokines into the blood stream, and negatively influence the systemic cardiovascular system and the brain^[32,44,82,92-94]. A number of studies have shown that elevations in levels of systemic inflammatory mediators such as adipokines, tumor necrosis factor- α , resistin, interleukin-6, plasminogen activator inhibitor-1, C-reactive protein, monocyte chemoattractant protein (MCP)-1 play a pivotal role in changing the physiology of the brain^[3,45,56,82,95-98]. Particularly, results of animal

and human studies have showed that insulin passes *via* the systemic circulation to the brain and it may have some physiologic actions which are different than its peripheral metabolic effects. Insulin resistance in peripheral tissues leads to the elevation of pro-inflammatory cytokines, neurotoxic ceramides, obesity-induced NADPH oxidase-associated oxidative stress in the brain, and insulin action on the brain is thought to be a regulator of peripheral glucose homeostasis in rodent studies *via* melatonin related mechanisms, increased unfolded protein response activation, mitochondrial and ER stress related overeating, leptin and insulin resistance, corticotropin-releasing factor-related islet cell control^[47,67,99-102]. Recent studies of the mouse brain have demonstrated that degenerative plaque formation observed in AD (AD is the most prevalent form of dementia) is associated with insulin resistance^[47,103]. Insulin regulates food intake and cognitive functions in the brain; however, deranged insulin signaling in the brain has also been implicated in neurodegenerative disorders^[104-107].

Insulin action in the brain is regarded as the main factor for maintaining DM patients in a healthy condition due to the interrelationship between peripheral and central insulin resistance.

ANIMAL MODELS IN DM RESEARCH

DM is a chronic disease that is characterized by a relative or absolute lack of insulin release, resulting in hyperglycemia. Since T1DM and T2DM, as endocrine disorders, represent quite complex diseases in which different organ systems are involved, animal models should be chosen carefully depending on what aspect of the disease is being investigated. On the other hand, for developing specific models of T1DM and T2DM, investigators should be aware of the different pathogenic mechanisms of DM that involve different inducible factors.

T1DM animal models

The main characteristic of T1DM is autoimmune destruction of the pancreatic β cells, leading to lack of insulin release. In animal models, investigators can induce this deficiency by chemical ablation of the beta cells in breeding animals that spontaneously develop the autoimmune diabetic condition. The representative chemicals that induce T1DM are streptozotocin synthesized by *Streptomyces achromogenes*^[108-110], and alloxan^[1,73,111,112] which causes poor β cell defense mechanisms against free radicals. Thus, these chemicals can be used for developing new insulin, transplantation models for testing treatments that may prevent beta cell death. However, the researchers should be aware that a number of studies using STZ did not consider the time period between chemical injection in animals and sacrifice. Thus, it is true that many researches on T1DM using STZ injection have ignored this factor. Shin *et al*^[110] and Yi *et al*^[113]

demonstrated chronological hippocampal changes in the brain at different time points of animal sacrifice after STZ injection. Therefore, researchers should remember that the results of T1DM *via* the chemical might be different based on how many days or weeks have passed following chemical administration in animals.

The non-obese diabetic mice, Biobreeding rats, and LEW. 1AR1/-iddm rats are the most commonly used animal models of spontaneous autoimmune diabetes showing beta cell destruction due to an autoimmune process^[1,73,75]. Akita mice, a genetically induced insulin dependent T1DM diabetic animal model, are characterized by beta cell destruction *via* ER stress. Lastly, T1DM can be induced by viruses such as Coxsackie B virus^[114], Encephalomyocarditis virus^[115,116], and Kilham rat virus^[116,117]. The virus-induced model can be complicated as the outcome is dependent on replication of the virus as well as timing of the infection^[118].

Several other large animal models except for rodent animals have been developed to study T1DM extensively. Since it is relatively difficult to expect the development of spontaneous diabetes in large animal models, induced models of T1DM are required. The most commonly used method of inducing T1DM in large animal models is by performing pancreatectomy and chemical ablation of beta cells (STZ)^[119-122]. The T1DM rodent models are summarized in Table 1.

T2DM animal models

The main characteristics of T2DM are insulin resistance and β cell dysfunction, and defective insulin secretion from β cells. Therefore, animal models of T2DM tend to include models of insulin resistance and/or β cell dysfunction. Most of the T2DM animal models are characterized by the obese phenotype, which reflects the human condition where obesity is closely related to T2DM development^[1]. The T2DM animal models are categorized according to the type of induction mechanism as follows: spontaneously obese models^[1], diet/nutrition induced obesity models^[123,124], non-obese models^[125], genetically induced models of β cell dysfunction^[126], and surgically induced diabetic animal models^[127]. The T2DM rodent models are summarized in Table 2.

DM AND THE NEURAL SYSTEM

DM and central nervous system

Diabetes is significantly related with brain microenvironments and functions. Diabetes is known to largely affect the intensely vascular organs such as kidneys, liver, and brain^[16,18-20,49,50,87,88,95,98,99,128-130]. Brain is the key organ that is involved in hormonal, sensory, and motor regulations so that living organisms can maintain homeostasis *via* the negative feedback system^[46,131]. However, diabetic condition can be a serious chronic stress factor, and its secondary negative effects can exert a bad influence on the

Table 1 Summary of animal models of type 1 diabetes mellitus

Induction	Models	Dose(s) (mg/kg)	Main characteristics	Model uses
Chemicals	Streptozotocin	Rat 35-65 (<i>iv</i> or <i>ip</i>) Mice 100-200 (<i>iv</i> or <i>ip</i>) Hamster 50 (<i>ip</i>) Dog 20-30 (<i>iv</i>) Pig 100-150 (<i>iv</i>) Primates 50-150 (<i>iv</i>)	Hyperglycemia	New formulations of insulin transplantation models
	Alloxan	Rat 40-200 (<i>iv</i> or <i>ip</i>) Mice 50-200 (<i>iv</i> or <i>ip</i>) Rabbit 100-150 (<i>iv</i> or <i>ip</i>) Dog 50-75 (<i>iv</i> or <i>ip</i>)		
	Multiple low dose Streptozotocin			Treatments prevent beta cell destructions
	NOD mice BB rats LEW.1AR1/-iddm rats			Understanding genetics of T1DM Understanding mechanism of T1DM Treatments prevent beta cell destruction Treatments manipulate autoimmune process
Genetically induced	AKITA		Beta cell destruction due to ER stress Insulin dependent	New formulations of insulin Transplantation models Treatments to prevent ER stress
Virally-induced	Coxsackie B virus Encephalomyocarditis virus Kilham rat virus		Beta cell destruction induced by viral infection of beta cells	Establish potential role of viruses in the development of T1DM

iv: Intravenous injection; *ip*: Intraperitoneal injection; T1DM: Type 1 diabetes mellitus.

Table 2 Summary of animal model of Type 2 diabetes mellitus

Induction	Model	Main characteristics	Model uses
Obese models	ob/ob mice db/db mice KK mice KK/Ay mice NZO mice TSOD mice Zucker fatty rat Zucker diabetic fatty rat	Obesity-induced hyperglycemia	Identifying factors involved in obesity-induced diabetes Some models show diabetic complications Treatments to improve beta cell function
	OLETE rat		
	GK rat		Treatments to improve beta cell function and beta cell survival
	Cohen diabetic rat		
Diet/nutrition induced obesity	High fat feeding (mice and rat) Desert gerbil Nile grass rat	Obesity-induced hyperglycemia	Treatments to improve insulin resistance Treatments to improve beta cell function Treatments to prevent diet-induced obesity
	VMH lesioned dietary Obese diabetic rat		Occurrence of hyperphagia Pancreatitis
	Partially pancreatectomized animals (dog, primate, pig and rats)		
Surgical diabetic animals	Uncoupling protein (UCP1) Knock out mice HiAPP mice	Poor activation of thermogenesis Amyloid deposition in islets	Treatments of obese conditions Increase obesity (energy storage) Treatments to prevent amyloid deposition

body^[10,46,113]. What is more important is that, since the brain is a very vulnerable and sensitive organ, the duration and severity of DM might result in serious neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease^[10,70,102]. The HPA axis regulates responses to various stress factors, digestion, immune response, mood and emotions, sexuality and energy expenditure/storage^[11,59,104,132-134]. In addition, since the HPA axis is also connected with the autonomic nervous system^[135,136], it is very important when the

brain orders right responses to diverse physiological conditions. If DM persists and/or is increased without any modification, the brain cannot maintain the normal HPA axis regulation, and the HPA axis based on the negative feedback system tends to be highly activated due to uncontrolled DM. However, sometimes exercise-induced stress might influence the beneficial effects of exercise are not observed in certain behavioral test. It is recognized that the amount of psychological stress that an animal encounters determines the degree of

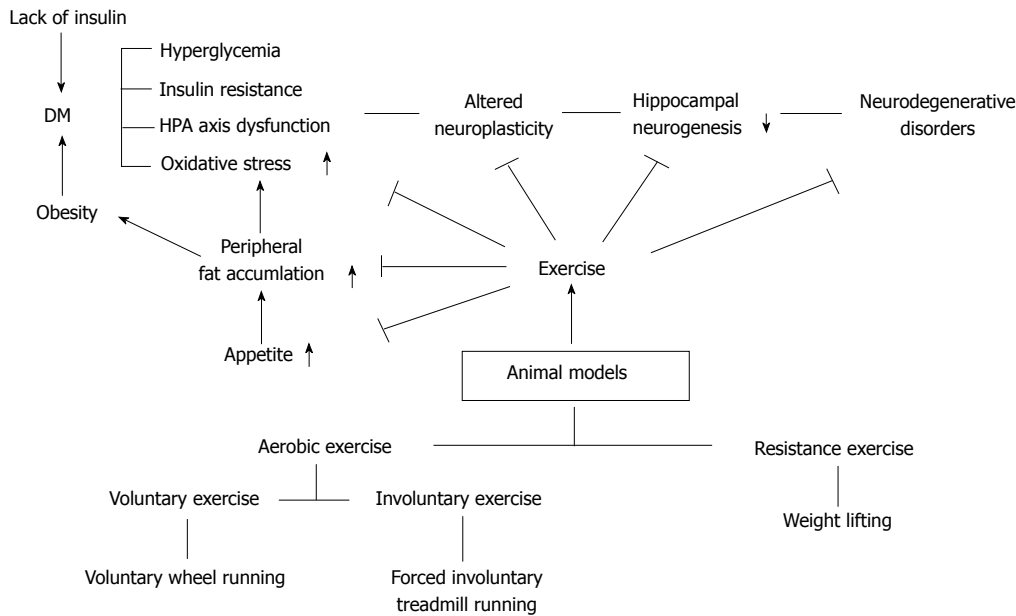


Figure 1 Exercise animal models can separate aerobic voluntary/involuntary exercises and non-aerobic resistance exercise. The exercise can attenuated many neuro-related disease followed DM and obesity. DM can develop many kinds of dysregulation such as hyperglycemia, increasing insulin resistance and HPA axis dysfunction and oxidative stress. Exercise reduces peripheral fat accumulation and appetite in animal models, and it has preventive and therapeutic effects for the many risks to develop obesity and DM. A number of studies about diabetes have been revealed the related mechanisms through exercise animal models. Non-aerobic resistance exercise described in Table 3. DM: Diabetes mellitus; HPA: Hypothalamo-pituitary-adrenal.

response of the HPA axis regulation^[137]. Moreover, it has been reported that animals performing an exercise at the stress-induced physiological and environmental factors can be strongly affected^[59,137-139]. Therefore, there would be enough possibilities to show different behavioral effects under various kinds of stress factors in exercise animal models such as metabolic DM and psychological depression/anxiety disorders. Cayado *et al.*^[137] reported that different training showed different exercise effects at the horse exercise training. Martínez-Mota *et al.*^[138] indicated that the HPA axis response can be different according to sex and age at the exercise animal model. Furthermore, since DM is defined as a chronic systemic inflammatory condition, the disease can contribute to the development of different metabolic disorders. The most significantly affected organs by the chronic inflammatory condition are the vascular converged areas, and thus cardiovascular system is mostly vulnerable and its vascular microenvironment is changed leading to profound damage. Particularly, the occurrence of T2DM is generally characterized by the development of chronic obese condition *via* overnutrition and/or increased hyperphagia^[123,124,133,140,141]. In addition, neuroinflammation and neurodegeneration have been known to be closely related with overnutrition-induced disease and diabetic animal models^[2,4,9,99] (Figure 1).

T2DM is commonly known to be the consequence of chronic obesity and it is usually accompanied by uncontrollable hyperphagia^[142-144]. Many factors contribute to pathologic overeating and mediate feeding behavior in humans and animals, and the most important factor is leptin^[104,145,146]. Leptin, which

is a cytokine originating mainly from white adipose tissue, plays an important role in regulating energy expenditure, food intake, and obesity^[45,71,91,98,104,145,146]. The mechanism by which leptin modulates these hypothalamic neurons involves the binding of leptin to the long form of leptin receptor (Ob-Rb) and subsequent intracellular signaling, initiated by autophosphorylation of Janus kinase 2 (JAK2) and activation of signal transducer and activator of transcription (STAT3). Following the translocation of STAT3 to the nucleus, suppressor of cytokine signaling-3 is activated, exerting feedback inhibition on JAK2. Leptin activation of insulin receptor substrates and the protein kinase B pathway inhibits food intake and modulation of extracellular regulated kinases has been demonstrated to play a role in the control of energy homeostasis^[147]. Obese patients and animals cannot regulate their hedonic appetite except for acceptable daily intake of calories. They have excessive WAT in the body and it secretes leptin in the blood; however, the appetite center does not recognize leptin and shows resistance to leptin. Therefore, leptin administration to obese rats and humans has elicited small effects on fat mass and appetite due to leptin resistance^[2,53,148]. Likewise, many neuropeptides located in hypothalamic nuclei transmit related anorexigenic or orexigenic signals^[104,146]. Furthermore, many kinds of neurotransmitters such as serotonin, dopamine, and norepinephrine participate in regulation of mood, emotions, and appetite^[149]. Particularly, specific serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) have been introduced and used as medical treatments to reduce food intake of overweight patients^[150-153]. These

drugs have been modified continuously to overcome their side effects or toxicities^[154]; however, chronic administration of SSRIs or SNRIs causes rebound body weight gain in the patients. The phenomenon has been observed for a while; however, the cause was not the patients' psychological drug dependence, and same results have been obtained in many studies performing animal experiments^[155,156]. Since these agents not only influence the appetite center but also the other areas in the brain, the environment of the brain gets affected^[157-159]. Therefore, more specific target regions in the brain and promising regulatory drugs are required. Finally, failure of appetite control can easily lead to T2DM, and then the continuous progression of this metabolic disorder harms the neuroenvironment of the brain and affects learning/memory and cognitive functions^[47,49,85,87,160]. According to the severity of DM, hippocampal neurogenesis in the dentate gyrus is significantly reduced and neuronal plasticity is also negatively influenced through reduction in neurotrophic factors^[31,130,160]. Uncontrolled DM with hyperglycemia can cause serious brain damage; therefore, appropriate therapies that can slow the progression of this disease are needed.

DM and peripheral nervous system

Diabetic neuropathy affecting the peripheral nervous and autonomic nervous systems is the most frequent complication of diabetes. The most common neuropathies are chronic sensorimotor distal polyneuropathy (DPN) and autonomic neuropathies^[161]. Morphologically, DPN is characterized by alterations in peripheral nerve fibers as well as degeneration and regeneration of both myelinated and unmyelinated fibers in humans, decreased axonal diameter of the sciatic nerve and myelin sheath thickness of the sural nerve, and alteration of the cytoskeletal component in dorsal root ganglia of rats^[162-164].

Currently, human life span has dramatically increased due to advances in medical science, and moreover, improving the quality of life has also received attention. Therefore, people are feeling the need to maintain their brain health throughout their life. However, DM poses a threat to the health of people and therefore it has become a problem that needs to be conquered.

EFFECTS OF EXERCISE ON THE BRAIN OF DIABETIC ANIMALS

It is already well known that regular physical activity has a tremendous impact on health and has protective effects against chronic diseases, including heart disease, stroke, hypertension, and DM. Over several decades, many evidences have demonstrated that exercise in human and animal models helps to maintain brain health such as cognitive performance, and it can even protect the central nervous system and improve

learning/memory functions following chronic exercise, both in animal models and humans^[59,61,104,165-171]. In recent years, many exercise and cognition studies have been carried out in adult rodents. These researches have provided insights into the underlying cellular mechanisms^[169,172]. Both voluntary and forced exercise enhanced spatial memory in Morris water maze, Y-maze, T-maze, and radial arm maze test^[65,169]. Particularly, running exercise improved performance in hippocampus-dependent tasks that require limited movement, and there were non-hippocampal dependent benefits from voluntary and forced exercise. Chronic involuntary treadmill exercise in an T2DM animal model (ZDF rats) reduced blood glucose levels, caused cell proliferation and an increase in neuroblasts in the hippocampal dentate gyrus; however, the onset of treadmill exercise in the severe chronic diabetic condition has a limitation in increasing neuroblast differentiation although it increases neural plasticity^[77,80]. Therefore, for achieving effectiveness of treadmill exercise in increasing neuronal differentiation in the hippocampus and for counteracting the negative effect of DM in the brain, the initiation time of exercise during the early stage of DM may be a very critical point to achieve the positive effects of exercise^[77]. Furthermore, Hwang *et al*^[77,173] reported that Cox-2 is very important factor for hippocampal neurogenesis in the T2DM animal exercise models. Griffin *et al*^[167] reported that voluntary exercise also increased volume of the hippocampus resulting in improved search strategies and decreased perseveration once the platform had been moved to a new location. Voluntary exercise results in elevation of levels of factors such as brain-derived neurotrophic factor (BDNF), whose levels increases with aerobic exercise, and enhances hippocampal function^[167].

Interestingly, Burghardt *et al*^[174] studied the behavioral effects of voluntary and involuntary running exercise with a battery of behavioral tests; they investigated the effects of 8 wk of forced treadmill running and voluntary wheel running on behavior measures in the elevated plus maze, open field, social interaction and conditioned freezing paradigms. They found that chronic voluntary running produces behavioral changes in the elevated plus maze and open field; however, chronic treadmill running failed to produce behavioral changes with their running protocol. Changes in opioidergic^[175], serotonergic^[176], GABAergic^[177], and catecholaminergic^[178] systems have also been observed after wheel running. Regular running exercise is closely associated with food preference and appetite depending on the volitional wheel running and involuntary treadmill exercise. Recently, attention has been paid to various causes of food preference and consumption according to a wide range of conditions for overcoming the obese and DM conditions^[179]. Diet composition may lead to changes in neuropeptides within brain nuclei regulating energy metabolism. Dietary manipulation has been thought to influence energy expenditure *via* changes in

central neuropeptide activity. Many studies report that medicines such as morphine, fenfluramine influence the neuro-regulatory systems and exercise can modify palatability in animals^[175]. Blundell *et al*^[37] asserted that changes in dietary preferences could be due to alterations in the hedonic properties of the food as a result of exercise in rodent models. Shin *et al*^[144] also indicated a possibility that treadmill exercise in animals inhibits diabetes-induced increment of the desire for food. Hormonal (leptin and insulin) and nutrient signals from the periphery are mainly integrated in the hypothalamus, and multiple factors regulate food intake. AMP-activated protein kinase (AMPK) is the downstream component of a kinase cascade that acts as a sensor of cellular energy charge, being activated by rising AMP coupled with falling ATP^[180]. Although the effects of AMPK on desire for food are still controversial, exercise may contribute to appetite suppressive actions in the hypothalamus due to the effects of leptin and in different causes in the rodent model^[147,179-182]. As mentioned above, alterations in opioid or inhibitory neurotransmission systems in both limbic and brainstem areas could be implicated, including the nucleus accumbens^[183]. Multiple mechanisms of action in the brain could be responsible for this behavioral difference and lack of gross metabolic difference^[171]. In humans, texture, temperature, color, and appearance all play a role in food acceptance^[184,185]; however, animals exhibit a wide range of food preferences and animal studies can eliminate the points of dispute in human studies. In addition, an important element in the study of effects of exercise on food preference is sex differences^[171,175,179]. Sex differences exist such that female rats tend to prefer carbohydrates over other macronutrients following exercise^[134,175]. Unfortunately, there is still no clear evidence on the effect of exercise on macronutrient or carbohydrate selection in different sexes in animal or human studies. Therefore, further research for assessing the sex differences in food preference after exercise is needed.

Chronic inflammation and increased oxidative stress are observed in the animals showing insulin resistance following diet-induced obesity^[36,44,45,62,131,186,187]. Indeed, since the brain tissue is highly sensitive to chronic inflammation and oxidative stress due to its high oxygen consumption, iron and lipid contents, and low activity of antioxidant defenses^[102,188], energy metabolism impairment and oxidative stress are important events that have been related to the pathogenesis of diseases affecting the central nervous system^[47,180]. Exercise has been known to decrease chronic systemic inflammatory response, show antioxidant effects and positive effects on synaptic plasticity in the obese and/or diabetic rodents^[55,60]. In the T1DM animal model, significant inflammatory responses are found and they showed different action in a time-dependent manner^[113]. These responses induced by DM lead to mitochondrial dysfunction, which can progress to various pathologies such as neurodegenerative diseases (dementia,

Alzheimer's disease, Parkinson's disease)^[33,34,47,61]. Both T2DM and neurodegenerative diseases are associated with impaired glucose tolerance and cognitive decline in the human and animal studies, and insulin resistance and subsequent hyperinsulinaemia have been found to increase the risk of Alzheimer's disease and promote decline in memory and cognitive dysfunction^[3,34,61,133,189]. Regular exercise and dietary restriction can attenuate the progression of metabolic and neurodegenerative disorders^[4,5,67,190]. Exercise (particularly vigorous aerobic exercise)^[111,167,191-193] and energy restriction (caloric restriction and intermittent fasting)^[143,194,195] can result in striking improvements in glucose and lipid metabolism, and can eliminate the need for medications. Exercise and dietary energy restriction activate a wide range of adaptive cellular responses in the peripheral organs (muscle, liver) and the brain, resulting in improved bioenergetics and brain function, and resistance to neurodegenerative disorders.

As mentioned previously, the causes of DM belong to different metabolic conditions and can show diverse pathologic phenotypes in a time-dependent manner^[113]. This review mainly focused on the changes in the brain caused by DM and exercise; however, changes in peripheral neuropeptides and organs are also significant. Adiposity, chronic inflammatory response, activation of oxidative stress, dysfunction of pancreatic islets, insulin and leptin resistance, dysfunction of the negative feedback mechanisms, and appetite disturbance constantly affect brain homeostasis. Indeed, exercise has been thought to attenuate brain damage caused by these risk factors; however, exercise during the early stage of diabetes is considered to be a critical factor for preserving brain function^[10,80]. The risk factors listed above can be therapeutic targets to treat and ameliorate DM; thus, refinements using various animal exercise models can give new insights into the treatment of DM.

It is well accepted that physical activity by contracting skeletal muscles (resistance exercise) secretes enhanced levels of myokines which have a beneficial endocrine effect on other organs, presenting novel targets for the treatment of metabolic diseases and T2DM^[70-72,94]. Pedersen hypothesized that physical inactivity leads to T2DM, depression, dementia, cancers, cardiovascular diseases, and asserted that skeletal muscle should be considered as an endocrine organ^[70]. Cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert paracrine or endocrine effects should be classified as myokines. Actually, since skeletal muscle is the largest organ in the human body, skeletal muscle should receive attention for identifying its new multiple functions in metabolic disorders and T2DM. Skeletal muscle has the capacity to express several myokines including IL-6, IL-8, IL-15, BDNF, FGF21, MCP-1, vascular endothelial growth factor, leukemia inhibitory factor, Irisin, and ANGPTL4^[71]. IL-6 was discovered as a myokine because of the observation that it increases

Table 3 Exercise animal models on brain function

Exercise type	Method	Measurement	Note ¹	Note ²
Aerobic exercise	Voluntary running	Freely access to running wheel		Cognitive performance
	wheel exercise	Exercise strength can be measured <i>via</i> digital counter. The running wheel was rotated by animal effort		Neurogenesis in subgranular zone or subventricular zone
	Involuntary treadmill exercise	Enforced running exercise Regularly enforced running exercise is enforced with constant speed on a motorized treadmill		Improvements of learning and memory
	Forced swimming	Animals are forced to swim in an acrylic glass cylinder filled with water	This test is used to see a rodent's response to the threat of drowning whose result has been interpreted as measuring susceptibility to negative mood. It is commonly used to measure the effectiveness of antidepressants	Neurophysiological development
Non-aerobic resistance exercise	Weight lifting	Kondziela's inverted screen test	The inverted screen is a 43 cm square of wire mesh consisting of 12 mm squares of 1 mm diameter wire	Relationship between Brain and Stress axis
		Weights test	Seven weights constitute the apparatus Ranging from 20 to 98 g	Feeding behavior
		Grip strength test	Forelimb grip strength is accessed using a digital Grip Strength Meter	

¹Principal; ²Uses.

up to 100-fold in the circulation during exercise. In particular, the identification of IL-6 production by skeletal muscle during physical exercise generated renewed interest in the metabolic role of IL-6 since it created a paradox^[70]. IL-6 can also alter brain function after peripheral administration, moreover, some myokines might be able to cross the blood-brain barrier^[196,197]. IL-6 is significantly produced and released in the post exercise period when insulin action is increased; on the other hand, IL-6 has also been associated with obesity and reduced insulin action. However, many researches during the past decade have reported that in response to muscle contraction, both type 1 and type 2 muscle fibers express the myokine IL-6, which subsequently exerts its effects locally and systemically in several organs^[70-72]. Within skeletal muscle, IL-6 acts to signal *via* AMPK and/or PI3-kinase to enhance glucose uptake and fat oxidation. In addition, muscular derived IL-6 mediates anti-inflammatory responses^[70].

A few researches on the relationship between myokines and the brain in animal models have just been published, and the effects of skeletal muscle derived myokines on brain function must be plausible enough directly and/or indirectly. Recently, Dun *et al*^[198] reported that myokine Irisin was detected in three types of cells; skeletal muscle cells, cardiomyocytes, and Purkinje cells of the cerebellum. Moreover, they reported that Irisin not only mediates the animal's movements but also regulates adipose tissue thermogenesis by neurons in the caudal ventrolateral medulla and rostral ventrolateral medulla that are an integral component of the medullary sympathetic circuitry and these neurons project their axons to spinal sympathetic premotor neurons^[198]. Similarly, it is known that resistance exercise improves body and brain bioenergetics for PD

risk reduction^[4], insulin and leptin signaling in obese rats^[2,45,82,104,199], and exerts antidepressant-like effects *via* improving the impaired neuroplasticity^[101,200]. Aerobic exercise and non-aerobic resistance exercise described in the Table 3.

Evidences of positive effects of resistance exercise on brain health in T2DM for therapeutic purposes with other aerobic exercises and pharmacologic treatments have been reported recently, and further studies on the mechanisms of treatment according to the severity of DM are needed.

CONCLUSION

It is confirmed that exercise is an incredible therapeutic option for treating DM patients. Animal exercise models are significant methods to study the network between central and peripheral organs. Brain is an extremely sensitive and soft tissue that can be damaged due to chronic insulin resistance, hyperglycemia, and chronic inflammation; however, various kinds of exercise can attenuate the brain damage and delay neurodegeneration caused by the risk factors. Many diabetic experimental animals with a genetic background and nutrition induced diabetic animals can be used in various DM studies; however, many physiopathologic conditions should be considered, and researchers should choose the animal models after giving careful consideration. Many aerobic running exercises and resistance skeletal muscle exercises have been performed recently in various animal models to study their therapeutic effect on brain function; however, more careful considerations reflecting the clinical conditions should be added in the animal models. Furthermore, it is important to study the therapeutic effects of exercise on brain health during

the stages of DM in animal models; however, dramatic effects of more prospective methods for maintaining brain health during DM seem to be achieved through development of various combinations of animal models in the pre-diabetic condition. A number of target signals from the exercise studies can also be the candidates for development of pharmacologic medicines.

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Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition

Iqra Hameed, Shariq R Masoodi, Shahnaz A Mir, Mudasar Nabi, Khalid Ghazanfar, Bashir A Ganai

Iqra Hameed, Mudasar Nabi, Khalid Ghazanfar, Department of Biochemistry, University of Kashmir, Srinagar 190006, India
 Shariq R Masoodi, Shahnaz A Mir, Department of Endocrinology, Sher-I-Kashmir Institute of medical Sciences, Srinagar 190006, India

Shariq R Masoodi, Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD 21201, United States

Bashir A Ganai, Centre for Research and Development, University of Kashmir, Hazratbal, Srinagar 190006, India

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Correspondence to: Dr. Bashir A Ganai, Professor, Director, Centre for Research and Development, University of Kashmir, Hazratbal Rd, Hazratbal, Srinagar 190006, India. bbcganai@gmail.com

Telephone: +91-979-7247851

Fax: +91-194-2415357

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target tissues and a relative deficiency of insulin secretion from pancreatic β -cells are the major features of type 2 diabetes (T2D). Chronic low-grade inflammation in T2D has given an impetus to the field of immuno-metabolism linking inflammation to insulin resistance and β -cell dysfunction. Many factors advocate a causal link between metabolic stress and inflammation. Numerous cellular factors trigger inflammatory signalling cascades, and as a result T2D is at the moment considered an inflammatory disorder triggered by disordered metabolism. Cellular mechanisms like activation of Toll-like receptors, Endoplasmic Reticulum stress, and inflammasome activation are related to the nutrient excess linking pathogenesis and progression of T2D with inflammation. This paper aims to systematically review the metabolic profile and role of various inflammatory pathways in T2D by capturing relevant evidence from various sources. The perspectives include suggestions for the development of therapies involving the shift from metabolic stress to homeostasis that would favour insulin sensitivity and survival of pancreatic β -cells in T2D.

Key words: Diabetes mellitus; Inflammation; Insulin resistance; β -cell dysfunction; Adipose tissue

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Core tip: Immuno-metabolism, the confluence of metabolism and immune system has emerged as a chief breakthrough especially in the field of diabetes mellitus; a metabolic disorder of great magnitude. Activation of immune system by metabolic stress has opened new insights in the pathogenesis and progression of type 2 diabetes (T2D). The link between metabolic overload and activation of the immune system form the core tip of this review. Metabolic stress can cause pathologic activation of the immune system, thus metabolic disorders like T2D manifest and progress as an inflammatory disorder with severe consequences thereof.

Abstract

Diabetes mellitus is increasing at an alarming rate and has become a global challenge. Insulin resistance in

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INTRODUCTION

Diabetes mellitus, a life style disease affecting 8.3% of the adult population of the world and increasing at an alarming rate, is one of the most common non-communicable diseases of current era^[1]. The burden of this disease is immense owing to transition in lifestyle and dietary habits, ageing of the population and urbanization in the setting of a genetically predisposed environment^[2]. The fact that the number of subjects with diabetes mellitus has doubled over the past three decades has made this disease a global challenge^[3]. The number of diabetes mellitus patients is projected to increase from 382 million in 2013 to 592 million by 2035, denoting a net increase of 55%^[1]. The predominant form is type 2 diabetes (T2D) which accounts for nearly 90% of all diabetes cases.

Diabetes mellitus-not so sweet

T2D is a metabolic disorder characterized by insulin resistance and pancreatic β -cell dysfunction as a consequence of unsettled hyperglycemia^[4,5]. In response to nutrient spill over in the setting of insulin resistance and eventual β -cell dysfunction, the general fuel homeostasis of body is altered^[2]. Insulin resistance in target tissues and a relative deficiency of insulin secretion from pancreatic β -cells are the major features of T2D. β -cell hyperplasia and hyperinsulinaemia in response to insulin resistance occur in the preclinical period of disease. Relative insulin deficiency as a consequence of failure of β -cells to compensate for insulin resistance, progresses into overt T2D^[6].

Metabolic alterations associated with T2D are well characterised by epidemiological and research based studies. The pathogenesis and progression of T2D is ascribed to four mechanisms; increased advanced glycation end product (AGE) formation, increased polyol pathway flux, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway flux^[7]. Till recently no common linking element was apparent for these mechanisms: however, recently production of superoxide emerged as a unifying mechanism for these four pathways. Downstream to oxidative stress, activation of inflammatory pathways has emerged as an imperative link between T2D and inflammation. Since, abundant data have elucidated the role of oxidative stress in T2D pathogenesis. In this review, we will evaluate the inflammatory component of T2D and underscore the link between metabolic alterations in T2D and inflammation.

T2D AS AN INFLAMMATORY CONDITION

Studies investigating the relation between inflammation and T2D have coalesced sufficient data implicating the role of inflammation towards the development of insulin resistance and pathogenesis of T2D^[8,9]. Metabolism and immune system were conventionally regarded as two distinctive mechanisms governing nutrient disposal and body defense, respectively. Typically, little was known about the coordination and interplay between these two systems. However, present research has led to combining these distinct entities as studies perceive pathological activation of the immune system as a regulatory mechanism associated with multiple disorders underlying the metabolic syndrome^[10]. Potency of steroid hormones as immune suppressors and hyperglycemic inductors, metabolic alterations associated with pyrexia, wasting syndrome initiated by chronic infections and of late, markers of acute-phase response have been associated with insulin resistance, insulin secretion defects, T2D and vascular complications of T2D^[8,11-15].

T2D encompasses colossal cellular factors characteristic of triggering inflammatory signalling cascades. A detailed analysis of these molecules cannot be underscored in this review, however their particular roles in T2D has been outlined in Table 1. Consequently, T2D at the moment is considered an inflammatory disorder triggered by disordered metabolism^[16]. The probable history of diabetes involves a more or less latent prodromal period followed by progressive deterioration of glucose tolerance culminating into explicit disease. Progression of islet β -cell failure results in hypertrophy of pancreatic islets and proliferation of β -cells. This phase is associated with an inflammatory response precipitating into reduction of cells by apoptosis and fibrosis of islets. In fact, an analogy of sequence of events involving an incipient inflammatory phase is associated with other T2D complications also^[17]. Hyperglycemia is regarded as the major upstream mechanism, and micro-inflammation is regarded as the subsequent downstream driving force of diabetes related complications^[17]. Epidemiological data advocate that markers of inflammation are predictive of T2D^[18]. The role of inflammation in insulin resistance is traced by the integration of metabolism and innate immunity *via* nutrient-sensing pathways mutual to pathogen-sensing pathways. Components of nutrition (free fatty acids, glucose, and amino acids) signal through collective receptors and pathways in a similar way as pathogens and/or cytokines. Cells of the immune system (macrophages) and metabolism (adipocytes) also share many functions like secretion of cytokines, and trans-differentiation into macrophages. Nutrients can activate macrophages and adipocytes through common receptors, such as toll-like receptors (TLRs) that sense broad classes of molecular structures common to pathogen groups, and are central to innate

Table 1 Role of various inflammatory molecules in type 2 diabetes

Category	Molecule	Role
Pro-inflammatory cytokines and signaling molecules	TNF- α	Increased levels related to IR and T2D
		Reduces insulin sensitivity by influencing the phosphorylation state of the insulin receptor
	IL-6	Major pro-inflammatory cytokine that induces inflammation and IR leading to T2D
	CRP	Elevated serum CRP associated with the incidence of T2D
	IL-1	Associated with obesity and IR
Transcription factors		Affects insulin signaling directly through the induction of SOCS-3
	IL-8	Leads to IR <i>via</i> the inhibition of insulin-induced Akt phosphorylation in adipocytes
	IL-1 β	Mediates auto-inflammatory process resulting in β -cell death
	NF- κ B	Increase the expression of genes encoding cytokines, chemokines, transcription factors and various receptors involved in IR and pathogenesis of T2D
	JNK	Promotes IR through phosphorylation of serine residues in IRS-1
Adipokines	IKK β	Leads to IR through transcriptional activation of NF- κ B
	Leptin	High leptin levels, reflecting leptin resistance predict increased risk of T2D
	Adiponectin	Low levels of this protective adipokine correlate with T2D. Adiponectin is downregulated by TNF- α
	Resistin	Promotes IR and decreases insulin-stimulated glucose transporters in adipose tissue
	Adipsin	Role in maintaining β cell function
Chemokines		Lower levels of adipsin found in T2D patient
	Visfatin	Visfatin binds to the insulin receptor at a site distinct from that of insulin and causes hypoglycaemia by reducing glucose release from liver cells and stimulating glucose utilization in adipocytes and myocytes
	MCP-1	MCP-1 expression in adipose tissue contributes to the macrophage infiltration into this tissue, IR and T2D
	IP-10/CXCL10	Downstream effector of pro-inflammatory cytokines involved in T2D-related complications
	CCR2	Imitates tissue inflammation and IR
Toll like receptor	TLR2 and TLR4	TLR2 and TLR4 play a critical role in the pathogenesis of IR and T2D
Adhesion molecules	E-selectin/P-selectin	Lead to leukocyte recruitment in local tissue and contributes to inflammation, IR and T2D
	ICAM-1/VCAM-1	Alters endothelial and sub-endothelial structure leading to reduced vascular permeability, reduced insulin delivery to peripheral insulin sensitive tissues and ultimately T2D
Nuclear receptors	PPAR α , PPAR γ , and PPAR β/δ	Mutations in PPAR genes associated with IR and T2D
	VDR	Regulates expression of insulin receptor preferentially by binding as a heterodimer with the RXR to VDREs in the promoter regions of insulin receptor gene

IR: Insulin resistance; CRP: C-reactive protein; T2D: Type 2 diabetes; SOCS-3: Suppressor of cytokine-3 signalling; NF- κ B: Nuclear factor κ B; JNK: c-Jun NH2-terminal kinase; IRS-1: Insulin receptor substrate; IKK β : Inhibitor of nuclear factor κ B kinase subunit β ; MCP: Monocyte chemoattractant protein-1; IP-10: Interferon gamma-induced protein 10; CXCL10: Chemokine (C-X-C motif) ligand 10; CCR2: Chemokine (C-C) motif receptor 2; ICAM-1: Intracellular adhesion molecule 1; VCAM-1: Vascular cell adhesion molecule 1; PPAR: Peroxisome proliferator activated receptor; VDR: Vitamin D receptor; RXR: Retinoid X receptor; VDREs: Vitamin D response elements.

immunity and inflammation.

ADIPOSE TISSUE AS A SITE OF INFLAMMATION

Clinical and experimental studies show that adipose tissue acts as a site of inflammation. The first insight came from the study on adipose tissue of obese mice exhibiting elevated production of TNF- α ^[11]. Consequently, increase in adiposity is associated with upregulation of genes encoding pro-inflammatory molecules and associated with accumulation of immune cells^[19-21]. Adipocytes hoard excessive nutrient load and become hypertrophic gradually. Events initiating a pro-inflammatory response involve synergistic contributions of various mechanisms like an increase in nuclear factor κ B (NF- κ B) and c-Jun NH2-terminal kinase (JNK) activity by hypertrophied adipocytes, endoplasmic reticulum (ER) stress causing altered unfolded protein response (UPR), hypoxic stress in adipose tissue, activation of TLR by excess free fatty acids (FFAs), or increased chylomicron-mediated transport from the gut lumen into the circulation in a lipid-rich diet^[16,22,23].

Stressed adipocytes produce various cytokines and chemokines promoting immune-cell activation and accumulation in adipose tissue^[24]. A pro-inflammatory loop is formed by several macrophages by clustering around adipocytes, particularly with dead adipocytes forming crown-like structures^[19,21,25]. Sustained accumulation of lipids in adipose tissues results in switching of macrophages from an anti-inflammatory "M2" (alternatively activated) to a pro-inflammatory "M1" (classically activated) phenotype^[19,21,26,27]. The skew in balance results in an increased secretion of inflammatory molecules that subsequently stimulate the hypertrophied adipocytes resulting into a pro-inflammatory response^[28]. The inflammatory response in macrophages is induced by adipocyte-derived FFAs *via* TLR or NOD-like receptor family, the pyrin domain containing 3 (NLRP3) dependent pathways^[29,30]. Local hypoxia as a result of vasculature insufficiency in hypertrophied adipocytes has been proposed to stimulate expression of inflammatory genes in adipocytes as well as immune cells^[31]. However, the hypothesis lacks confirmation in the situation of human obesity^[32]. Instead, mechanisms like ER stress and

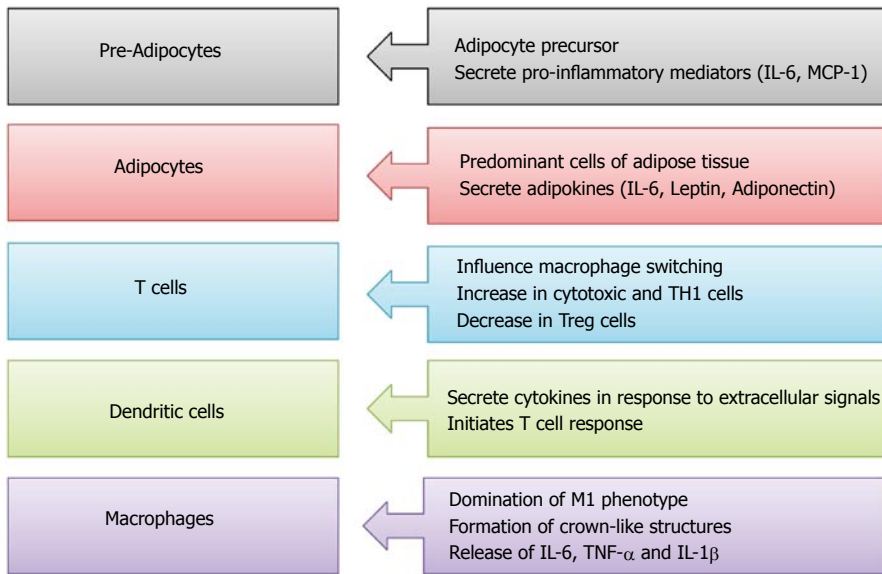


Figure 1 Functions of various immune cell types in pathogenesis of type 2 diabetes. IL: Interleukin; MCP-1: Monocyte chemoattractant protein-1; TNF- α : Tumor necrosis factor α .

autophagocytosis have been proposed as origin of local inflammatory signalling pathways in adipose tissue^[22,33]. Recently, the role of the incretin hormone glucose-dependent insulintropic peptide has also been implicated^[34,35]. In addition to adipose tissue, a pro-inflammatory state in liver and skeletal muscle result in disruption of systemic insulin sensitivity and glucose homeostasis that are characteristic of T2D^[36-38].

Metabolic inflammation is regulated by critical orchestration of innate and adaptive immune cell interactions^[39,40]. Studies investigating immuno-metabolism have recognised that the inflammatory status of immune cells is dictated by their metabolic programming, mitigating the progression of T2D. T2D is preceded by an extensive period of disease development, and inflammation has been shown to be a precipitating factor underpinning insulin resistance, preceding T2D^[41,42]. The progression of T2D involves an intricate interplay between metabolism and immunity. The progression of T2D has been causally linked to various types of immune cells but the primary sources of inflammatory effectors contributing to insulin resistance are macrophages^[43-45]. Among various cell types, pre-adipocytes, adipocytes, T cells, dendritic cells and macrophages are major cell types involved in obesity-induced inflammation and insulin resistance^[46]. Their prime functions are shown in Figure 1. The key inducers of cytokine release in metabolic organs leading to impaired insulin action are tissue-resident macrophages^[47].

Nutrient overload corresponds to increased infiltration of macrophages in metabolic tissues promoting a pro-inflammatory environment characterised by augmented TNF- α , IL-1 β and inducible nitric oxide synthase (iNOS) levels. The accrual of these pro-inflammatory macrophages in metabolic organs like liver, adipose tissue and muscle directly suppresses

insulin action, thereby promoting hyperglycemia^[48].

ROLE OF INFLAMMATION IN INSULIN RESISTANCE

Insulin is a key endocrine hormone produced by β -cells of pancreatic islets. Insulin is regarded as "hormone of abundance" owing to the array of functions it performs, the effects of which extend from metabolic to mitogenic activity (Figure 2). It is likely that disruption of insulin-mediated pathways will have pleiotropic effects that are not confined to carbohydrate metabolism only. Various mechanism working separately or in synergy have been linked to the development of insulin resistance among which chronic inflammation represents as a triggering point^[8].

Inflammation is an important component linking insulin resistance with nutrient overload and increased visceral adipocyte mass^[42]. During an insulin-sensitive state, the signalling cascade of insulin upon binding to its receptor results in phosphorylation of tyrosine residues of the insulin receptor substrate 1 (IRS-1) ensuing in downstream insulin signalling^[49]. However, in an insulin-resistance state, pro-inflammatory molecules activate various other serine kinases like JNK, inhibitor of NF κ B kinase subunit β (IKK- β), extracellular-signal regulated kinase (ERK), ribosomal protein S6 kinase (S6K), mammalian target of rapamycin (mTOR), PKC and glycogen synthase kinase 3 β ^[50]. The activation of these kinases inhibits insulin action by phosphorylating serine residues instead of tyrosine residues in the insulin signalling pathway^[49].

The development of insulin resistance is linked to two prime transcription factor-signalling pathways: JNK and IKK β /NF- κ B^[51]. Activation of these two pathways involves a series of proinflammatory stimuli, many of which comprise of both activators and upregulators of

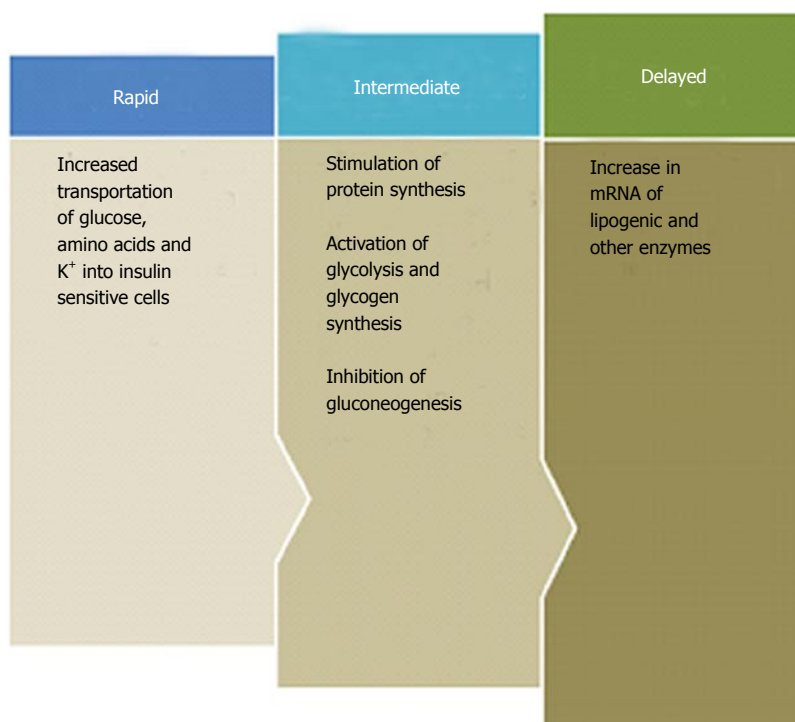


Figure 2 Various hormone functions of insulin.

NF- κ B. In addition, these pathways are also activated by pattern recognition receptors like TLRs and receptors for advanced glycation end products (RAGE). Elevated levels of FFAs result in an increase in diacylglycerol (DAG) that activates PKC isoforms leading to concomitant activation of JNK and NF- κ B pathways^[52]. Further stimuli involve production of reactive oxygen species (ROS), ER stress and changes in adiposity^[53-55].

The mechanisms in development of inflammation-induced insulin resistance are different for JNK and IKK β . Unlike JNK that phosphorylates the serine residues of IRS-1, IKK β induces insulin resistance by transcriptional activation of NF- κ B^[56-59]. The physiological substrates of IKK β are I κ B protein inhibitors of NF- κ B. IKK β phosphorylation promotes proteosomal degradation of I κ B α liberating NF- κ B for nuclear translocation where it stimulates the expression of several target genes (Figure 3)^[9]. The products of these target genes of NF- κ B induce insulin resistance. The production of inflammatory molecules further activates JNK and NF- κ B pathways promoting a vicious loop of insulin resistance by feed-forward mechanism.

PANCREATIC ISLET INFLAMMATION IN T2D

Increasing evidence suggests the presence of an inflammatory milieu in pancreatic islets in T2D, such as increased cytokine levels, chemokine levels and immune cell infiltration. Evidence of islet inflammation was initially observed in hyperglycemia induced β -cell apoptosis^[60]. Recent studies on human islets

and monocytes have shown that the combination of hyperglycemia and elevated FFAs induces a more efficient pro-inflammatory phenotype^[61,62]. Various T2D experimental animal models like db/db mice and Goto-Kakasaki rats showed increased infiltration by immune cells in the pancreatic islets^[63]. Studies on experimental animal models elucidated islet inflammation and macrophage infiltration as an event occurring as early as eight weeks before the onset of frank diabetes^[63]. Recruitment of macrophages is a consequence of phagocytic clearance owing to the death of islet β -cells^[64]. Alternately, in a diabetic milieu endocrine cell-derived inflammatory molecules like IL-6 and IL-8 produced in islets are also attributed to increased macrophage infiltration^[63]. Production of pro-inflammatory cytokines and secretion of chemokines by β -cells results in a vicious cycle speeding up islet inflammation. In humans, IL-1 β secreted by infiltrating immune cells is related to the pathogenic process of T2D, as blockade of IL-1 has been associated with reduced hyperglycemia, improved β -cell function and reduced expression of inflammatory markers^[65]. However, recent studies involving human islets have shown that induction of IL-1 β plays a role in precipitating the clinical features of diabetes and is unlikely involved in initial pathogenesis^[66-68]. The first study demonstrating the hyperglycemia-induced IL-1 β secretion documented a pro-inflammatory response induced by a non-autoimmune mechanism in β -cells^[12]. *Ex vivo* experiments on isolated human islets exposed to high glucose levels showed increased IL-1 β production preceding activation of NF- κ B, upregulation of Fas, fragmentation of DNA, and reduction

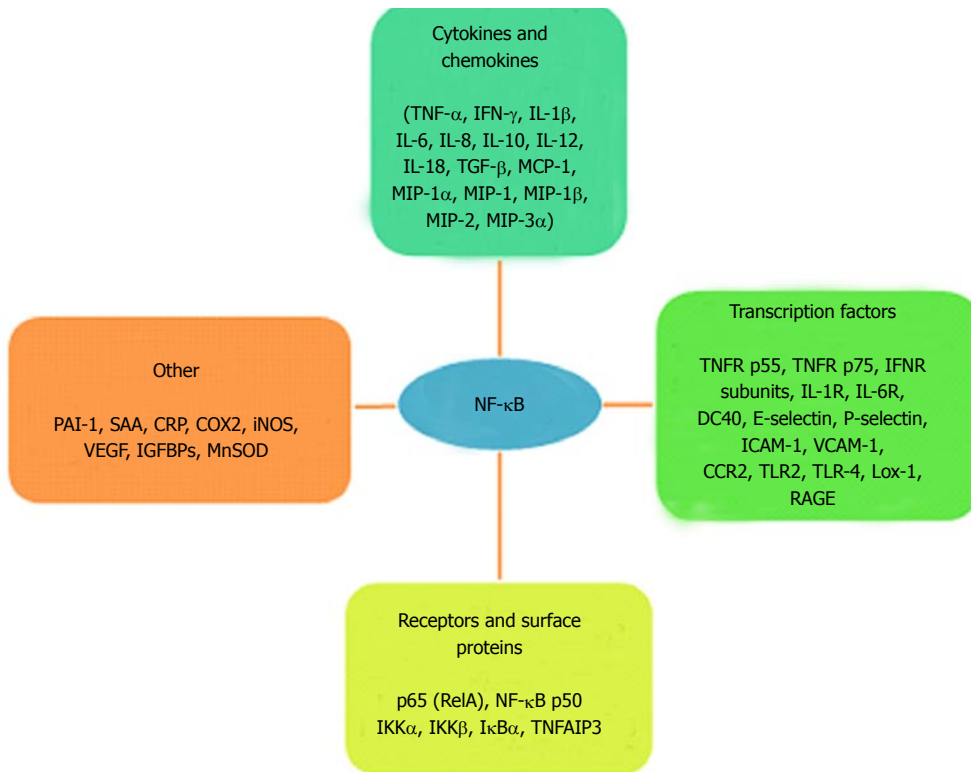


Figure 3 Target genes activated by NF- κ B. TNF- α : Tumor necrosis factor-alpha; IFN- γ : Interferon-gamma; IL: Interleukin; TGF- β : Tumor growth factor-beta; MCP-1: Monocyte chemoattractant protein-1; MIP: Major intrinsic protein; TNFR: Tumor necrosis factor receptor; INFR: Interferon receptor; IL-R: Interleukin receptor; CD: Cluster of differentiation; ICAM: Intracellular cell adhesion molecule; VCAM: Vascular cell adhesion molecule; CCR: Chemokine CC receptor; TLR: Toll-like receptor; Lox: Lysyl oxidase; RAGE: Receptor advanced glycation end product; PAI: Plasminogen inhibitor activator; SAA: Serum amyloid; CRP: C-reactive protein; COX: Cyclo-oxygenase; iNOS: Inducible nitric oxide synthase; VEGF: Vascular endothelial growth factor; IGFBPs: Insulin-like growth factor binding protein; MnSOD: Manganese superoxide dismutase; RelA: Reticuloendotheliosis viral oncogene homolog A; NF- κ B: Nuclear factor-kappa B; IKK: Inhibitor Kappa B kinase; I κ B α : Inhibitor of NF- κ B; TNFAIP3: TNF- α induced protein 3.

of insulin secretion^[69]. Upregulation of IL-1 β plays a predominant role as a major cytokine regulating other chemokines and cytokines in islets of T2D patients^[12,66,70,71]. This master cytokine elicits a broader response by recruitment of various immune cells and also by induction of IL-1 β in β -cells, provoking a vicious inflammatory cycle^[66]. The critical role of IL-1 β in islet inflammation was recently confirmed by analysing global gene expression in pancreatic islets of humans that showed an association of a group of co-expressed modules enriched for IL-1 related genes with T2D and insulin resistance^[72]. *SFRP4* gene encoding the secreted frizzled-related protein 4 was one of the interesting genes that were overexpressed, likely mediating the effect of IL-1 β on islets^[72]. In islets of both T2D subjects as well as in animal models, an eminent number of immune cells along with cytokines and chemokines has been observed^[63,66,73]. In fact, T2D animal models invariably exhibit islet immune cell infiltration^[63,71].

ISLET INFLAMMATION AND β -CELL DEATH

Islet tissue sections of T2D subjects show well-defined fibrosis which is a hallmark of the late stage of a chronic inflammatory process. In clinically overt T2D subjects

a decreased β -cell mass has been reported indicating a probable role in its pathogenesis^[4,74]. Decreased β -cell mass in T2D has been attributed to pancreatic β -cell apoptosis and to β -cell dedifferentiation^[75]. In slowly progressing T2D, the probability of detecting β -cell damage in pancreatic sections is low, thus very few studies on this aspect have been reported^[4,76]. Several mechanisms like amyloid deposition in islets, presence of long-chain FFAs^[77], and chronic hyperglycemia^[60] has been implicated in β -cell apoptosis. Sustained gluco-lipotoxic conditions amplifies the β -cell stress responses by potentiating effects of elevated levels of FFAs, glucose causing ER stress and mTORC1 activation^[78,79,54]. The underlying mechanism for hyperglycemia-induced β -cell apoptosis is attributed to the glucose-induced IL-1 β production that upregulates the Fas receptor^[80,81,12]. FFAs act as important effector molecules causing β -cell dysfunction by lipoapoptosis (a metabolic cause of programmed cell death). The most abundant saturated FFA in blood is palmitate that has direct lipotoxic effects on β -cells by inducing ER stress and ROS^[82-85]. Ceramide, an effector molecule responsible for inducing lipoapoptosis of β -cells, is a metabolic product of FFAs that activates JNK^[86-88]. Likewise, incomplete β -cell oxidation of fatty acids resulting in metabolites like DAG and triglycerides (TGs) also elicits final effector molecules contributing to FFA-

induced lipotoxicity as well as insulin resistance^[89-91]. In addition to this, FFA-induced activation of JNK by Src has also been reported in a recent study^[92]. These studies show that islet inflammation contributes to β -cell dysfunction.

TRIGGERING OF THE INNATE IMMUNE SYSTEM IN T2D

Nutrient excess in metabolic tissues resulting in metabolic inflammation, *i.e.*, a low-level pro-inflammatory milieu, has emerged as an important factor underlying the development of T2D^[11-15,93,94]. Activation of innate immunity in T2D is linked to the activation of TLRs. These receptors have been implicated in diabetes-induced inflammation and vascular complications^[95]. TLRs comprise the pattern-recognition receptors characteristic of the innate immune system. Various pathogen-associated molecular patterns (PAMPs) encompassing carbohydrates, proteins, nucleic acids and lipids, are recognised by TLRs followed by initiation of an immune response. TLR2, a receptor for pathogen lipoproteins and TLR4, a receptor of lipopolysaccharides, are activated by FFAs^[96,97]. Binding of FFAs to TLRs has been postulated to directly induce a pro-inflammatory response^[97,98]. Also, various indirect ways of TLR activation by FFAs has been postulated recently^[99]. *In vitro* studies have demonstrated that, unlike the short chain FFAs, the long chain palmitate and oleate that comprise 80% of circulating FFAs are pro-inflammatory in various cell types^[29,96,98,100,101]. Contemporary studies report the activation of TLR signalling by FFA-induced formation of lipid rafts that favour TLR dimerization in cell membranes^[92,102]. Recently, fatty acid transporter CD36 binding to TLR2 and liver-derived glycoprotein fetuin-A binding to TLR4 were identified as endogenous ligands linking FFAs to TLRs, eliciting inflammation and prompting insulin resistance^[103,104]. In addition, damage-associated molecular patterns (DAMPs) like high-mobility group box 1 (HMGB1) and AGEs also act as endogenous ligands which are recognised by TLRs, thereby activating pro-inflammatory pathways^[105]. TLR2 is responsible for upregulation of inflammatory molecules like NF- κ B, myeloid differentiating factor 88 (MyD88) and chemokine (C-C motif) ligand 2 (CCL2)^[106]. TLR4 knockout mice have been shown to be protected from insulin resistance as well as from fat-induced inflammation^[106]. TLR4 silencing by siRNA technology has been shown to attenuate the hyperglycemia-induced activation of I κ B/NF- κ B^[107]. TLR5 is a receptor for bacterial flagellin that controls metabolic pathways through sensing gut microbiota. TLR5 knockout mice have been reported to exhibit increased adiposity along with hyperphagia, hypertension, hyperlipidemia and insulin resistance^[108]. Activation of inflammatory pathways in a TLR-independent mechanism by metabolic stress involves generation of ROS that induce stress kinases and NLRP3 inflammasome (multiprotein complexes responsible for production of bioactive IL-

1 β) formation^[109].

Both TLR-dependent and TLR-independent mechanisms function in concert. This finding is demonstrated by animal models of diabetes in which there is partly protection of pro-inflammatory cytokine production in case of deficiency of TLR2 or TLR4, whereas deficiency of a universal intracellular docking protein MyD88 required for TLR signalling, exerted total protection^[61]. Apart from FFAs, systemic inflammatory responses are also elicited by elevated glucose levels^[110]. Sustained hyperglycemia results in non-enzymatic glycation of lipids and proteins resulting in the formation of AGEs. AGEs stimulate the pattern recognition receptor RAGE. Numerous cell types, like macrophages, T cells, smooth muscle cells, neuronal cells, podocytes and cardiomyocytes, express RAGE^[111]. RAGE activates the pleiotropic pro-inflammatory transcription factor NF- κ B along with stress kinases ERK1 and ERK2^[112]. Excessive glucose metabolized by oxidative phosphorylation to ATP results in ROS generation that tends to activate the NLRP3 inflammasome concomitantly with FFAs^[67]. This results in release of active IL-1 β along with IL-1-dependent cytokine and chemokine production^[61].

FROM INNATE TO ADAPTIVE IMMUNITY IN T2DM

The role of specific or adaptive immunity comes from the recent clinical overlap between type 1 diabetes (T1D) and T2D such as younger age of onset in T2D and increasing body mass index (BMI) coinciding with increased incidence in T1D. Moreover, progressive decrease in β -cell mass observed in T2D and evidence of insulin resistance in T1D has blurred the etiology^[113]. The argument supporting the involvement of autoimmunity in islets of T2D patients is evident from the presence of β -cell specific antibodies in nearly 10% of T2D patients and presence T cells reactive to β cell antigens in some patients^[114]. The number of autoantigen-responsive T lymphocytes in islets from T2D patients has been reported to correlate with disease progression^[114], however the exact role of islet autoimmunity in T2D requires further studies. A monogenic form of diabetes characterised by typical features of T1D like lean body mass, young age of onset, autoantibodies to β -cells, rapid disappearance of C-peptide and insulin requirement concomitantly with T2D-associated insulin resistance provides genetic support for the overlap between T1D and T2D^[115]. The genetic alteration is attributed to an autosomal-dominant mutation in the *SIRT1* gene, and the pathogenesis involves β -cell impairment and death, paralleling a state of activation of immune system^[115]. As a consequence of insulin resistance, stress induced β -cell death results in the release of autoantigens along with alarmins (endogenous molecules released by necrotic cells causing activation of immune system). Alarmins have potentiating effects of promoting pathologic self-antigen presentation, resulting in enhanced adaptive

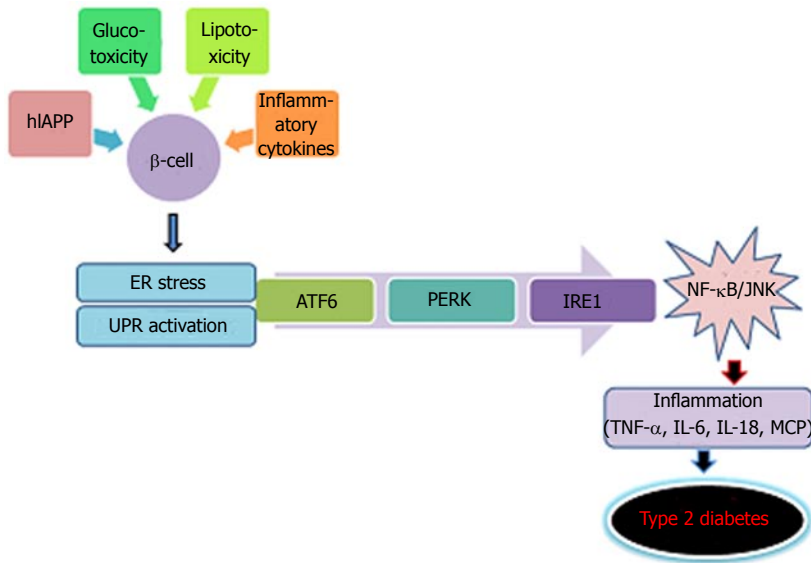


Figure 4 Mechanism of endoplasmic reticulum stress. ER: Endoplasmic-reticulum; ATF6: Activating transcription factor 6; PERK: Double-stranded RNA-activated protein kinase (PKR)-like ER kinase; IRE1: Inositol-requiring kinase 1; IL: Interleukin; MCP: Monocyte chemoattractant protein; TNF- α : Tumor necrosis factor α ; JNK: c-Jun NH2-terminal kinase; NF- κ B: Nuclear factor κ B.

immune response^[116]. In light of these observations, sirtuins are recognised as novel regulators of immuno-metabolism in humans. Apart from SIRT1, SIRT2 has been recently linked to cytoskeleton remodeling and activation of NLRP3 in intracellular pathways^[117]. Apart from the activation of innate immunity, the contribution of adaptive immune cells in inducing inflammation is now established in T2D at the cellular level.

Experimental animal models of insulin resistance have demonstrated a Th2/Th1 shift in favour of Th1, shifting the T_{reg}/Th17 shift towards Th17 and shifting the CD8/CD4 ratio in favour of CD8 and finally reduction of T-cell receptor (TCR) diversity^[118-121]. These studies have recently been extrapolated to human subjects^[122] and confirm the observation that an increase pro-inflammatory stimuli (IFN- γ) causing M1 phenotype switching of adipose tissue macrophages result in the activation of a Th1 type response^[121]. IFN- γ and IL-17 produced by these T cell populations interact directly with adipocytes in addition to contributing to a pro-inflammatory loop in cells of innate immunity. IFN- γ inhibits the JAK-STAT pathway, and IL-17 induces the secretion of IL-6 from adipocytes^[123]. β -cells isolated from T2D patients exhibit increased IL-8 and decreased IL-10 secretion^[124]. Recent studies regard the contribution of B-cell humoral immunity in adipose tissue inflammation. A study on experimental mice models involving B-cell knockout mice and anti-CD20 therapy showed a significantly improved metabolic phenotype and adipose tissue inflammation^[120].

LINK BETWEEN ER STRESS AND INFLAMMATION IN T2D

Activation of ER stress and the UPR forms a convincing hypothesis for the induction of inflammatory pathways

in T2D. ER stress in T2D occurs by virtue of nutrient overload, hypoxia and accumulation of unfolded proteins in metabolic organs^[22]. Under normal conditions, the flux of proteins through ER is high, and in the setting of insulin resistance or glucotoxicity, a prolonged state of insulin need generates ER stress^[125].

Three ER localized sensors control the activation of ER stress and UPR (Figure 4): (1) the double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK); (2) inositol-requiring kinase 1 (IRE1); and (3) activating transcription factor 6 (ATF6). ER stress by protein overload or accumulation of unfolded proteins causes dissociation of GRP78, and the subsequent binding to unfolded proteins in ER prevents their transport to cis Golgi.

Prominently, UPR activation stimulates inflammatory stress kinases like JNK and IKK and their critical downstream transcriptional targets; activator protein 1 (AP-1) and NF- κ B, respectively^[126,127].

These transcription factors control the induction of inflammatory cytokines and chemoattractants that are known to have a direct link with the development of insulin resistance^[128,129]. ER stress can also impair insulin signalling by activation of stress kinases (JNK, IKK) that can inhibit insulin receptor substrates by direct phosphorylation. Recently, death protein 5 (DP5) and p53-upregulated modulator of apoptosis (PUMA) have been reported as inducers of β -cell apoptosis by mediating ER stress^[130]. ER stress can also cause induction of lipogenic genes that promote lipid accumulation and thereby contributes to the development of lipid-induced insulin resistance^[131].

ER stress and UPR pathways

Triggering of inflammatory signals by three pathways of UPR is initiated by activation of JNKs and NF- κ B in B

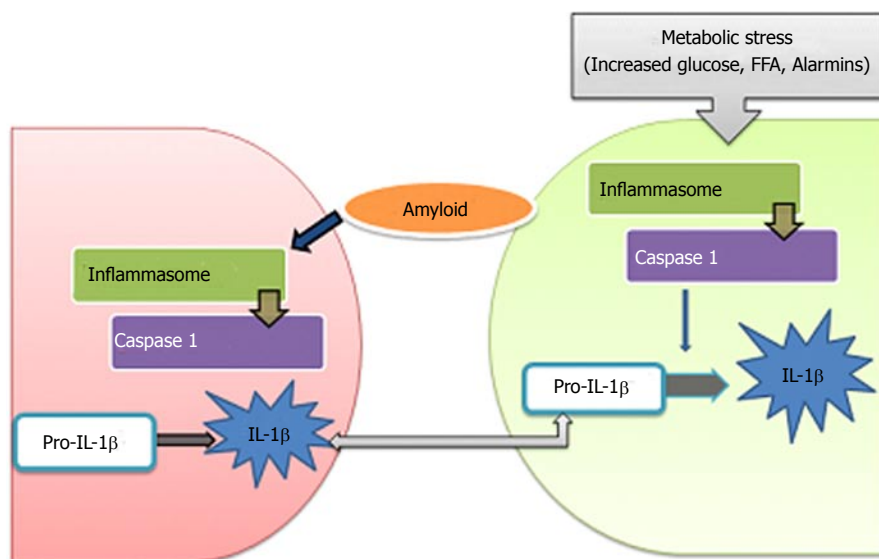


Figure 5 Activation of inflammasomes in type 2 diabetes (metabolic stress activates multiprotein complex, inflammasome in β -cells that induce caspase-1 to cleave pro-interleukin-1 β (pro-IL-1 β) into active IL-1 β . β -cell-derived IL-1 β promote the release of chemokines and recruitment of macrophages that are activated by human islet amyloid polypeptide, leading to deleterious concentrations of IL-1 β . FFA: Free fatty acid; IL: Interleukin.

cells. This activation acts as the linkage point between metabolic and immune pathways since the activation of these very kinases is analogous to that elicited by an immune response^[94,132]. JNKs play an important role in T2D, as increased activity has been shown to promote insulin resistance^[56,133].

The first responses for opposing ER stress involve decreasing the translation of proteins. This involves phosphorylation of α subunit of eIF2 by PERK. In humans and mice, loss of PERK expression is linked to dysregulation of the UPR response which is fundamental to ER stress, resulting in increased cell death and T2D^[134]. A permanent form of neonatal diabetes in humans is related to elevated ER stress markers as a result of a mutation in PERK, confirming the pivotal role of PERK in regulating ER stress during fetal development^[135-137].

A factor in the second pathway of UPR, IRE1, is a prime regulator of ER stress and is highly expressed in the pancreas. An *in vitro* knockdown study on IRE1 signalling showed a decreased synthesis of insulin^[138,139]. Upon activation, IRE1 initiates activation of X-box binding protein 1 (XBP1) that leads to upregulation of ER expansion and biogenesis^[140]. The critical role of XBP1 in achieving an optimal insulin secretion and glucose control was demonstrated in β -cell-specific XBP1-deficient mice that exhibited impaired pro-insulin processing and secretion, reduced β -cell proliferation and hyperactivation of IRE1^[141].

The third pathway of UPR involves the activation of ATF6, the basic leucine zipper domain protein, that upregulates PERK1 and IRE1 pathways by suppressing the apoptotic UPR signalling cascade under chronic ER stress. The role of ATF6 activation in β -cell dysfunction has been concluded in studies that showed decreased expression of insulin gene by ER stress-induced ATF6

activation and a decrease in ER chaperones along with induction of apoptosis in ATF6 knockdown insulinoma cells^[142,143].

ACTIVATION OF INFLAMMASOME IN T2D

Inflammasomes are multiprotein complexes in the intracellular machinery responsible for production of bioactive IL-1 β in response to multiple stimuli^[144]. NLRP is a subfamily of Nod-like receptors containing a central nucleotide binding and oligomerization (NACHT) domain with flanking C-terminal leucine-rich repeats (LRRs) and N-terminal caspase recruitment (CARD) or pyrin (PYD) domains^[145]. The NOD-like receptor family, the pyrin domain containing 3 (NLRP3) inflammasome is in a pathway that controls the production of IL-1 β and IL-18^[146-148]. Unlike TLR, a potential role of NLR in metabolic abnormalities has not been extensively investigated. NLRP forms a constituent of the inflammasomes responsible for maturation and release of IL-1 β , and thus is a relevant candidate for metabolic disorders and T2D^[149]. NLRP3-dependent activation of inflammasomes in diabetes was proposed by studies implicating the release of IL-1 β as a consequence of elevated levels of glucose, FFAs and human islet amylopolypeptide (hIAPP)^[16,150,151]. However, the effective metabolites involved in activation of inflammasomes are not clearly elucidated yet (Figure 5).

The NLRP3 inflammasome is a general metabolic alarmin stimulated by different endogenous and exogenous stimuli^[152]. NLRP3 inflammasome activation is augmented in T2D patients^[153]. Dysregulation of lipid metabolism, paving the way to aberrant lipid accumulation, as well as formation of oxidized LDL and cholesterol, triggers NLRP3 activation^[30,153,154] similar

to ER stress that acts as one of the important factors triggering NLRP3 activation^[155,156]. In T2D subjects, increased oxidative stress also contributes to NLRP3 inflammasome activation^[157,158].

Studies on obesity-induced inflammation and insulin resistance are also indicative of the role of NLRP3. In experimental models of calorie-restricted mice, a positive correlation has been observed between IL-1 β /NLRP3 mRNA and body weight^[30] whereas disruption of *NLRP3* gene in obese mice has revealed changes in metabolic profiles. Insulin resistance as a consequence of inflammasome activation is directly related to FFAs and LPS^[109]. Apart from Insulin resistance, activation of inflammasomes is related to β -cell dysfunction, as NLRP3-knockout mice exhibit improved glycemic profiles after consumption of a high-fat diet, likely due to attenuation of IL-1 β ^[67]. In response to hyperglycemia-induced increased production of ROS, NLRP3 activation occurs as a result of dissociation of thioredoxin interacting protein (TXNIP) from thioredoxin and its subsequent binding to NLRP3^[67]. Nevertheless, shortage of TXNIP has shown effects on glucose metabolism in addition to the NLRP3 activation^[159]. A substantial role of inflammasome activation in β -cell dysfunction was recently reported by ablation of NLRP3 that conferred protection to β -cell function and structure from injury inflicted by metabolic stress^[160].

Secretion of IL-1 β requires two induction stimuli; the first stimulus induces pro-IL-1 β expression and the second inflammasome activation. Inflammasome activation triggers caspase-1 resulting in cleavage of pro-IL-1 β and release of mature IL-1 β . In T2D, the first stimulus comes from minimally-modified LDL in islets which prime the macrophages for processing of IL-1 β by activation of TLR4 signalling. Recently, the second stimulus was recognized to regard islet hIAPP, secreted by β -cells in response to high glucose levels^[151]. hIAPP was shown to direct NLRP3 activation by inducing β -cell injury. In islets, interaction of macrophages and β -cells is essential for the activation of inflammasomes. hIAPP, a soluble oligomer induces activation of NLRP3 and subsequent release of IL-1 β from macrophages and dendritic cells which are primed with TLR4 agonists like LPS or modified LDL molecules^[151]. The macrophages are attracted to islets by hIAPP-induced synthesis of chemokines (CCL2 and CXCL1). It has been reported that overexpression of hIAPP in islet grafts increases the recruitment of macrophages by 50%^[161]. Recently the activation of inflammasomes in myeloid cells in T2D patients was elucidated. A study on untreated T2D subjects showed upregulation of IL-1 β production and maturation in macrophages^[153]. Treatment of macrophages with various alarmins like FFA, hIAPP, HMGB1 and ATP resulted in release of inflammasome products. Studies have shown that T2D subjects exhibit elevated levels of circulating alarmin molecules thereby advocating a possible role of these molecules in NLRP3 inflammasome activation in myeloid cells^[162].

PERSPECTIVES

The concept of chronic low-level inflammation in T2D has given an impetus to the field of immune-metabolism. Elucidation of various cellular mechanisms linking inflammation to insulin resistance and β -cell dysfunction has revolutionized insights in the molecular pathogenesis of diabetes. Insights into intricate pathways provide a platform to tackle the distinct pathway without compromising immuno-surveillance. Nutritional and therapeutic interventions aimed at controlling/inhibiting the escalating pro-inflammatory response can help in attenuating the pathogenesis and progression of T2D. Well-designed studies should offer the development of novel targeted therapeutics to deal with the disease burden of T2D and its associated complications.

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Lean diabetes mellitus: An emerging entity in the era of obesity

Amrutha Mary George, Amith George Jacob, Leon Fogelfeld

Amrutha Mary George, Amith George Jacob, Leon Fogelfeld, Division of Endocrinology, John H Stroger Hospital of Cook County, Chicago, IL 60612, United States

Author contributions: George AM, Jacob AG and Fogelfeld L contributed to this work.

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Correspondence to: Leon Fogelfeld, MD, Head Division of Endocrinology, John H Stroger Hospital of Cook County, Professor of Medicine, Rush University Medical Center, Division of Endocrinology, John H Stroger Hospital of Cook County, 1901 W Harrison Street, W.Polk (room 811), Chicago, IL 60612, United States. lfogelfe@cchil.org

Telephone: +1-312-8640539

Fax: +1-312-8649734

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Abstract

Much has been published on the characteristics of type 2 diabetes mellitus and its association with the epidemic of obesity. But relatively little is known about the incidence of lean diabetes, progression of disease and fate of the patients with low-normal body mass index (< 25). Studies in developing countries have shown that the clinical characteristics of these patients include history of childhood malnutrition, poor socioeconomic status, relatively early age of onset and absence of ketosis on withdrawal of insulin. In the United States, recent studies showed that the lean,

normal weight diabetes is not rare especially among minority populations. They showed that these patients are mainly males, have higher prevalence of insulin use indicating rapid beta cell failure. They might have increased total, cardiovascular and non cardiovascular mortality when compared to obese diabetic patients. In this review, the epidemiologic and clinical features of lean diabetes are presented. The potential causal mechanisms of this emerging diabetes type that may include genetic, autoimmune, acquired and behavioral factors are discussed. The need for studies to further elucidate the causation as well as specific prevention and treatment of lean diabetes is emphasized.

Key words: Lean diabetes; Beta cell failure; Ketosis resistant diabetes of young; Obesity paradox; Sarcopenic obesity

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Core tip: Little is known about lean diabetes (patients with low-normal body mass index). Studies in developing countries have shown that these patients have history of childhood malnutrition, poor socioeconomic status and early age of onset with absence of ketosis. In the United States, recent studies showed that the lean, normal weight diabetes is not rare especially among minorities. These patients are mainly males and have higher prevalence of insulin use indicating rapid beta cell failure. They might have increased total, cardiovascular and non cardiovascular mortality when compared to obese diabetic patients. The potential causal mechanisms of this diabetes type may include genetic, acquired and behavioral factors.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) has increased exponentially over the past decade globally. In the United States, nearly 26 million adults have diabetes with over 9 million being prediabetic. The numbers are expected to double by 2050. Nearly 35% of American adults are obese putting them at an increased risk for the development of Diabetes. Healthy people 2020 initiative has stressed the importance of reduction in obesity to lower the incidence of this morbid condition among the United States population^[1].

Since early times obesity has been a well known risk factor for diabetes. However, beyond the classical obesity related type 2 diabetes and other well defined types of diabetes like type 1, Maturity onset Diabetes of the Young, Gestational diabetes, *etc.*, there is a renewed interest in the underweight or normal weight lean diabetes that is emerging also in the developed countries like United States. This review will focus on the low-normal weight diabetes that is so far not well characterized and defined. More importantly, this review will highlight the differences in the clinical features of diabetes mellitus in normal body weight patients in the United States in comparison to the classical obese diabetes. The evidence related to the profile, peculiarities, morbidity, mortality and pathogenesis associated with lean type diabetes will be described.

DIABETES IN LOW BODY WEIGHT GROUP (BODY MASS INDEX < 18 KG/M²)

Though recognized as a distinct entity early on, very little attention has been paid to the qualitative changes associated with it. Any "atypical diabetes" syndrome which did not meet the classical American Diabetes Association or World Health Organization Classification suffered from imprecise definitions which led to a group of complex phenotypes^[2]. Further studies have been undertaken only recently shedding much insight into their disease burden, progression and natural history. Adult onset diabetes with body mass index (BMI) < 25 was initially placed under the category of "malnutrition related diabetes mellitus" in a subcategory termed "protein deficient pancreatic diabetes"^[3]. Later this syndrome was noted to be similar to that originally described as "Jamaica type Diabetes", a term used to represent around 5% of Caribbean diabetics^[4]. Similar clinical syndromes were subsequently described in regions of south Asia and Africa and has acquired various names; "Tropical Diabetes, Mixed onset type Diabetes, Phasic insulin dependent Diabetes, J type Diabetes, Z type Diabetes, M type or type 3 Diabetes, Ketosis resistant growth onset type Diabetes"^[5].

Many of the overlapping categories described above could be lumped together under the term

coined by Ahuja^[6] as "Ketosis Resistant Diabetes of the Young (KR DY)". This category includes a broad subset of patients mostly of Asian and African ethnicity. The following criteria were suggested for the diagnosis of KR DY^[6]: (1) blood glucose > 200; (2) onset < 30 years of age; (3) BMI < 18 kg/m²; (4) absence of ketosis on insulin withdrawal; (5) poor socio-economic status or history of childhood malnutrition; and (6) insulin requirement > 60 units/d or 1.5 units/kg.

Although early age of onset and low BMI may raise suspicion for type 1 diabetes, the presence of islet cell specific antibodies has consistently been lower than those with type 1 diabetes across multiple population groups. It can be argued that the wasting or leanness noted in around 25%-50% of patients could represent the effects of long standing glycosuria^[7]. Though BMI mostly improved with weight gain following optimal glycemic control, the mean value remained within the definition of low body weight among both genders^[8]. Phenotypic similarities have been described from various other regions of the world with nearly all of them demonstrating a male preponderance with the most extensive data being described from Ethiopia^[9,10].

A study from India on around 10000 type 2 diabetics revealed that around 3.5% patients were lean with a BMI < 18.5, with the larger share of around 63% patients having ideal body weight at diagnosis. Age of diagnosis (45 ± 13) and smoking patterns were not significantly different among the lean, ideal body weight and obese groups, although a male preponderance was noted only in the former two. This study also highlighted the fact that HbA1c, fasting and postprandial blood glucose levels were higher among those in the lean group. Micro-vascular complications of Diabetes such as retinopathy, nephropathy and neuropathy were more common among the lean male patients presumed to be related to the higher plasma glucose and HbA1c levels^[11]. Other studies have also highlighted the higher incidence of peripheral neuropathy among lean diabetic males^[12], whereas hypertension and coronary artery disease tend to be more common in the obese group^[13]. A limitation of these studies were that auto antibodies were measured only in a very small number, thereby leaving a chance that the lean diabetics could represent type 1 diabetics^[14], although patients who had an abrupt onset, ketosis or ketoacidosis at any time or required insulin at time of diagnosis were excluded from the study to avoid bias. In whom C-peptide levels were measured, they were found to be significantly higher compared to the type 1 diabetics being followed at the respective centers. Moreover the results of islet cell antibodies and antibodies against Glutamic acid decarboxylase were not significantly different between the 3 groups^[15]. Nearly 48% of the lean NIDDM patients responded to diet or oral hypoglycemic agents after a mean duration of 9.2 ± 8.1 years, which is a clear distinction from type 1 diabetics. Symptomatic ketoacidosis was absent in this group^[11].

The pathophysiology and its distinction from classic

type 2 diabetes is still unclear and a subject of much debate. The key feature appears to be a defect in insulin secretory capacity as opposed to peripheral insulin resistance as noted in classical diabetes. Multiple studies have shown an association between lean diabetes, malnutrition in early years of life and poor socioeconomic status. Although prospective human studies are lacking, experiments done on rats and primates have shown that low protein diet in early life leads to decreased beta cell mass and insulinopenia. Insulin mediated glucose disposal appears to be similar in KRDY and type 1 Diabetic patients^[16]. These patients have fasting C-peptide levels intermediate between type 1 and type 2 diabetics^[17,18]. Despite the "decent" C-peptide levels suggesting a good beta cell reserve, the circulating insulin levels at baseline and post stimulation with insulin secretagogues (glucose, tolbutamide and amino acids) have been consistently lower in lean diabetics when compared to their obese counterparts^[8,19-24]. The 2 mechanisms that have been postulated to cause this are excessive extraction of insulin in the porto-hepatic circulation from raised glucokinase activity and hyperactive futile cycles of carbohydrate metabolism^[8]. Resistance to ketosis noted in these cases is due to a small but sufficient insulin secretory reserve which is absent in type 1 diabetics^[2]. They have also been noted to have lower fasting plasma free fatty acid and ketone levels and a blunted response to catecholamines further delaying the development of ketoacidosis^[19-21]. The occurrence of fat malabsorption in a small subset of patients contributes evidence to an exocrine defect in KRDY patients^[25].

Auto-antibodies can be present in anywhere between 2%-25% of KRDY patients. Glutamic acid decarboxylase (GAD), tyrosine phosphatase like protein (IA-2) or high mobility group box transcription factor SOX-13 (ICA-12) antibodies are commonly found, though co-occurrence of GAD and IA-2 was observed in only 4.7% of lean patients compared to 22% type 1 diabetics^[26]. Any single antibody by itself is neither sensitive nor specific to distinguish between the phenotypes of type 1 or type 2 diabetes as GAD or ICA positivity has been reported even in 4%-13% of obese type 2 diabetics^[8]. In summary the primary etiology appears to be depressed beta cell function most likely due to malnutrition in utero and early infancy in addition to autoimmune modulations. However more studies are needed on this front to unearth the metabolic, hormonal and immunological characteristics of lean type 2 diabetics.

DIABETES IN NORMAL BODY WEIGHT GROUP (BMI 18-24.9)

The term "lean" has been described variously in different studies. The major distinction seems to originate from the geographic region where the study was conducted. Those from developing countries use a BMI < 18 to describe leanness whereas studies

performed in the United States describe lean patients to have a BMI ranging from 18-24.9.

A study cohort of 18000 patients with type 2 diabetes in Chicago, United States showed that around 13% belonged to this group, with ideal body weight defined as a BMI ranging from 17-25. The study failed to demonstrate a significant difference between the age of diagnosis (43 ± 13) between the lean and obese diabetics, and corroborated the previous finding of male preponderance among the lean group (62%). Asians were shown to have a five-fold higher prevalence in the lean group (17% vs 4%). Environmental insults such as use of alcohol and cigarette smoking were more common among lean diabetics. As confirmed by various other studies, glycemic control was worse among lean diabetics and coronary complications more prevalent among the obese with no significant difference noted among micro-vascular complications^[27].

The major pathophysiology in this group appears to be rapid beta cell failure as opposed to insulin resistance. This was highlighted by the fact that lean individuals had both a higher prevalence and early initiation of insulin use^[27]. They were also noted to have lower TG/HDL ratios, which is an indirect marker of lower insulin resistance^[28-30]. In diabetics, central obesity (waist circumference > 102 cm in males and 88 cm in females by NCEP) correlates with the degree of insulin resistance^[31]. In the above cohort, 96.9% of the lean males did not have central obesity which points away from insulin resistance causing hyperglycemia^[27].

The key defect responsible for hyperglycemia in the lean diabetics is impaired pancreatic insulin secretion^[32-34] which is partly due to a reduced beta cell mass as demonstrated *via* autopsies^[35,36]. The more severe beta cell dysfunction in these patients may be functional rather than structural as beta cell mass was noted to be equally reduced in both lean and obese patients^[34]. Lean healthy Caucasian subjects born with a low birth weight have been demonstrated to develop several physiological defects of type 2 diabetes such as decline of insulin secretion, reduced muscle glucose uptake, reduced insulin stimulated glycolysis, lower fasting plasma glycerol levels and increased fat accumulation more prematurely than expected^[37-40]. Dutch Famine Study has also proven that a brief period of malnutrition during postnatal period or early childhood increases the risk of diabetes^[41]. These findings have been further validated in Asian populations too.

In addition, there was a higher prevalence of smoking, alcoholism and pancreatitis in the lean group in the Chicago study^[27]. Chronic alcohol consumption induces pancreatic beta cell dysfunction and apoptosis^[42]. Exposure to passive and active smoking are positively and independently associated with the risk of diabetes^[43]. Whether the pronounced male prevalence among the lean is due to inherent genetic differences or unhealthy life style that can promote beta cell failure in comparison to females is still a matter of debate.

Genetic modulators might also predispose to reduced beta cell function in the lean body weight group. Polymorphisms of transcription factor FL2 gene (TCF7L2) and a genetic defect of ATP sensitive potassium channel Kir6.2 (or KCN JII) are associated with defective insulin secretion. Carriers of TCF7L2 gene polymorphism were also shown to be leaner and more insulin sensitive as compared with other type 2 diabetics^[44]. Genetic scores for insulin resistance have shown association of lower subcutaneous fat mass and ectopic fat deposition highlighting the role of impaired adipose expandability and body fat distribution even among lean type 2 diabetic individuals^[45].

Higher prevalence of Asians in the lean group could be from greater intrauterine insults and under-representation of overweight and obesity amongst them based on standard Body Mass Index definitions available. GAD antibodies though less frequently noted^[46,47] may play a role in the autoimmune destruction of beta cells. It remains to be proven whether occurrence of these antibodies is merely secondary to the loss of beta cell function as compared to an etiological agent in itself^[48]. The role of genetics and autoimmunity in lean diabetics needs to be further elucidated prior to drawing more concrete conclusions in this group.

THE OBESITY PARADOX AND SARCOPENIC OBESITY

Another interesting aspect is the occurrence of complications and increased rate of mortality in certain normal weight diabetes patients in comparison to their obese counterparts. This phenomenon is called the "obesity paradox". A pooled analysis done on 2625 participants from 5 longitudinal cohorts showed that normal weight adults at the time of incident diabetes had higher mortality than adults who were overweight or obese^[49]. This "obesity paradox" has also been previously noted in other studies on diabetics and in various other chronic conditions such as hypertension, end stage renal disease and heart failure. It is likely that lower body weight in the presence of obesity related metabolic disorders may just be a reflection of preexisting illness that may predispose to mortality^[49]. It is also known that despite having a leaner body mass, cigarette smokers are more insulin resistant, more likely to develop diabetes, and have a higher mortality from chronic lung disease and malignancies as compared with non-smokers. Carnethon *et al.*^[49] concluded that the elevated mortality in normal weight participants could not be entirely attributed to smoking, though a subgroup analysis demonstrated that there is no statistically significant difference between mortality in non-smoker adults of either cohort. A main limitation of this analysis was inability to assess the smoking burden and relatively low statistical power that limited the body mass index classification to 2 broad and

heterogeneous groups (BMI 18.5-24.9 and BMI \geq 25).

Further research into this obesity paradox by Tobias *et al.*^[50] in over 11000 participants, showed a J-shaped association between body mass index among all participants and in current or previous smokers. A direct linear relationship was observed in those who had never smoked. The lower mortality was observed among participants with a BMI of 22.4-24.9. The obesity paradox was therefore not observed in this study^[50]. The increased mortality among lean diabetic smokers has been observed in the general population as well^[51-54] and further studies are needed to address whether they represent an effect modification or is secondary to bias^[55]. In participants 65 years or older, a null or weaker linear association was observed, which was probably due to increased prevalence of co-existing chronic diseases and decreased validity of BMI as a measure of adiposity due to age related decline in muscle mass and wasting^[50,56].

A possible explanation for the observed obesity paradox could be sarcopenic obesity, defined as the presence of high body fat with reduced or normal lean body mass^[1]. The localization of adipose tissue particularly abdominal obesity, independent of total obesity is associated with increased risk of cardiovascular disease. Sarcopenic obesity reduces the cardio-pulmonary fitness and physical functioning possibly leading to premature death and could account for the higher mortality eventually seen in individuals who are normal weight at the time of diabetes onset^[23]. The sarcopenic obesity may also indicate a catabolic underlying illness that leads to increased mortality. This was further supported by a systematic review, which showed that all cause mortality was lower among those with a high body mass index and good aerobic fitness as compared with individuals of a normal body mass index and poor fitness^[57]. A study on veterans has also supported this view^[58]. Hence questions can be raised regarding the sufficiency of such a simplistic classification of diabetes into obese or non-obese groups solely based on BMI.

IS FURTHER WEIGHT LOSS RECOMMENDED IN LEAN DIABETICS?

Weight loss is recommended for all overweight or obese diabetics. Studies such as the diabetes prevention program outcome study (DPP) and Look AHEAD^[59] have primarily focused on weight management in overweight and obese individuals^[1]. Could such recommendation be also effective in lean diabetics? Some concerns may be raised that additional weight loss could worsen both bone loss and decrease further the lean body mass contributing further to sarcopenic obesity. In the Chicago study, it appeared that leaner individuals had worse beta cell failure. One can then postulate that lean diabetics is a special variant of type II diabetes whereby the failing beta cell cannot even

Table 1 Clinical differences between type 1, type 2, ketosis resistant diabetes of the young and lean type 2 variant diabetes

Clinical features	Type 1 diabetes	Type 2 diabetes	KRDY	Lean type 2 variant
Age of diagnosis	Can occur at any age, usually < 25	Usually > 25 yr, but increasing prevalence in adolescents	< 30 yr	Average age around 40 with male preponderance
Weight	Usually lean	Overweight and obesity	BMI < 18	BMI 18-25
Autoantibodies	Present	Absent	Variable	Absent
Family history of diabetes	5% to 10%	75% to 90%	Unknown	Around 50%
Insulin sensitivity	Normal	Decreased	Normal	Normal, in females might be decreased
Insulin dependent at diagnosis	Yes	No	40% at diagnosis	35% at diagnosis
Risk of ketoacidosis	High	Low	Low	Low

KRDY: Ketosis resistant diabetes of the young; BMI: Body mass index.

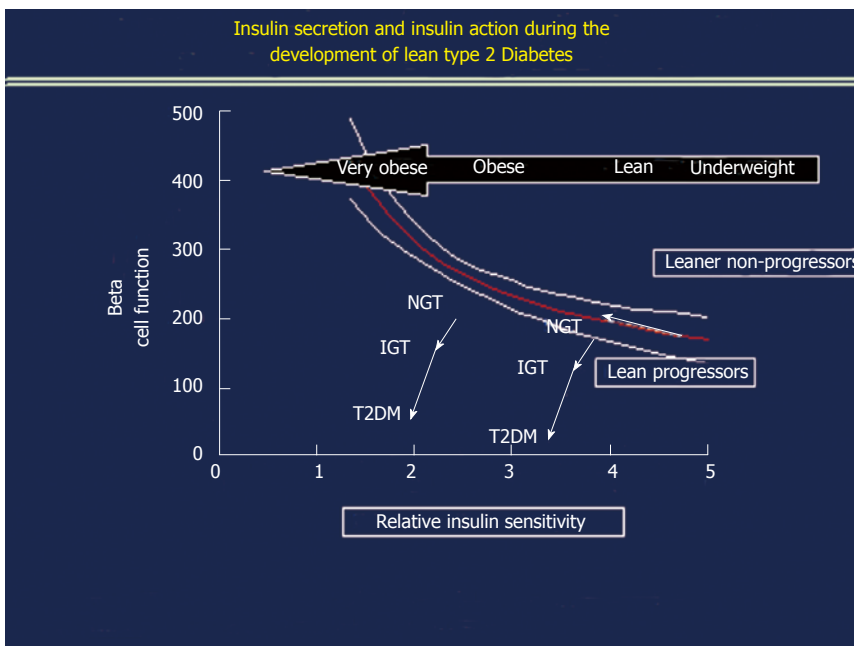


Figure 1 Pathogenetic model of development of lean type 2 diabetes. In the obese individuals the diabetes develops once the beta cell cannot cope with the insulin resistance conferred by the growing obesity. In the lean diabetes the early failure of the beta cells results in development of diabetes at much lower insulin resistance. It might be speculated that in individual with similar beta cell dysfunction but lower insulin resistance (lower weight) diabetes might not develop. NGT: Normal glucose tolerance; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes; Modified from Weyer *et al. J Clin Invest* 1999; 104: 787–794.

cope with the small amount of insulin resistance that lean body weight confers. Could then achievement of a lower body weight or lower adiposity in these patients prevent diabetes? Currently there is no answer to this intriguing hypothesis (Figure 1).

CONCLUSION

The patients with lean diabetes in comparison to classical obese type 2 diabetes (Table 1) are characterized by younger age at onset, earlier and more prevalent use of insulin, higher prevalence in males and higher rates of cigarette smoking and alcohol abuse. It was initially shown that patients with diabetes who are leaner have higher mortality rate in comparison to the obese (the obesity paradox). This was explained by the concept of sarcopenic obesity whereby these patients are metabolically obese with excess of adiposity but have decreased muscle mass (sarcopenia) which is the predisposing factor for the increased mortality. The obesity paradox however could not be confirmed in a most recent study in a larger cohort.

The underlying pathogenetic mechanism of lean diabetes has not yet been clarified and more studies are needed for its elucidation. It could be a completely new pathogenic entity, however there is a possibility that it may just be a variant of type 2 diabetes. In type 2 diabetes, the beta cells that are genetically destined to fail gradually over the years cannot cope with the increasing insulin resistance that is conferred by obesity. Lean diabetes might be a variant of these main operating pathogenic mechanisms. The difference is the much more pronounced beta cell failure that occurs earlier and results in more rapid exhaustion. Several potential mechanisms could be involved in the beta cell failure. The initial predisposing factors may be an adverse intrauterine or early postnatal environment with insufficient nutrients that result in a smaller beta cell mass. Then genetic predisposition of a more fragile beta cell mass may cause early destruction and apoptosis. Studies have showed that genetic markers of such fragility are more common in the lean than in obese diabetics. In addition, in developed countries like the United States, acquired insults like cigarette

smoking and alcoholism might be newly defined and significant pathogenetic contributors that could further weaken the beta cells. In these circumstances, even the little insulin resistance associated with lean body weight could precipitate diabetes (Figure 1). Recent study has also shown that genetically determined insulin resistance may also play a role in the pathogenesis of lean diabetes.

Considering the inadequacy of BMI in distinguishing leanness, future studies should investigate the complex interaction between body composition, amount and distribution of adipose tissue and physical functioning in determining the development of lean diabetes. In the meantime emphasis on modifiable risk factors like smoking and alcohol abuse that may further accelerate beta cell failure in lean patients may prevent further progression of the disease.

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Psychological themes that influence self-management of type 1 diabetes

Clare Shaban

Clare Shaban, Bournemouth Diabetes and Endocrine Centre, Royal Bournemouth Hospital, BH7 7DW Bournemouth, United Kingdom

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Correspondence to: Clare Shaban, Consultant Clinical Psychologist, Bournemouth Diabetes and Endocrine Centre, Royal Bournemouth Hospital, Castle Lane East, BH7 7DW Bournemouth, United Kingdom. clare.shaban@rbch.nhs.uk
Telephone: +44-1202-704888

Fax: +44-1202-704759

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Abstract

It has long been accepted that psychological factors adversely influence efforts to optimise glycaemic control. These are often unrecognised in terms of clinical assessment and therefore under reported. This essay presents an introduction to psychological issues that interact with psychiatric co-morbidities and diabetes-specific distress, and a case scenario illustrating the interconnectedness of presenting problems and themes. In the way that we cannot separate carbohydrate counting, blood glucose monitoring and insulin dose

adjustment in the understanding of a presenting problem such as poor control, so we cannot separate the concurrent thoughts, feelings, and behaviours. Each of these emotional aspects are self-managed either through avoidance, or by delayed disclosure and are frequently associated with poor health outcomes. There is a requirement for the healthcare team to be sensitised to these issues and to develop styles of communication that are empathic, reflective and non judgemental. A brief outline of evidence-based psychotherapy treatments is given.

Key words: Psychological factors; Glycaemic control; Anxiety; Depression; Eating disorder; Diabetes distress; Maladaptive coping; Psychotherapy

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Core tip: Psychological factors adversely influence efforts to optimise glycemic control. The focus on psychiatric diagnosis has done a disservice to people with diabetes who experience significant levels of sub-clinical distress and it is essential to develop an understanding of the psychological issues that underpin poor self-management of type 1 diabetes. The diabetes healthcare team needs to be sensitive to the underlying issues and to be confident in the use of consultation styles that facilitate recognition and appropriate signposting for specialised support and treatment.

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INTRODUCTION

It has long been accepted that psychological factors

adversely influence efforts to optimise glycemic control and for many years these have been addressed in the context of psychiatric diagnoses anxiety^[1], depression^[2,3] and eating disorders^[4,5]. Until recently the Quality Outcomes Framework^[6] has been used to remunerate United Kingdom General Practitioners for recording assessments of anxiety and depression in those living with long-term conditions. This focus on diagnosis has been to the detriment of people with diabetes who experience significant levels of distress, visible in terms of poor control but unrecognised in terms of clinical assessment and therefore under reported. More recently attention has been given to the need to differentiate between clinical diagnoses and diabetes-emotional distress^[7-9]. This article, informed by literature and the clinical experience of the author, presents an introduction to psychological issues that interact with psychiatric co-morbidities and diabetes-specific distress, and a case scenario illustrating the interconnectedness of presenting problems and themes. A brief outline of psychotherapeutic models available to treat these difficulties is given.

Management of type 1 diabetes requires optimising a sequence of actions or behaviours that include blood glucose monitoring, carbohydrate counting, insulin administration and physical activity, in the context of cognitions and emotions (thoughts and feelings). On the face of it the most straightforward approach to management is to advise people what to do, and expect it will be done. Living with and managing diabetes in the context of education, employment, recreation, ill health and relationships to name a few aspects of daily living, can be challenging. Diabetes is not an exact science and day to day life, early learning, emotions and motivation can, and do emerge as barriers to the consistent application of the "straight-forward" approach.

EMOTIONS AND COGNITIONS ASSOCIATED WITH DIABETES

Diabetes is the ultimate gatecrasher. There is no satisfactory explanation to answer the question "why me?" and at best glycaemic control can be managed, but unlike the party gatecrasher, diabetes cannot be sent away. Clinicians are familiar with the lexicon offered by people living with diabetes in response to the question "How does diabetes make you feel?" that includes words like anxious, worried, afraid, mood swings, depressed, euphoric, shame, guilty, angry and frustrated. Similarly when asked about thoughts triggered by living with diabetes the responses reflect what it means to live with the condition and its impact on quality of life, and the difficulties of management. This thinking interacts with beliefs about the self, the reaction of others and unconscious motives derived from early experience.

TOWARDS AN UNDERSTANDING OF THE MISMANAGEMENT OF DIABETES

In the way that we cannot separate carbohydrate counting, blood glucose monitoring and insulin dose adjustment in the understanding of a presenting problem such as poor control, so we cannot separate the co-existing thoughts, feelings, and behaviours.

Psychiatric diagnoses of anxiety, depression and eating disorders are frequently listed as psychological aspects of diabetes, and improvements in glycaemic control (HbA1c) reported as the primary outcome measure of treatment. As stated in the introduction, in recent years there has been increasing attention given to the need to differentiate psychiatric comorbidities and diabetes-related emotional distress. Identifying the factors that influence the decisions for particular actions that contribute to poor control is the crux of psychological formulation. Assessment of the presenting problem and the context in which it occurs offers a pathway to a personalised understanding that informs the treatment of choice. This might include further diabetes education, pharmacological treatment, appropriate psychological therapy, or indeed any combination of these.

Assessment of an individual presenting with poor control may reveal distress associated with, for example, a fear of hypoglycaemia. Thoughts might reflect a fear of loss of control, or of drawing unsolicited attention giving rise to emotions such as anxiety, frustration or guilt. Actions to elevate blood glucose such as reducing insulin or eating to maintain a "safe" blood glucose level result in both a reduction of risk of hypoglycaemia and a reduction in anxiety. Alternatively, high blood glucose readings frequently trigger guilt and fears about long term health. Evidence of high blood glucose is denied by avoidance of blood monitoring, and the emotional equilibrium thereby maintained. These are examples of the mismanagement of diabetes being used to manage emotional distress.

Managing diabetes requires multifactorial consideration of food choice and quantity, activity, blood glucose, ill health, ambient temperature, alcohol consumption, and menstrual cycle to name a few of the factors. The psychological responses are equally complex and influence actions with consequences on a continuum ranging from subtle to extreme outcomes on glycaemic control and psychological well-being.

PRESENTING PROBLEM AND UNDERLYING PSYCHOLOGICAL ISSUES

The following case history is used to illustrate psychological issues underlying poor control evidenced by elevated HbA1c and frequent episodes of hypoglycaemia. It invites consideration as to whether the presenting problem requires direct treatment or

is a symptom of underlying difficulties that need to be addressed.

CASE STUDY (REPORTED WITH PERMISSION)

Sam is a 60-year old post menopausal woman approaching retirement. She has been married for 38 years and although she experienced difficulty with conception she has one adult child. Her family of origin practised strict religious beliefs and she was brought up to be sensitive to the needs of others before her own. She had little preparation for independent living and intimate relationships: indeed such topics were taboo and usually associated with duty, guilt, shame and blame. There was an expectation that she would be self-sufficient and undemanding which contributed to considerable reluctance to seek help and resulted in her needs not being met. She suffered a sexual assault in her late teens not disclosed at the time, for which she did not receive emotional support and which subsequently influenced intimate relationships and generated a sense of shame. Her predominant employment has involved looking after others in a variety of roles, consistently prioritising the needs of others above her own. Sam has had type 1 diabetes for 20 years with significant fluctuations in control over that time and frequent hypoglycaemia, severe episodes occurring most frequently during sleep. Weight and body-image concerns are relevant in terms of her self-perception as unloveable and unattractive. She engages in regular exercise and has the not uncommon challenge of balancing energy needs, insulin dose and extremes of blood glucose levels. Whilst there is no indication that Sam uses insulin omission to influence her weight she tends to maintain elevated blood glucose to protect against hypoglycaemia. Pervading themes reflect guilt, shame, blame, a sense of being undeserving and either not good enough or a failure.

On the face of it a diabetes-specific intervention might focus on insulin adjustment, reducing episodes of hypoglycaemia, optimising exercise and blood glucose control or, less likely, weight management. The brief history offers insight into interconnecting psychological factors that contribute to poor glycemic control (Figure 1).

We can map the interlinking of the psychological factors, many of which are derived from early experience, others of which are accentuated by the role of diabetes and its impact on daily living. The experience of hypoglycaemia potentially predisposes fear of further episodes interconnecting with blame from others for "getting it wrong", shame and personal failure for "getting it wrong" and embarrassment and a fear of being out of control. Maladaptive coping behaviours include overeating and/or the reduction of insulin to elevate blood glucose, avoiding any physical exertion that may result in hypoglycaemia and avoiding social

situations. The awareness that the coping mechanism results in short term relief with adverse consequences on long-term health perpetuates the cycle with resulting feelings of guilt, shame, and body-image concerns.

The spider's web gives some indication of why managing diabetes can be so challenging. Advice to adjust insulin:carbohydrate ratios, conservative treatment of hypoglycaemia, dietary advice and information about women's sexual function and diabetes may all be appropriate but do not address the psychological themes underpinning the maladaptive behaviours contributing to poor control.

PSYCHOLOGICAL ISSUES AND MALADAPTIVE COPING

The earlier paragraphs describe circumstances and events with psychological consequences, in the context of diabetes, that lead to maladaptive coping and mismanagement of diabetes. The following is a brief account of some of these with reference to the literature for more detailed exploration. Evidence has been provided for different components of type 1 diabetes distress: emotional burden, interpersonal and social distress, regimen related, and health care related^[9-12]. The psychological issues are inextricably interconnected and related to specific aspects of diabetes-distress and presenting problems such as hypoglycaemia, fear of complications and body-image concerns.

Fear and anxiety

Fear and anxiety are the cognitive and emotional responses to threat. In order to reduce emotional discomfort individuals either "overdo" in an attempt to prevent the feared event or "under do" (avoid) the action in the misapprehension that by not addressing it, it will go away. The lack of exposure to the feared event (*e.g.*, hypoglycaemia) means that the individual reinforces the avoidance behaviour and does not learn how to cope were the threat to occur.

Blame and shame

Blame and shame indicate perceived negative judgement. Both emotions result from and give rise to the instigation of thoughts of not being good enough, having done wrong, or having failed, and are consistent with the experience of distress. "Shame plays a major role in the eventual consequences of diabetes self-management"^[13]. Embarrassment and shame are also associated with specific diabetes symptoms which are both embarrassing to experience and for which to seek help^[14].

Stigma

"Health related stigma is a negative social judgement based on a feature of a condition or its management that may lead to perceived or experienced exclusion, rejection, blame, stereotyping and/or status loss"^[15].

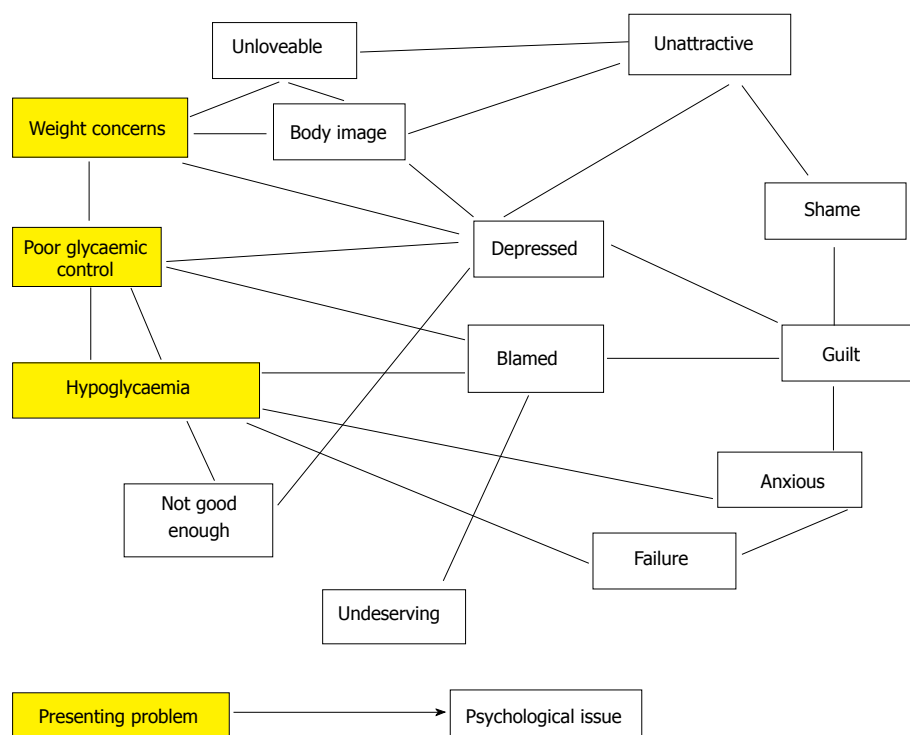


Figure 1 The spider's web: Visual representation of interconnecting psychological issues associated with the presenting problems of a female with type 1 diabetes.

Consequences of stigma span emotional, behavioural and social domains with specific implications of an unwillingness to disclose the condition which may compromise care, and fear of being judged or blamed for suboptimal diabetes management. A model is proposed to understand diabetes-related stigma^[15].

Guilt

Guilt is a personal emotion experienced when there is recognition that something has not been done as believed it should have been, or something has been done that should not have been. It is similar to shame in its negative impact on self esteem but tends to relate to a specific action whereas shame is more to do with the perception of self. It evokes efforts to correct or make reparation, however this can result in perpetuating the negative feelings as a consequence of negative thoughts about self worth.

Each of these emotional aspects are self managed either through avoidance, striving to maintain invisibility, with poor outcomes or by delayed disclosure which can intensify the distress, at least in the short term, and frequently are also associated with poor outcomes.

There is a requirement for the healthcare team to be sensitised to these issues and to develop styles of communication that are empathic, reflective and non judgemental.

Models of therapy

Assessment and formulation guide treatment plans and until there is a robust evidence base specific to

people with diabetes that suggests otherwise there is a choice of psychological therapies available for accredited practitioners to use. Behaviour therapy^[16] focuses on modification of observable behaviours without taking into account "invisible" emotions and cognitions and is rarely an appropriate model to use with people with type 1 diabetes.

Cognitive behavioural therapy

Traditional cognitive behavioural therapy is a goal oriented, problem focussed therapy that combines behavioural and cognitive models. It is a collaborative approach that focuses on current problems rather than past issues. It has evolved as a specific treatment for symptoms associated with specific diagnoses, and is used to challenge cognitive distortions thereby promoting behaviour change^[17].

Acceptance and commitment therapy

Acceptance and Commitment Therapy is an example of "Third Wave Therapies". It is particularly relevant in the context of fluctuating and frequently negative thoughts and feelings associated with diabetes. Rather than focus on the influence of emotions and cognitions as drivers for behaviour, the emphasis is on promoting value driven behaviour. The treatment facilitates a willingness to step back, notice and accept thoughts as they occur without becoming ensnared by the emotional response. Individuals commit to value driven actions whilst noticing the thoughts and feelings that invite self-sabotaging behaviour. For a detailed review of the full range of Third Wave therapies the reader is

invited to a recent review article by Kahl *et al*^[18].

Cognitive analytic therapy

Cognitive analytic therapy is a time limited therapy which integrates concepts from cognitive and psychodynamic models^[19]. The treatment involves the identification of sequences of thoughts and emotions that explain how a problem is established and maintained. The recognition of unhelpful self-sabotaging patterns of interaction derived from early experience, replayed in later life are interpreted in the context of diabetes management.

CONCLUSION

Non-psychiatric psychological aspects of living with type 1 diabetes interconnect with the day to day self-management tasks and related diabetes distress. There is a need for the diabetes healthcare team to be sensitive to the underlying issues and to be confident in the use of consultation styles that facilitate recognition, assessment, and appropriate signposting for specialised support and treatment. There is an unequivocal need for the multi-disciplinary team to include experienced psychological-therapists with considerable knowledge of diabetes management so that difficulties can be addressed as an integral part of the diabetes care available.

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Insulin action in muscle and adipose tissue in type 2 diabetes: The significance of blood flow

Vaia Lambadiari, Konstantinos Triantafyllou, George D Dimitriadis

Vaia Lambadiari, Konstantinos Triantafyllou, George D Dimitriadis, Second Department of Internal Medicine, Research Institute and Diabetes Centre, Attikon University General Hospital, Athens University Medical School, GR 12462 Haidari, Greece

Author contributions: Lambadiari V conceived the idea, drafted the manuscript and approved the final version of the manuscript; Triantafyllou K scanned the literature, reviewed the draft and approved the final version; Dimitriadis GD reviewed the draft, approved the final version and he is the guarantor of the manuscript.

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Correspondence to: Dr. Konstantinos Triantafyllou, Assistant Professor of Gastroenterology, Second Department of Internal Medicine, Research Institute and Diabetes Centre, Attikon University General Hospital, 1 Rimini Street, GR 12462 Haidari, Greece. ktriant@med.uoa.gr

Telephone: +30-210-5832087

Fax: +30-210-5326454

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Abstract

Under normal metabolic conditions insulin stimulates microvascular perfusion (capillary recruitment) of skeletal muscle and subcutaneous adipose tissue and thus increases blood flow mainly after meal ingestion or physical exercise. This helps the delivery of insulin

itself but also that of substrates and of other signalling molecules to multiple tissues beds and facilitates glucose disposal and lipid kinetics. This effect is impaired in insulin resistance and type 2 diabetes early in the development of metabolic dysregulation and reflects early-onset endothelial dysfunction. Failure of insulin to increase muscle and adipose tissue blood flow results in decreased glucose handling. In fat depots, a blunted postprandial blood flow response will result in an insufficient suppression of lipolysis and an increased spill over of fatty acids in the circulation, leading to a more pronounced insulin resistant state in skeletal muscle. This defect in blood flow response is apparent even in the prediabetic state, implying that it is a facet of insulin resistance and exists long before overt hyperglycaemia develops. The following review intends to summarize the contribution of blood flow impairment to the development of the atherogenic dysglycemia and dyslipidaemia.

Key words: Insulin resistance; Muscle blood flow; Glucose uptake; Adipose tissue blood flow; Diabetes

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Core tip: Insulin resistance and type 2 diabetes present with diminished glucose transport and disposal in muscles and fat and inadequate inhibition of lipolysis after meal ingestion or during physical exercise. This defect lies mainly in the cellular and subcellular level of insulin action. However, the resistance in the haemodynamic properties of insulin is another facet of type 2 diabetes and the metabolic syndrome. In this review, we intend to summarize the contribution of this impairment to the development of the atherogenic dysglycemia and dyslipidaemia.

Lambadiari V, Triantafyllou K, Dimitriadis GD. Insulin action in muscle and adipose tissue in type 2 diabetes: The significance of blood flow. *World J Diabetes* 2015; 6(4): 626-633 Available

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INTRODUCTION

The role of insulin in regulating glucose disposal in peripheral tissues, such as skeletal muscle and adipose tissue is well established. In the 50s, when it was discovered that insulin stimulated glucose uptake and disposal into the muscles, this effect was thought to be the most important way by which insulin regulated glycaemia *in vivo*. When the glucose/fatty acid regulatory cycle was discovered in the 60s a new insight of the way that insulin regulates glucose metabolism was introduced^[1]. However, apart from its direct action on cells, insulin is also a vasoactive hormone, and it is now recognized that its vascular and metabolic actions are closely linked. Baron *et al*^[2-4] originally introduced the concept that insulin might control its own access and that of other substrates - like glucose, lipids and several signalling molecules- to peripheral tissues, by increasing blood flow, and that this effect is compromised in states of insulin resistance.

In states of metabolic dysregulation, as in diabetes and obesity, there is deterioration in the cellular effects of insulin in peripheral tissues, which leads to a reduced ability of the latter to stimulate glucose uptake from the skeletal muscle and adipose tissue, as well as to inhibit lipolysis in fat depots^[5]. Apart from the defects at the cellular level, the metabolic derangement could also be a result of the inability of insulin to cause vasodilatation and delivery of substrates to peripheral tissues especially in the postprandial period. This could contribute to the progression to type 2 diabetes as well as to the development of atherosclerosis, which is often evident even before overt hyperglycaemia develops^[6].

In this review we summarize the current understanding of insulin action on peripheral blood flow and its implications on metabolic impairment both under fasting and postprandial conditions in type 2 diabetes.

INSULIN AND THE VASCULATURE IN NORMAL METABOLIC PHYSIOLOGY

Skeletal muscle

In skeletal muscle, insulin promotes the rate of glucose transport and the activities of hexokinase and 6-phosphofructokinase and subsequently the rate of glycolysis. In terms of protein metabolism, insulin increases synthesis and decreases degradation of proteins, in favour of an anabolic process^[1]. Insulin also enhances vasodilatation and capillary recruitment, consequently increasing the flow of nutrients in peripheral tissues and especially in skeletal muscle^[7]. It acts through traditional insulin receptors on the vascular endothelium to stimulate production of nitric

oxide and induce vasodilatation^[8]. The endothelial insulin response is mediated through a PI3-kinase pathway, which after several intermediate steps ends up activating endothelial nitric oxide synthase (eNOS)^[6].

Blood flow is highly important for the metabolic function of skeletal muscle and under normal conditions increases after meal ingestion and during exercise and a correlation between the rate of insulin stimulated glucose uptake and the extent of vasodilatation seems to exist^[9].

Insulin stimulates skeletal muscle glucose disposal and total muscle blood flow in a time- and dose-dependent fashion. *In vivo*, it enhances nitric oxide synthase-dependent vascular actions, in order to increase total skeletal muscle blood flow and to recruit muscle capillaries (by relaxing resistance and terminal arterioles, respectively). It is speculated that enhancing blood flow in this way on resistance vessels may induce the delivery of glucose and insulin to peripheral tissues and thus contribute to overall glucose disposal.

Capillary blood volume increases when precapillary arterioles dilate, thus increasing the flow to previously unperfused or underperfused areas, and total blood flow to skeletal muscle increases when larger resistance vessels relax^[10].

Insulin increases tissue perfusion by augmenting microvasculature and, at normal concentrations, the rise in total muscle blood flow follows 60-90 min later^[11,12].

Both haemodynamic effects of insulin, muscle blood flow increase and capillary recruitment seem to be independent of each other. Capillary recruitment occurs earlier *in vivo*, and at lower doses of insulin^[13].

Insulin resistance may correlate to endothelial dysfunction in many ways, including dysregulation of sub-cellular signalling pathways that influence both insulin action and nitric oxide production^[14,15].

Adipose tissue

Subcutaneous adipose tissue represents about 85% of whole body fat stores in subjects with various degrees of adiposity. Its main metabolic role is the storage of triglycerides which derive from energy overflow, and the release of stored lipids when other tissues are in need. Adipose tissue metabolism is under distinct control: usually, when a person consumes a meal, within the first hour postprandially, fat catabolism converts to fat storage, while the opposite happens in the case of physical activity. Adipose tissue interacts with the circulation by providing or drawing triglycerides and non-esterified fatty acids depending on metabolic needs. There are two kinds of triglyceride-rich lipoproteins: (1) chylomicrons, the largest particles, that carry the fat from absorbed nutrients within the intestine; and (2) very-low-density lipoproteins, that carry "endogenous" triglycerides and are released by the liver. Chylomicron- triglycerides are preferably stored within adipose tissue, and the fatty acid composition of adipose tissue (*i.e.*, the kind of fatty acids that form its triglycerides) usually represents the

composition of a person's dietary fat intake, suggesting that adipose tissue triglycerides derive mainly from the ingested fat through diet. However, a proportion of plasma triglycerides are endogenously produced from non-lipid substrates (*de novo* lipogenesis) in adipose tissue^[16-22].

In terms of metabolic regulation, adipose tissue can be divided into central (abdominal) and peripheral (lower body) depots. An unfavourable metabolic profile has been related to central fat accumulation (visceral and subcutaneous, each with distinct metabolic, endocrine and paracrine characteristics and blood flow rates)^[23,24].

Adipose tissue regulates its metabolism, at least in part, by increasing its blood flow rate mainly in the early postprandial period^[16]. Capillary perfusion is essential for that function. In the case of increased energy demands, as in physical activity, blood flow increases to facilitate the delivery of lipolytic products to peripheral tissues. Furthermore, after meal consumption, it helps the delivery of ingested substrates to fat depots for storage^[16]. Adipose tissue blood flow responses are subject to adrenergic stimulation or inhibition. Adrenaline administration stimulates postprandial increases whereas beta-blockers inhibit the latter. Genetic studies in subcutaneous adipose tissue biopsies have identified expression of the type A receptor of A natriuretic peptide and of the synthase of nitric oxide, and have found an association of those with post-challenge blood flow responses^[16,25-28].

Lipid kinetics and subcutaneous adipose tissue blood flow alterations are closely linked. More specifically, blood flow rises in response to an increased demand for lipolytic products as energy, or that for cleavage of free fatty acids from the circulation. In euglycaemic subjects with normal weight blood flow peaks within the first hour after a glucose load or a mixed meal. This facilitates the postprandial delivery of energy substrates and insulin to the fat depot, leading to adipose tissue lipoprotein lipase stimulation which stores circulating triglycerides and the suppression of hormone-sensitive lipase, which results in the inhibition of endogenous lipolysis^[16,29-31].

On the other hand, visceral adiposity exerts even more unfavourable metabolic actions. Increased visceral fat has been associated with atherogenic dyslipidaemia and the development of atherosclerosis, even in non-diabetic individuals^[32]. Although increased abdominal fat is in general positively associated with markers of inflammation and atherosclerosis, visceral fat is more strongly correlated with C-reactive protein, monocyte chemoattractant protein-1, interleukin-6 and isoprostanes independently of total adiposity, indicating a major role in systemic inflammation^[33]. Furthermore, visceral fat has been more strongly related to hypertension both in men and women, and provides information towards the latter above BMI and waist circumference. However, subcutaneous adipose tissue is also contributing to vascular dysfunction,

possibly through the actions of leptin apart from the presence of insulin resistance^[34]. Both adipose tissue beds' size has been found correlated to adipose tissue blood flow, independently of BMI, leptin or adiponectin concentrations^[35].

INSULIN AND BLOOD FLOW IN TYPE 2 DIABETES

Skeletal muscle

Insulin provokes microvascular recruitment in skeletal muscle^[10]. Impaired muscle blood flow as a facet of insulin resistance in subjects with either dysglycaemia or diabetes is well recognized in the literature. In the early 90s Steinberg *et al.*^[36] have shown that obese insulin resistant subjects present with an endothelial dysfunction and during a euglycaemic hyperinsulinaemic clamp they fail to increase endothelium-dependent vasodilation. In these trials, catheterizations of the femoral artery was used to measure the response to an intra-arterial vasodilator stimulus, comparing control to euglycemic-hyperinsulinemic clamp conditions^[36].

Thereafter, it was suggested that since insulin exerts its vasodilatory effects through endothelial nitric oxide release, *in vivo* stopping nitric oxide production could inhibit insulin's vasoactive actions in skeletal muscle and consequently reduce glucose uptake^[37]. Moreover in obese insulin resistant subjects, insulin resistance in skeletal muscle was promoted by the increased endogenous endothelin action^[38].

A rat model of insulin resistance has shown that endothelial-dependent vasodilation is blunted, in part due to an unresponsive nitric oxide synthase to insulin, leading to decreased nitric oxide levels in the endothelial cells^[39,40].

In type 2 diabetes and other insulin-resistant states, impaired suppression of adipose tissue lipolysis and postprandial hyperglycemia favour non-esterified fatty acid utilization and oxidation and increase glucose uptake from insulin independent tissues (like liver). Dyslipidaemia, usually related to lack of insulin sensitivity, enhances atherosclerosis and triggers inflammation in endothelial cells^[41].

In insulin-resistant patients basal blood flow is generally not altered^[42-44]. Laakso *et al.*^[45] demonstrated that insulin cannot effectively increase muscle blood flow in type 2 diabetic patients, using the combined euglycemic clamp and leg balance techniques during different insulin infusions. They also concluded that impaired insulin-dependent rise in skeletal muscle blood flow can be attributed to the diabetic milieu and not to obesity, in a study of obese diabetic patients^[45].

Lambadiari *et al.*^[46] studied simultaneously lean subjects with insulin sensitivity varying from normoglycaemic insulin-resistant first-degree relatives of diabetic subjects to prediabetic and diabetic patients with either isolated postprandial hyperglycaemia or overt diabetes^[46]. They demonstrated that using a

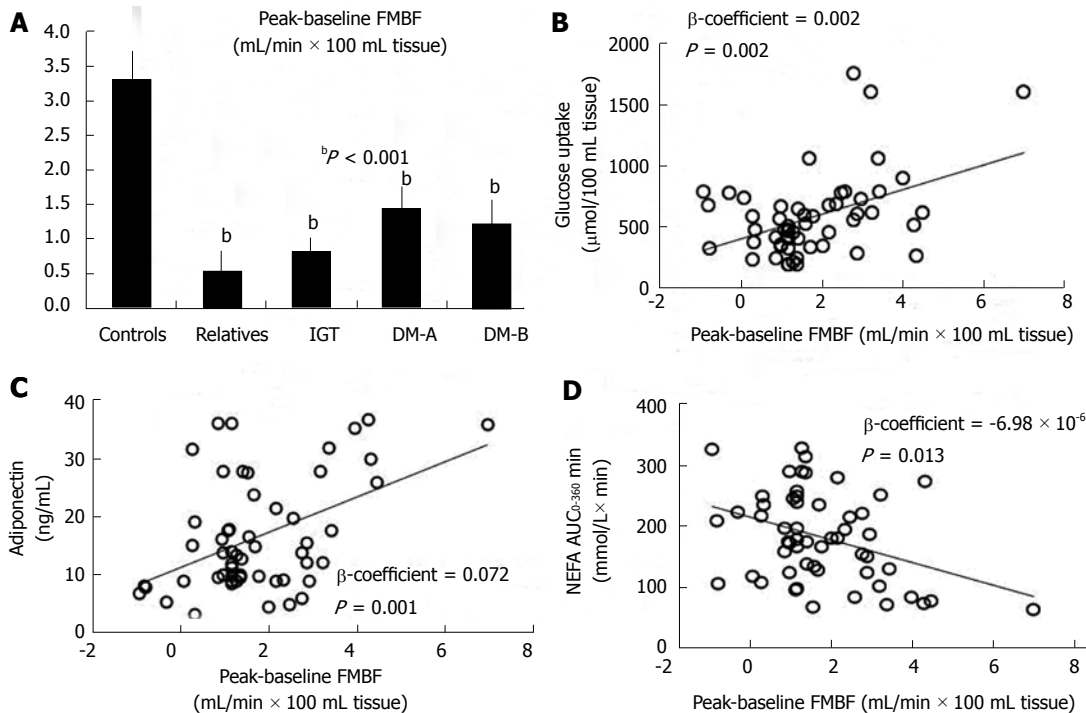


Figure 1 Forearm muscle blood flow peak-baseline values in all groups (A), and associations between peak-baseline forearm muscle blood flow and forearm muscle glucose uptake (B), plasma adiponectin (C) and postprandial non-esterified fatty acids (D), in subjects at all stages of type 2 diabetes. A: bP overall < 0.001; B: Forearm muscle glucose uptake = $427.9 + 101.4$ peak-baseline FMBF, $P = 0.001$; C: Adiponectin = $12.17 + 3.05$ peak-baseline FMBF, $P < 0.001$; D: Postprandial NEFA (AUC₀₋₃₆₀) = $209.5 - 18.52$ peak-baseline FMBF, $P = 0.005$. FMBF: Forearm muscle blood flow; NEFAs: Non-esterified fatty acids. IGT: Impaired glucose tolerance; DM: Diabetes mellitus. Adapted from Lambadiari *et al.*^[46].

physiological mixed meal as a stimulus, the postprandial augmentation in forearm muscle blood flow is blunted throughout all stages of metabolic impairment compared to controls; this occurs even before overt hyperglycaemia develops. The latter affected glucose disposal in muscle, which was also unresponsive after meal delivery and was also positively correlated to the post-load muscle blood flow differences. Lipid substrates affected blood flow peak as well. Triglyceride levels had a negative impact on blood flow responsiveness in the fed as well as in the fasting period. Post-challenge non-esterified fatty acids levels exhibited a negative effect on blood flow responsiveness, suggesting a possible mechanism for the decrease in muscle glucose clearance after the meal. A lower serum adiponectin level was also seen in the diabetic and the prediabetic insulin-resistant subjects, with the latter being positively related to the decreased postload blood flow rise^[46] (Figure 1).

In subjects with morbid obesity postprandial muscle blood flow was also blunted in a study by the same group and this contributed to the decrease in muscle glucose uptake postprandially^[47]. The same was observed by the same group in another insulin resistant state, such as hypothyroidism, in which a decreased postprandial blood flow response was coupled with an impairment in muscle glucose uptake^[48]. In a study by Magalhães *et al.*^[49] administration of metformin to non-obese type 2 diabetic patients increased post-load forearm muscle blood flow and lowered free fatty acids,

thus improving glucose oxidation and insulin sensitivity in the muscle bed.

However, there is not universal agreement with the above mentioned results, since numerous studies have failed to reveal a defect in insulin-mediated blood flow in type 2 diabetic patients^[42,50]. There is a certain discrepancy since the literature either confirms or not a substantial^[45,51], or an unimportant correlation between insulin kinetics, muscle blood flow and glucose disposal^[52,53].

Some of these discrepancies may at least partially explained by the different studies populations and by the experimental protocol used. The commonly used clamp technique is not physiological, because these exceptionally high insulin concentrations are not normally present for long after meal consumption. Hence, one could question the physiological significance of such an increase in blood flow rates. A normal stimulus, such as a mixed meal, can provide evidence of a real life metabolic state^[46,54]. However, not only the type of meal but the method for the detection of blood flow is important in this evaluation.

Adipose tissue

In lean insulin-sensitive subjects, abdominal adipose tissue blood flow increases by two- to four-times in response to feeding. The same seems to be true for blood flow in lower body fat depots (thigh) and forearm tissues. Physiologically, adipose tissue blood flow peaks within half to one hour after nutrient ingestion. This rise

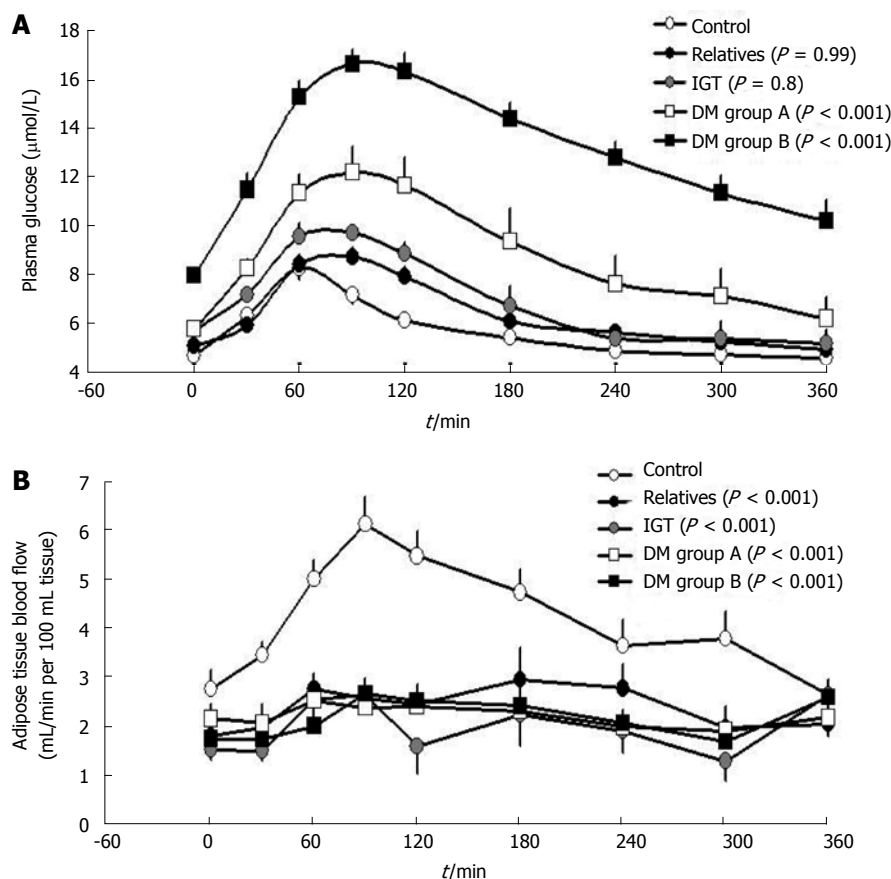


Figure 2 Plasma glucose, and adipose tissue blood flow in healthy subjects (control), first degree relatives of subjects with type 2 diabetes (relatives), subjects with impaired glucose tolerance, subjects with type 2 diabetes with postprandial hyperglycemia and normal fasting plasma glucose levels (diabetes mellitus group A) and subjects with type 2 diabetes with both postprandial and fasting hyperglycemia (diabetes mellitus group B). " P " stands for overall comparison (repeated measures ANOVA) between control and patient groups. IGT: Impaired glucose tolerance. Adapted from Dimitriadis *et al*^[62].

coincides with plasma insulin peak and the inhibition of lipolysis^[23].

By studying obese or diabetic individuals in the 90s, Jansson *et al*^[55,56] detected impairment in adipose tissue blood flow response as a facet of insulin resistance coupled with hypertension and elevated lipolysis products.

Since then, numerous studies have shown that in states of decreased insulin sensitivity, as in "diabesity", the postprandial increase in adipose tissue blood flow is reduced^[57-60]. Karpe *et al*^[61] showed that the postprandial blood flow rise is associated with insulin sensitivity independently of weight. Moreover, they showed that hyperinsulinaemia affects adipose tissue blood flow indirectly by stimulation of sympathetic activity^[61].

Previous reports in healthy subjects by the same research group have demonstrated that nitric oxide determines the actual rate of adipose tissue blood flow, whereas postprandial augmentation of it is mainly under adrenergic regulation *in vivo*, and that blood flow regulation and lipolysis are co-regulated^[25].

Dimitriadis *et al*^[62] showed an altered fasting and postprandial adipose tissue blood flow in all stages of metabolic regulation, from the prediabetic state to clinical diabetes, even in lean first-degree relatives of

diabetic patients. This study, using a mixed meal as a stimulus, showed significant association of postprandial adipose tissue blood flow with insulin sensitivity. Basal and post-challenge triglycerides were negatively correlated to the responsiveness of adipose tissue blood flow; the same was true for postprandial non-esterified fatty acids but not for fasting values^[62] (Figure 2).

Fatty acid overflow (mainly palmitic acid), a well recognized factor to interfere with insulin sensitivity, causes both cellular and vascular insulin dysfunction^[63]. The increased rate of lipolysis in diabetes may result in increased lipid oxidation and a decreased glucose oxidation rate^[61,64].

Impairment in blood flow response of adipose tissue has been found in other insulin resistance states. Mitrou *et al*^[47] study in morbidly obese subjects, shows a drop in postprandial adipose tissue blood flow response and in glucose disposal per 100 mL fat tissue. However, glucose fractional extraction from subcutaneous fat depot was unaltered and glucose uptake per total fat mass was increased. Thus, it seems that although an expanded adipose tissue causes insulin resistance, total fat mass provides a buffer for glucose overflow and compensates for insulin resistance.

Diabetic subjects fail to increase adipose tissue blood flow during prolonged exercise of moderate

intensity, in combination to the inability to regulate non-esterified fatty acid mobilization and adipose tissue glucose clearance^[65]. Exercise augments adipose tissue lipolysis in diabetic patients, but due to an impaired blood flow response, a high proportion of free fatty acids that come from lipolysis cannot be released into the circulation. Visceral glucose release is lower than whole-body glucose utilisation during exercise and post-exercise recovery^[66].

The cause of the impairment in postprandial adipose tissue blood flow reactivity in insulin resistance is still obscure. One potential explanation is the downregulation of the adrenergic receptor during chronic sympathetic stimulation in a milieu of long-standing hyperinsulinaemia. Sympathetic nervous system overactivity induces oxidative stress. Increased levels of circulating free oxygen radicals consumes nitric oxide, and inhibits physiological insulin-dependent vasodilatation^[23]. Interestingly, the transcription of eNOS and natriuretic peptide receptor-A, which are expressed in adipose tissue and interfere with vasoactive actions, was associated with adipose tissue blood flow responsiveness to feeding. This finding suggests that part of blood flow regulation is at a transcriptional level and it is independent of adiposity^[28].

At the bottom line, adipose tissue is an important buffer against the postprandial spill-over of nonesterified fatty acids in the circulation, thus protecting other peripheral tissues. This buffering effect is dysregulated in states of an over-expanded inflammatory, hypoxic adipose tissue, where the postprandial blood flow response is minimized, potentially leading to atherogenic dyslipidaemia^[67].

CONCLUSION

Resistance in the haemodynamic actions of insulin is essential for the development of type 2 diabetes and insulin resistant states as well as their complications, namely cardiovascular disease, the development of which often precedes overt hyperglycaemia and which is the primary cause of mortality within the diabetic population.

Insulin normally stimulates microvascular perfusion (capillary recruitment) of skeletal muscle and subcutaneous adipose tissue and thus increases blood flow mainly after meal ingestion or physical exercise. This effect is impaired in insulin resistance and type 2 diabetes early during metabolic dysregulation development and reflects early-onset vascular dysfunction. Failure of insulin to increase muscle blood flow results in the inability to regulate its own delivery and that of other substrates and hormones and consequently to a decrease in glucose disposal. In fat depots blood flow is closely related to triglyceride clearance and non-esterified fatty acid kinetics. Therefore, we may speculate that dysregulation of post-challenge blood flow responsiveness in skeletal muscle and adipose tissue may together underlie some of the detrimental

aspects of insulin resistance.

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Assessment of cardiovascular risk in diabetes: Risk scores and provocative testing

Teresa Lam, Kharis Burns, Mark Dennis, N Wah Cheung, Jenny E Gunton

Teresa Lam, Kharis Burns, N Wah Cheung, Jenny E Gunton, Department of Diabetes and Endocrinology, Westmead Hospital, Sydney 2145, Australia

Kharis Burns, Mark Dennis, N Wah Cheung, Jenny E Gunton, Sydney Medical School, the University of Sydney, Sydney NSW 2006, Australia

Mark Dennis, Department of Cardiology, Royal Prince Alfred Hospital, Sydney 2050, Australia

Jenny E Gunton, Faculty of Medicine, Westmead Hospital, University of Sydney, Sydney 2145, Australia

Jenny E Gunton, St Vincent's Clinical School, University of New South Wales, Sydney 2010, Australia

Jenny E Gunton, Diabetes and Transcription Factors Group, Garvan Institute of Medical Research, Sydney NSW 2010, Australia

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Correspondence to: Jenny E Gunton, Professor, Faculty of Medicine, Westmead Hospital, University of Sydney, Room 2040, Clinical Sciences Corridor, Sydney NSW 2145, Australia. j.gunton@garvan.org.au

Telephone: +61-2-98458089

Fax: +61-2-92958404

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morbidity and mortality among patients with diabetes mellitus, who have a risk of cardiovascular mortality two to four times that of people without diabetes. An individualised approach to cardiovascular risk estimation and management is needed. Over the past decades, many risk scores have been developed to predict CVD. However, few have been externally validated in a diabetic population and limited studies have examined the impact of applying a prediction model in clinical practice. Currently, guidelines are focused on testing for CVD in symptomatic patients. Atypical symptoms or silent ischemia are more common in the diabetic population, and with additional markers of vascular disease such as erectile dysfunction and autonomic neuropathy, these guidelines can be difficult to interpret. We propose an algorithm incorporating cardiovascular risk scores in combination with typical and atypical signs and symptoms to alert clinicians to consider further investigation with provocative testing. The modalities for investigation of CVD are discussed.

Key words: Diabetes; Cardiovascular risk; Risk scores; Provocative testing; Silent ischaemia; Atypical symptoms

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Core tip: Current guidelines focus on testing for cardiovascular disease in symptomatic patients. However, patients with diabetes often present with atypical features of underlying vascular disease. An individualised approach to cardiovascular risk estimation and management is needed in patients with diabetes. We propose an algorithm incorporating cardiovascular risk scores in combination with typical and atypical signs and symptoms to alert clinicians to consider further investigation with provocative testing. The modalities for investigation of cardiovascular disease are discussed.

Abstract

Cardiovascular disease (CVD) is the leading cause of

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of cardiovascular risk in diabetes: Risk scores and provocative testing. *World J Diabetes* 2015; 6(4): 634-641 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i4/634.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i4.634>

INTRODUCTION

The incidence of diabetes mellitus is increasing globally. The World Health Organisation estimated there were 30 million people who had diabetes worldwide in 1985. This number increased to 217 million in 2005, and by the year 2030, it is predicted this number will increase to at least 366 million^[1].

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among people with diabetes mellitus, who have a risk of cardiovascular mortality two to four times greater than that of people without diabetes^[2]. Diabetes is commonly associated with other cardiovascular risk factors, interacting with these to accelerate atherogenesis^[3-6]. Multifactorial interventions, such as those targeting hyperglycaemia, hypertension and hypercholesterolaemia, significantly reduce the risk of both fatal and non-fatal CVD^[7]. The National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATPIII) has listed diabetes as a coronary heart disease (CHD) equivalent, which would obviate the need for risk stratification. However, clearly not all patients with diabetes have the same cardiovascular risk. An individualised approach to cardiovascular risk estimation and management is needed^[8]. Furthermore there is a high prevalence of asymptomatic coronary artery disease (CAD), and higher incidences of silent ischaemia and of atypical symptoms^[9].

Over the past two decades, there has been a significant reduction in the incidence of diabetes-related complications. The greatest absolute decline was in the number of cases of acute myocardial infarction, likely reflecting a combination of enhanced awareness, detection and early management of risk factors^[10]. The development of statistical models, such as the Framingham equations, has allowed the probability of future cardiovascular events to be calculated based on multiple risk factors^[11]. This allows targeted preventative therapy for those with highest absolute risk^[12]. However, the majority of these risk equations have not been validated enough in the diabetic population, and either overestimate or underestimate cardiovascular risk.

USE OF CARDIOVASCULAR RISK SCORES IN DIABETES MELLITUS: PREDICTORS, VALIDATION AND IMPACT ON CLINICAL OUTCOME

There have been a multitude of risk scores developed

over the past decades, but only a few have been specifically developed for use in the diabetic population. In a systematic review of prediction models for CVD risk in type 2 diabetes^[13], 12 of 45 prediction models were specifically developed for patients with type 2 diabetes. The majority of these predicted 5-year risk of CHD or total CVD, with the most commonly used predictors being age, sex, duration of diagnosed diabetes, HbA1c (glycosylated haemoglobin A1c) and smoking. Non-traditional risk factors, such as novel biomarkers and low birthweight, have generally not been incorporated into these models, and are of questionable clinical significance^[14,15]. Prediction models derived from the general population, in which diabetes was used as a predictor, included other risk factors such as age, sex, systolic blood pressure, smoking and cholesterol. Of the risk scores, only a third had been externally validated in a diabetic population^[13].

The International Diabetes Federation recommends calculating cardiovascular risk in patients with type 2 diabetes with prediction models that can be applied to the diabetes population, including the United Kingdom Prospective Diabetes Study (UKPDS) risk engine^[16]. This risk engine provides a comprehensive model for predicting CHD risk in patients with type 2 diabetes. The Australian National Vascular Disease Prevention Alliance^[17] recommends using both the Framingham prediction model and UKPDS risk engine. However, certain subgroups (Table 1) are at high risk of cardiovascular events because of their comorbidities, and a calculation of absolute CVD is not considered necessary^[17].

Kengne *et al.*^[11] evaluated the performance of the Framingham and UKPDS models in a cohort of patients with established type 2 diabetes, and found both models to overestimate the 4-year risk of CHD; by 146% and 198% respectively. The Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled Evaluation model^[18] was developed from a contemporary multinational cohort of diabetic patients, and includes both retinopathy and microalbuminuria as risk predictors. They are both significantly associated with CVD. It has largely outperformed the Framingham models in validation studies, with only a modest risk underestimation^[19]. Similarly, the Fremantle prediction model^[20], developed from a type 2 diabetic cohort, had good positive and negative predictive values, but requires further validation^[13].

Very few studies have examined the impact of applying a prediction model in clinical practice. In a cohort of patients with type 2 diabetes at high risk of CVD, clear documentation of a cardiovascular risk prediction score on patient medical records was associated with more intensive intervention through prescription of lipid-modifying or antihypertensive medications^[21]. Furthermore, use of risk scores has resulted in improvements in lipid profiles and significant reductions in risk of CHD^[22].

The use of cardiovascular risk scores has been

Table 1 Clinical features suggesting diabetes patients at high risk

Diabetes and age > 60 yr
Diabetes and microalbuminuria (> 20 mcg/min or urine albumin to creatinine ratio > 2.5 mg/mmol for males, > 3.5 mg/mmol for females)
Diabetes and moderate or severe chronic kidney disease (persistent proteinuria or eGFR < 45 mL/min per 1.73 m ²)
Diabetes and a previous diagnosis of familial hypercholesterolaemia in the individual
Diabetes and systolic blood pressure ≥ 180 mmHg or diastolic blood pressure ≥ 110 mmHg
Diabetes and serum total cholesterol > 7.5 mmol/L

Albumin to creatinine ratio - confirmed on second test and not due to another cause (*e.g.*, urinary tract infection).

Table 2 American Diabetes Association guidelines on stress testing in diabetic patients^[23]

Typical or atypical cardiac symptoms
Resting electrocardiogram suggestive of ischaemia or infarction
Peripheral or carotid occlusive arterial disease

Table 3 Signs and symptoms of concern in an otherwise asymptomatic patient

Symptoms suggestive of cardiovascular autonomic neuropathy
Resting tachycardia
Postural hypotension
Signs/symptoms suggestive of coexisting vascular disease
Erectile dysfunction
Claudication symptoms
Carotid bruit
Diminished/absent peripheral pulses
Inappropriate exercise tolerance
Shortness of breath without clear pathology

incorporated into multiple guidelines, and may be a useful initial step towards CVS risk stratification. However, given the modest performance of most prediction models, and need for more extensive validation studies, further decision-making may be useful before proceeding to provocative testing.

PROVOCATIVE TESTING: FACTORS INFLUENCING DECISION MAKING

The onset of microvascular and macrovascular complications in diabetic patients is frequently insidious, with the absence of typical symptoms often delaying diagnosis. Studies have demonstrated that a significant percentage of patients with diabetes who have no symptoms of CAD have abnormal stress tests, either by stress electrocardiogram (ECG), stress echocardiogram or stress nuclear perfusion imaging^[23]. CAD in patients is often silent, more advanced and associated with less favourable prognosis than those in the non-diabetic population^[23]. Diabetic cardiovascular autonomic neuropathy (CAN) resulting in damage to the neural fibres responsible for innervation of the heart and cardiac vessels can lead to atypical clinical manifestations, hence the concept of screening an asymptomatic patient is complex^[24]. However, the American Heart Association recommends against routine screening in diabetic patients who are asymptomatic, as there is currently no outcome data to support stress

testing in this group of patients^[25]. In contrast, the American Diabetes Association (ADA) recommends exercise stress testing in both symptomatic and asymptomatic patients with specific criteria (Table 2).

There are further specific guidelines for screening for CVD before beginning moderate to vigorous exercise training program which expand to include the length of disease; 15 years for type 1 diabetes and 10 years for type 2 diabetes, and age ≥ 35 for type 2 diabetes. Given we encourage all our patients to exercise as part of a general care plan for diabetes, it may be argued that all patients should be screened prior to this recommendation.

Furthermore, given symptoms may be atypical in the diabetic patient, there may be some clues to the presence of CVD to alert the treating clinician to investigate (Table 3). Symptoms of exercise intolerance and erectile dysfunction may suggest underlying coronary artery disease and may prompt further investigation. Peripheral arterial disease and the presence of Q waves and or ST/T wave abnormalities on ECG have also been shown to predict presence of coronary artery disease^[26].

Erectile dysfunction

Erectile dysfunction may be the manifestation of endothelial dysfunction in many cases and is recognised to represent the coexistence of vascular disease in other areas^[27]. It has been documented that men with

Table 4 Sensitivity and specificity of provocative tests in patients with diabetes

Diagnostic test	Sensitivity (%)	Specificity (%)
Exercise stress test ^[33]	47	81
Stress echo ^[35]	82	54
Stress nuclear perfusion study ^[36]	86	56
CT coronary angiogram ^[40]	76	90
Coronary calcium score ^[39]	64-75	75-83

CT: Computed tomography.

no cardiac symptoms and erectile dysfunction have increased risk of cardiac events over the following 3-5 years^[28]. Furthermore a large meta-analysis found patients with erectile dysfunction have an increased risk of CVD, cerebrovascular disease, stroke and all-cause mortality independent of traditional risk factors^[29]. The suggestion that patients with erectile dysfunction are likely to be vasculopathic validates the investigation of cardiovascular and peripheral vascular disease, even in the absence of typical symptoms. We therefore propose that patients with a history of erectile dysfunction be investigated further for underlying vascular disease.

Exercise tolerance and CAN

Autonomic dysfunction in diabetes leads to exercise intolerance. Suboptimal cardiac output in times of exertion can be a result of CAN as well as vascular disease and silent ischaemia^[30]. Signs and symptoms of CAN may include resting tachycardia due to impaired vagal tone or orthostatic hypotension^[30]. CAN significantly increases the risk of fatal or non-fatal cardiovascular event^[24]. The suspicion of CAN may therefore justify further investigation for coronary vascular disease.

Claudication symptoms

Symptoms of claudication in the diabetic patient justify consideration of investigating other vascular disease including coronary artery disease, even in the absence of symptoms. Patients with peripheral vascular disease have increased mortality from cardiovascular causes^[31]. Assessment of peripheral pulses should be performed in all patients, given this is a simple method of screening. If abnormal, further investigation with ankle brachial indices and provocative testing for cardiac ischaemia may be warranted^[31].

CHOICE OF INVESTIGATION FOR RISK STRATIFICATION IN DIABETIC PATIENTS WITH SUSPECTED CVD

The choice of investigation will depend on a number of factors including mobility, exercise tolerance, plans for future increases in exercise and potentially gender. As a baseline investigation, the American Heart Association recommends that a resting electro-

Table 5 Sensitivity and specificity of provocative testing in women^[44]

Diagnostic test	Sensitivity (%)	Specificity (%)
Exercise electrocardiogram	31-71	66-78
Exercise echocardiogram	80-88	79-86
Pharmacological echocardiogram	76-90	85-94
Nuclear perfusion study	78-88	64-91
Computed tomography coronary angiogram	97	79

cardiogram (ECG) is a reasonable tool for risk assessment in asymptomatic adults with diabetes^[32]. Beyond this, the factors influencing selection of a particular modality for provocative testing are similar between diabetic and non-diabetic patients and include availability, sensitivity and specificity and risk. Each modality has varying performance accuracy in terms of sensitivity and specificity with some specific differences in patients with diabetes (Table 4).

Exercise ECG

Exercise ECG (stress testing) is widely regarded as the first line test in mobile patients with a normal baseline electrocardiogram and it has been found to have similar predictive value between diabetic and non-diabetic populations^[33]. However sensitivity is variable, and in some studies is less than 50%^[33]. A positive test will identify the majority of patients with left main or significant multi-vessel coronary artery disease^[33]. One study found a positive predictive value of 94% in a cohort of asymptomatic older males with poorly controlled diabetes^[34].

Stress ECG is less sensitive and specific in asymptomatic populations, *i.e.*, where there is a lower pre-test probability. The test is highly dependent on the patient's capacity to exercise long enough to provide a valid test. Whilst a patient reaching above expected exercise capacity provides useful prognostic and clinical information, many diabetic patients with obesity, peripheral neuropathy, decreased physical conditioning or other co-morbidities are unable to exercise long enough to determine low cardiovascular risk. It can therefore be argued that this form of investigation is suboptimal for patients with diabetes who are unlikely to be able to reach an appropriate workload owing to co-morbidities. In women, the test may also be less useful, with quoted sensitivities of 31%-71% (Table 5).

Stress echocardiography

In the general population addition of imaging modalities such as echocardiography to stress testing provides greater diagnostic accuracy. Addition of echocardiography gives additional information about regional wall motion abnormalities (suggesting prior infarcts) and ventricular dysfunction, both of which are more common in people with diabetes. However data regarding diagnostic accuracy of stress echocardiography specifically in diabetic populations is relatively limited. Hennessy *et al.*^[35] evaluated

dobutamine stress echo in 52 patients with diabetes, finding a sensitivity of 82% but a specificity of only 54%. The positive predictive value was 84% with a poor negative predictive value of 50%^[35]. Availability may be limited by cost and operator expertise.

Nuclear perfusion scans

Stress nuclear imaging has been the most widely investigated modality for the detection of CAD in people with diabetes. The sensitivity of this tool has been quoted as 86% with a specificity of 56% in patients with diabetes^[36]. Wackers *et al.*^[37] examined asymptomatic patients with diabetes using adenosine Single Photon Emission Computed Tomography (SPECT) imaging and found positive test results for CAD in 22%. Interestingly, 41% of these patients with abnormal imaging findings would not have met usual criteria for further investigation of coronary disease according to previous ADA guidelines. Thus, use of stress imaging in selected people with diabetes who have high absolute cardiovascular risk is reasonable even if they are asymptomatic. Nuclear imaging studies can be performed with exercise, or in subjects with limited exercise capacity with other modalities to increase coronary flow such as adenosine. This modality provides information about coronary flow at rest, with exercise or stimulated stress, as well as regional wall motion, although the last is much less precise than the information obtained with echocardiography.

Computed tomography coronary angiogram and coronary calcium score

Computed tomography (CT) coronary angiogram (CTCA) may provide information on the vascular lumen and the arterial wall^[38]. In people without diabetes it has been reported to have high sensitivity^[39]. However, a study comparing the use of CTCA in diabetic vs non-diabetic patients found reduced sensitivity and specificity in people with diabetes, due to differences in artefacts and calcification^[40]. While coronary calcium score may be able to predict coronary disease beyond standard risk factors, significant stenosis can occur in the absence of calcification, so this tool should not be used in isolation^[31,41,42].

A study by Maffei *et al.*^[42] showed that coronary plaque burden and coronary calcium scores were higher in diabetic vs non diabetic patients. Furthermore it has been shown that asymptomatic patients with diabetes with high coronary artery calcium scores have a high prevalence of inducible ischaemia on stress imaging^[43]. The American Heart Association acknowledges that measurement of coronary artery calcium score is reasonable for cardiovascular risk assessment in patients with diabetes who are asymptomatic and age over 40^[32]. The efficacy of this test in women with diabetes is less clear, see below.

Gender effects

Both symptoms and pathophysiology of coronary

artery disease can differ between males and females. Women, whether diabetic or not, are more likely to have atypical symptoms and are often older at the time of onset of disease or events. Prognosis is poorer in women than men with higher mortality rates from acute myocardial infarction^[44]. Detection of disease in women is more difficult given the lower likelihood of obstructive coronary disease and apparently lower levels of clinical suspicion^[45].

As well as these issues, currently available provocative tests are both less sensitive and less specific in women^[44]. Information regarding the characteristics of coronary artery disease in diabetic women vs the general female population is surprisingly sparse. To date, guidelines suggest the use of exercise ECG testing as first line investigation in women with symptoms of coronary disease with a normal baseline ECG^[46]. If either the baseline ECG or exercise ECG is abnormal, the addition of stress testing with imaging is recommended^[46]. However, these investigations are well known to have limitations in the female population due to interference from breast soft tissue and differences in coronary anatomy in women^[45].

Stress SPECT and stress echo are considered superior to exercise ECG in women for both sensitivity and specificity. Adenosine stress nuclear imaging has similar prognostic ability in men and women, though it has been shown that women have worse clinical profiles for the same degree of imaging abnormality^[47]. However, the ultimate decision may be limited by cost and local expertise. Table 5 summaries the sensitivity and specificity of the different provocative investigations in women.

Risks associated with different testing modalities

There are risks associated with each of the tests discussed. For non-invasive stress testing such as exercise stress tests and exercise stress echo, 5% of patients may experience mild angina, shortness of breath or musculoskeletal pain. Less commonly (< 5%) chest pain, hypotension or syncope may occur and rarely (< 1%) there is a risk of acute myocardial infarction, stroke or arrhythmia^[45]. Investigations requiring contrast such as CTCA carry risks of associated renal toxicity or allergic reaction, and exposure to significant radiation with resultant cancer risk. Nuclear perfusion scans may employ the use of agents such as adenosine, which are known to induce asthma in some individuals and also involve some radiation exposure. Each of these factors must to be considered in the decision to utilise a certain modality. In women who are considering pregnancy, stress ECG or stress echocardiography are radiation-free, which is an important consideration.

SUGGESTED ALGORITHM

The decision to proceed with provocative testing should be based on a combination of cardiovascular risk

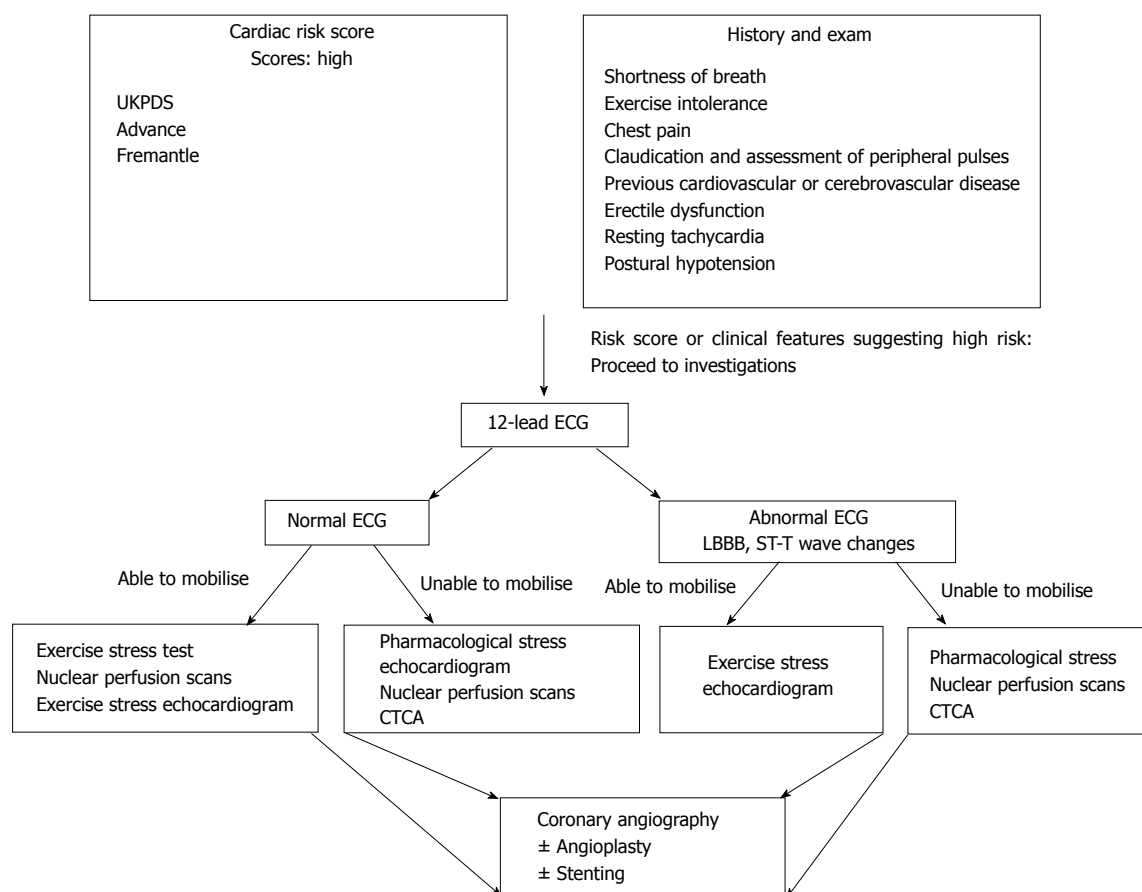


Figure 1 Suggested algorithm for investigation of cardiovascular disease in patients with diabetes. Since a 12-lead ECG is a safe and cheap test, it should be performed in people with diabetes with a low threshold. At each layer of testing, if the test is normal or unchanged from previous testing, consider whether the next level of testing is needed. ECG: Echocardiogram; LBBB: Left bundle branch block; CTCA: CT coronary angiogram; UKPDS: United Kingdom prospective diabetes study.

score and suspicious features on clinical history and or examination. As discussed above, risk calculators do not consider specific features such as erectile dysfunction or cardiac autonomic neuropathy. Therefore, risk calculators may fail to identify potential high risk features when used in isolation.

Firstly, a baseline 12-lead ECG should be performed in all patients considered at risk. Following this, the choice of modality for provocative testing will depend on factors such as abnormal resting ECG (left bundle branch block or ST-T wave changes at baseline), mobility including ability to perform exercise testing, gender, cost and access to local expertise. There is a need to highlight and alert the treating clinician to recognise novel markers of disease that have been previously under-recognised by traditional risk scores. Considering these risk factors, we propose the algorithm in Figure 1.

CONCLUSION

Patients with diabetes are at high risk of mortality from CVD. Given this group of patients often present with atypical symptoms and silent ischaemia, traditional recommendations for screening in symptomatic individuals may not be applicable. National guidelines

recommend incorporation of a cardiovascular risk score in risk stratification. Risk scores have arguably suboptimal performance when used in isolation and have not been extensively validated. Additionally, to date such clinical signs as erectile dysfunction or autonomic neuropathy have not been incorporated into cardiovascular risk prediction models, though it is well recognised that these pathologies represent underlying cardiac disease. We propose the use of a combination of a risk score and relevant clinical findings in the overall assessment of cardiovascular risk. The algorithm (Figure 1) presented may provide treating clinicians with various clues to prompt further investigation with provocative testing. There is an ongoing need for re-evaluation of guidelines for screening in this high risk patient group.

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Gene-gene, gene-environment, gene-nutrient interactions and single nucleotide polymorphisms of inflammatory cytokines

Amina Nadeem, Sadaf Mumtaz, Abdul Khaliq Naveed, Muhammad Aslam, Arif Siddiqui, Ghulam Mustafa Lodhi, Tausif Ahmad

Amina Nadeem, Department of Physiology, Army Medical College, National University of Sciences and Technology, Islamabad 46000, Pakistan

Sadaf Mumtaz, College of Medicine, Dammam, University of Dammam, Dammam 31451, Kingdom of Saudi Arabia

Abdul Khaliq Naveed, Department of Biochemistry, Islamic International Medical College, Rifah University, Rawalpindi 44000, Pakistan

Muhammad Aslam, Department of Physiology, University of Health Sciences, Lahore 54000, Pakistan

Arif Siddiqui, Department of Physiology, Islamic International Medical College, Rifah University, Rawalpindi 44000, Pakistan

Ghulam Mustafa Lodhi, Department of Physiology, AL-Nafees Medical College, Isra University, Islamabad 46000, Pakistan

Tausif Ahmad, Dean - Faculty of Pharmacy, Margalla College of Pharmacy, Margalla Institute of Health Sciences, Rawalpindi 44000, Pakistan

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Correspondence to: Dr. Amina Nadeem, Associate Professor, Department of Physiology, Army Medical College, National University of Sciences and Technology, NUST Campus, H-12, Islamabad 46000, Pakistan. aminanadeem@amcollege.nust.edu.pk
Telephone: +92-321-5231807
Fax: +92-51-9272502

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Abstract

Inflammation plays a significant role in the etiology of type 2 diabetes mellitus (T2DM). The rise in the pro-inflammatory cytokines is the essential step in glucotoxicity and lipotoxicity induced mitochondrial injury, oxidative stress and beta cell apoptosis in T2DM. Among the recognized markers are interleukin (IL)-6, IL-1, IL-10, IL-18, tissue necrosis factor- α (TNF- α), C-reactive protein, resistin, adiponectin, tissue plasminogen activator, fibrinogen and heptoglobins. Diabetes mellitus has firm genetic and very strong environmental influence; exhibiting a polygenic mode of inheritance. Many single nucleotide polymorphisms (SNPs) in various genes including those of pro and anti-inflammatory cytokines have been reported as a risk for T2DM. Not all the SNPs have been confirmed by unifying results in different studies and wide variations have been reported in various ethnic groups. The inter-ethnic variations can be explained by the fact that gene expression may be regulated by gene-gene, gene-environment and gene-nutrient interactions. This review highlights the impact of these interactions on determining the role of single nucleotide polymorphism of IL-6, TNF- α , resistin and adiponectin in pathogenesis of T2DM.

Key words: Cytokines; Gene-environment interaction; Diabetes mellitus; Single nucleotide polymorphism; Gene-gene interaction

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Core tip: Single nucleotide polymorphisms (SNPs) in inflammatory cytokines play role in insulin resistance and type 2 diabetes mellitus (T2DM). These SNPs are found to be correlated with cytokine serum levels, body mass index, insulin resistance and dyslipidemia although these findings are challenged by other studies. Gene-gene, gene-environment and gene-nutrient interactions alter the impact of these SNPs in pathogenesis of T2DM. These interactions may explain the inter-ethnic variations in role of inflammatory cytokines in T2DM reported in international studies. This mini-review highlights these gene-genes, gene-environment and gene-nutrient interactions and their impact on inflammatory cytokine SNPs.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) has increased globally over decades and is projected to continue increasing^[1]. The etiology of DM is multi-factorial and inflammation plays a role in its pathogenesis. DM is considered as chronic low grade inflammatory state and markers of sub clinical inflammation increase in type 2 diabetes mellitus (T2DM) years before diagnosis of the disease^[2]. They have a role in pathogenesis of the disease, obesity, insulin resistance and apoptosis of beta cells of endocrine pancreas by various mechanisms. Among the recognized markers are interleukin (IL)-6, IL-1, IL-18, tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), tissue plasminogen activator (tPA), heptoglobulins, fibrinogen, resistin and adiponectin. The single nucleotide polymorphisms (SNPs) of pro- and anti-inflammatory cytokines have been found to influence the cytokines at translational level and modify serum levels. Inter-ethnic variations have been reported regarding association of SNPs of cytokines with T2DM and serum levels (Table 1). Pro-inflammatory cytokines induce glucotoxicity and lipotoxicity which in turns leads to mitochondrial damage, oxidative stress and beta cell apoptosis^[2].

Although the relationship between single nucleotide polymorphisms of cytokines/adipokines and risk of T2DM has been robustly proven, inter-ethnic and intra-ethnic variations in this relationship cannot be explained in generalized terms. A number of factors including demographic features, sample size, gene-gene, gene-environment and gene-nutrients interaction, average age of onset, duration of disease, life style, degree of obesity, glucose tolerance and pathogenesis of disease can confound association studies.

GENE-GENE INTERACTION

Variable results regarding association of cytokines SNPs with T2DM in different ethnic groups have been reported in international studies^[1-7]. Even different studies in the same ethnic group have also reported varying results^[8,9]. This variation is also found in association of cytokines SNPs with serum levels of respective cytokines, insulin resistance, serum insulin, lipid profile and body mass index (BMI). Gene-gene interaction is an established fact and a few studies have reported this factor as a contributing one in inter-ethnic variations in various parameters. There might be another unidentified functional gene polymorphism in close linkage disequilibrium with cytokine SNPs.

It has been found that presence or absence of a minor allele of inflammatory cytokine genotype may influence the binding of transcription factors to promoter region altering the promoter activity from almost non-existent to augmented by many folds^[4]. Similarly gene-gene interactions may also influence either the binding of transcription factors or translational activity. Polymorphism of a stress protein gene; P2/P2 genotype of heat shock protein 70-2 is found to be close to and in linkage disequilibrium with TNF- α promoter area and is statistically associated with obesity in Tunisian subjects and may influence the impact of TNF- α polymorphism^[3].

IL-6 -174 G/C SNP is in the promoter region (-173 to -145) that contains multiple response elements. These functional sites respond to many factors including IL-1, TNF- α , NF- κ B and glucocorticoids^[6]. Presence or absence of a minor allele may influence the binding of transcription factors to DNA response elements. Moreover, *IL-6* gene promoter function is also effected by a variable run of A and T bases (-257 to -276)^[4]. *IL-6* promoter variants; G and C are also affected by the *IL-6* haplotype, age and sex of the individual and presence of sex steroids^[5]. Estrogen regulates protein synthesis at transcriptional levels, particularly those proteins which are involved in glucose and lipid metabolism.

In overweight IGT Finnish subjects, simultaneous polymorphism *C-174G* in *IL-6* and the *G-308A* polymorphism in TNF- α showed a 2.2 fold increased risk of T2DM than any other SNP^[6] neither of SNP, although risk was not higher in simultaneous SNPs as compared to the *G-308A* SNP alone showing a gene-gene interaction^[6].

Individually, *IL-6-597 G/A*, *TNF- α -308G/A* and *IL-10 -592C/A* do not show any association between SNP and risk of T2DM in Indian population but combined genotypes of *IL-6 -597 GA* and *TNF- α -308 GG* increased the risk of T2DM up to 21 times, while triple combination of *IL-6 -597 AA*, *TNF- α -308 GG* and *IL-10 -592 CA* increased the risk to 314 times. Presence of minor allele A in all 3 genes increased the risk up to 1.41 times in Indian population, showing strong gene-gene interaction^[7].

Table 1 Frequencies of single nucleotide polymorphisms of cytokines in various ethnic groups

S no	Ethnic group	n	IL6 -174G/C genotype frequency			Ref.
			GG (%)	GC (%)	CC (%)	
1	Diabetic Turkish	96		83.9	16.1	Karadeniz <i>et al</i> ^[5]
2	Diabetic Indians	40	57	28	15	Mukhopadhyaya <i>et al</i> ^[33]
	Healthy Indians	40	30	33	37	
3	Diabetic Finnish	737		61	39	Razquin <i>et al</i> ^[27]
4	Diabetic Mexicans	90	76.7	20	3.3	Guzmán-Guzmán <i>et al</i> ^[34]
IL6 -572 G/C allele frequency						
			GG (%)	GC (%)	CC (%)	
5	Healthy Chinese	581	3.74	39.8	56.4	Zhou <i>et al</i> ^[35]
6	Diabetic Mexicans	90	61.1	30	8.9	Guzmán-Guzmán <i>et al</i> ^[34]
7	Healthy Caucasians	677	6.2	39	54.8	Paik <i>et al</i> ^[36]
TNFα -308G/A allele frequency						
			GG (%)	GA (%)	AA (%)	
8	Diabetic Mexicans	278		76	24	Pérez-Martínez <i>et al</i> ^[31]
9	Diabetic Caucasians	350		87	13	Chan <i>et al</i> ^[37]
10	Diabetic Indians	40	87.5	5	7.5	Mukhopadhyaya <i>et al</i> ^[33]
	Healthy Indians	40	92.5	0	7.5	
TNFα -238 G/A allele frequency						
			GG (%)	GA (%)	AA (%)	
11	Healthy Iranians	202	79.3	19.2	1.5	Hedayati <i>et al</i> ^[38]
12	Diabetic Canadians	123	85.4	14.6		Fontaine-Bisson <i>et al</i> ^[14]
13	Diabetic Chileans	230	90	10	0	Santos <i>et al</i> ^[30]
RETN -420 G/C allele frequency						
			CC %	GC%	GG%	
14	Diabetic Japanese	161	47.2	46	6.8	Hishida <i>et al</i> ^[39]
	Healthy Japanese	2491	42.6	43.7	13.7	
15	Diabetic Han Chinese	318	60	40		Chi <i>et al</i> ^[40]
	Healthy Han Chinese	370	61.5	38.5		
16	Diabetic Finnish	258	65	35		Kunnari <i>et al</i> ^[41]
	Healthy Finnish	494	73	27		
ADIPOQ -11377 G/C allele frequency						
17	Diabetics White	503	79	21		Chiodini <i>et al</i> ^[42]
18	Diabetic Han Chinese	212	68.6	31.4		Yang <i>et al</i> ^[43]
19	Diabetic Germans	365	71	29		Schwarz <i>et al</i> ^[44]

Impact of SNPs in the resistin promoter region is also found to be influenced by gene-gene interaction. Presence of Pro/Pro genotype of PPAR gamma acts synergistically with *RETN* -420 G allele in augmenting serum resistin levels in Japanese population^[8]. SNPs in regions outside the coding region may influence transcription or mRNA stability and thus affect the expression of the gene. Nuclear proteins are specifically recognized with a single base difference at SNP-358 in *RETN* gene but not at SNP *RETN* -638. Therefore, A at *RETN* -358 is required for G at *RETN* -420 to confer the highest plasma resistin in the general Japanese population^[9]. In Caucasians, the association between SNP *RETN* C -420G and plasma resistin is not strong, and A at *RETN* -358 may not exist, suggesting that SNP *RETN* -358 could explain this ethnic difference^[9].

Similarly, in Filipino population, common SNPs in *ADIPOQ* gene were found to be in linkage disequilibrium with a rare coding variant in *R221S* at *ADIPOQ* locus. This variant was unique to Filipino population and was not found in 12514 European individuals^[10].

PPAR-γ agonists are identified transcription factor for *ADIPOQ* expression. In differentiated 3T3L1 mice adipocytes cell line, the haplotype AC or AG both at -11426 and -11377 position results in 2-3 fold increase in rosiglitazone induced promoter activity whereas

GG haplotype result in almost non-existent promoter activity. The result indicates that inducibility of *ADIPOQ* promoter activity by rosiglitazone (PPAR-γ agonist) depend on SNP variant combination rather than a particular allele^[11].

Genome-wide association studies (GWAS) on large sample size and various ethnic groups are required to reach a definitive conclusion about gene-gene interaction.

GENE-ENVIRONMENT INTERACTION

T2DM is a disease in which environmental factors play a very significant role. Physical activity, BMI, physical and mental stress, dietary habits, smoking and life-style not only influence the pathogenesis of the disease but also the age of onset of disease, response to treatment, and onset of complications of the disease. These environmental factors are not functioning alone and there is strong gene-environmental interaction.

Adipose tissue is the source of 30% of IL-6. Some studies suggested the role of adiposity in modulating the association between IL-6 genetic variability and T2DM risk^[12,13]. Association of IL-6 SNP with S. IL-6 levels has also been found highly variable in different ethnic groups. IL-6 C-174G SNP is suggested to

enhance IL-6 expression, IL-6 mRNA levels and thus serum levels but these effects are cell-type specific^[12]. Various IL-6 producing cell types may respond differently to various risk factors like inflammation, obesity, insulin resistance or yet unidentified parameters and thus altering the impact of SNP. This hypothesis is supported by the finding that TNF- α -308A allele was associated with significant elevation of TNF- α expression but when catering for genotypes, A allele association with insulin resistance in presence of obesity but not in absence of it, has been reported in diabetic Canadians^[14]. Interestingly not many studies were found in literature indicating positive association of A allele presence with higher insulin resistance even in absence of obesity. In one study, insulin resistance was found greater in both obese and lean Romans^[15] in either allele carriers. It may indicate the possibility that insulin resistance may be secondary to raised BMI, in all obese irrespective of genotype; a fact supported by studies on obese Australian^[16], obese Americans^[17], overweight IGT Finnish subjects^[6] and obese female Polish Caucasians^[18], where insulin resistance was present in obese subjects carrying either allele. TNF- α polymorphism may act as a genetic factor enhancing the insulin resistance in presence of obesity, irrespective of serum TNF- α levels as found in many studies. Why in some studies, this association between polymorphism and insulin resistance is not found even in presence of obesity is not known.

Smoking is a known factor inducing oxidative stress and increased levels of inflammatory cytokines are found in smokers. Genotype-smoking interaction has been found statistically significant in Korean healthy subjects. IL-6 -174C allele was associated with significant elevation of IL-6 expression after coronary artery bypass surgery, although IL-6 levels were same in either C or G allele carriers before surgery. It indicates that probably stress led to an altered impact of the IL-6 G-174C SNP on IL-6 expression^[19]. Similarly, C allele was found to be associated with reduced levels of IL-6 after long term exercise although no difference was reported between genotypes before exercise training program^[4,20]. Although, the low level of physical activity may increase the risk of T2DM in the absence of the risk allele, presence of the risk allele may not always assure the protection from the disease with exercise.

Association of TNF- α -308 G/A polymorphism with T2DM is explained by increased insulin resistance caused by raised serum levels of TNF- α . Despite quite a large number of studies on association of T2DM with this SNP, insulin resistance and BMI; very few studies correlated the TNF- α SNPs with serum levels of TNF, insulin resistance, BMI. Association of A allele with higher serum levels of TNF has been reported various ethnic groups such as in overweight Finnish subjects^[6], Danish Caucasians^[21] and in Chinese^[22]. Negative association has been reported in healthy controls, impaired glucose tolerant and diabetic Czech

Caucasians^[23] and in healthy Chinese^[24].

Homozygous IL6 -572GG genotype results in higher serum IL-6 in smokers as compared to GC and CC genotype (P value = 0.04)^[25]. Evidences indicate that environmental factors not only alone through metabolic derangement but also through gene-environmental interaction influence the impact of cytokine SNPs on pathogenesis of T2DM.

GENE-NUTRIENT INTERACTION

Single nucleotide polymorphisms in cytokines also interact with dietary factors influencing the cytokine expression induced by diet. IL-6 -174G allele has been found to be associated with higher energy expenditure as compared to C allele which could be one of the possible causes of lower BMI in G allele carriers in this cohort^[26]. A recent study indicate the higher BMI in IL-6 -174 CC genotype in Koreans but greater reduction in weight in CC genotype as compared to GG genotype by low fat diet/virgin olive oil diet for 3 years. The effect of gene variant on obesity indices was reversed by low fat diet^[27].

TNF- α -238 A allele alter the post-prandial suppression of FFA and levels remain high in obese, but not in non-obese A carriers. High TNF-expression in obese due to presence of A allele but also due to larger adipocytes may explain the absence of this effect in non-obese despite having A allele in Canadians^[14].

A recent study highlighted the strong gene-nutrient interaction affecting the serum levels of adiponectin. Marine fish oil contains unsaturated fatty acids which are ligands for transcription factor; PPAR γ which enhances the adiponectin expression thus increasing its serum level. The presence of SNPs in ADIPOQ fosters the effect of dietary marine fish oil on adiponectin expression^[28].

Another example of gene-nutrient interaction is dependence of insulin sensitivity on plasma saturated fatty acid (SFA) levels in the presence of homozygous minor alleles ADIPOQ -11377 GG. Insulin resistance was higher in GG carriers with high SFA levels. In the presence of homozygous major alleles; ADIPOQ -11377 CC and heterozygous CG genotypes, the insulin sensitivity was not altered by plasma concentrations of SFA^[29]. ADIPOQ -11377 SNP also influences the extent to which energy is derived from dietary fat in obese women^[30]. ADIPOQ CC homozygotes men have less insulin resistance after consumption of a monounsaturated fatty acid and carbohydrate diet as compared to saturated fatty acid diet; an effect not seen in females^[31]. On the other hand, serum adiponectin was reduced after α -linolenic acid supplementation in obese individuals irrespective of C or G allele at -11377 ADIPOQ^[29].

Heterogeneity in serum levels of cytokines in response to lipid lowering drugs in various patients may be due, in part to genotypes of inflammatory cytokines. IL-6 -572 GG is associated with higher S.

IL-6 levels as compared to GC and CC genotypes in those who are not taking lipid-lowering drugs while levels are comparable in GG, GC and CC genotypes who were taking those drugs. It is possible that statins might reduce the augmented effect of GG genotype on inflammation^[32].

CONCLUSION

Pathogenesis of T2DM is multi-factorial. Genetic background has a profound effect especially in T2DM. The genetic makeup itself is governed by many factors; some of which are still unidentified. Contribution of T2DM susceptibility genes in insulin resistance and beta cell failure and their interaction with cytokine genes, environment and nutrients need to be explored. There could be additional, still unidentified risk factors which obscure the impact of SNP with specific genetic/environmental/nutrient background in various ethnic groups in population. Single nucleotide polymorphism may be considered as a susceptibility factor in certain population segments based on other risk factors; both genetic and environmental. GWAS with large number of sample sizes may indeed be required to statistically manifest SNP-related risk factors. Most of the studies are done in relatively small population groups in a particular ethnic group. Sampling might include patient selection inherent biases and do not reflect the general risk of population for the disease.

Gene-gene, gene-environment and gene-nutrient interactions strongly influence the impact of cytokine polymorphisms on not only serum levels of cytokines but also on the insulin sensitivity, susceptibility to disease, response to weight reduction and lipid lowering drugs, energy expenditure and energy derivation from dietary fats. Such multi-dimensional regulatory factors can explain the wide variations in the role of single nucleotide polymorphisms in cytokines in pathogenesis of T2DM and MS reported in various ethnic groups.

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Attenuating type 2 diabetes with postpartum interventions following gestational diabetes mellitus

Sudharshani Wasalathanthri

Sudharshani Wasalathanthri, Department of Physiology, Faculty of Medicine, University of Colombo, Colombo 00800, Sri Lanka

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Correspondence to: Sudharshani Wasalathanthri, MBBS, PhD, Senior Lecturer, Department of Physiology, Faculty of Medicine, University of Colombo, Kynsey Road, Colombo 00800, Sri Lanka. sudharshaniw@gmail.com

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Abstract

Women with a history of gestational diabetes should be screened during and after the postpartum period because of a high risk for developing type 2 diabetes mellitus. Although differences exist between guidelines practiced throughout various parts of the world, all recommend the use of cutoffs for fasting and/or post-load plasma glucose to diagnose diabetes or pre-diabetes. The use of these glycemic parameters could be optimized when a trend is observed, rather than considering them as isolated values at various time points. As the presence of insulin resistance and beta-cell dysfunction start before glycemic changes are

evident, the estimation of insulin sensitivity and beta-cell function by Homeostatic Model Assessment is suggested for women who have additional risk factors for diabetes, such as obesity. Disease-modifying lifestyle intervention should be the first-line strategy to prevent or delay the onset of diabetes in women with a history of gestational diabetes mellitus. Intensive lifestyle interventions are designed to decrease caloric intake and increase physical activity in order to reduce body weight and fat, which will in turn reduce insulin resistance. This article also reviews unique problems of postpartum women, which should be considered when designing and implementing an intervention. Innovative "out of the box" thinking is appreciated, as continued adherence to a program is a challenge to both the women and the health care personnel who deal with them.

Key words: Gestational diabetes mellitus; Glycemic parameters; Lifestyle intervention; Screening; Type 2 diabetes mellitus

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Core tip: This article reviews and highlights important areas concerning diabetic risk during and after the postpartum period in women with gestational diabetes mellitus. Optimizing the use of glycemic parameters and assessing beta-cell function, particularly in high-risk women, will facilitate early recognition of those on the path to pre-diabetes and diabetes. Lifestyle interventions designed to attenuate the progression should be carefully planned, taking into consideration the unique set of problems in these women. "Out of the box" thinking is necessary to design lifestyle intervention protocols that will have high acceptance by these women.

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DIABETIC RISK FOR WOMEN WITH GESTATIONAL DIABETES MELLITUS

Gestational diabetes mellitus (GDM), which occurs and is diagnosed during pregnancy^[1], is a condition that increases the risk of developing type 2 diabetes mellitus (T2DM)^[2,3]. In a large meta-analysis of 20 cohort studies in 2009, Bellamy *et al*^[4] showed that women with GDM have a more than seven-fold increased risk of developing T2DM when compared to women with normoglycemic pregnancies. However, the incidence of diabetes in these women varies, with relative risks ranging from 6^[5] to 12^[6], possibly due to differences in screening and diagnostic criteria, associated risk factors^[7], and inclusion of subjects with overt diabetes uncovered by pregnancy^[8]. Feig *et al*^[6] further demonstrated an increase in the probability of developing diabetes from 3.7% at 9 mo to 18.9% at 9 years after delivery, suggesting the need for long-term follow-up and monitoring of women with a history of GDM.

The development of peripheral insulin resistance during pregnancy is facilitated by the increased maternal adiposity and release of insulin-desensitizing hormones from the placenta^[9]. The secretion of insulin is increased to compensate, and women with a deficit in this secretion can develop GDM. The effects of pregnancy on glucose homeostasis are alleviated following delivery of the offspring and removal of the placenta, such that the glycemic profile should return to normal within 6-12 wk postpartum.

POSTPARTUM SCREENING OF PATIENTS WITH A HISTORY OF GDM

Despite the lack of a consensus concerning precise recommendations for postpartum screening of women with a history of GDM^[10], the importance of optimal screening is universally accepted. The American Diabetes Association recommends using the oral glucose tolerance test (OGTT) to screen these women for persistent diabetes at 6-12 wk postpartum, and lifelong screening for development of diabetes or pre-diabetes at least every three years^[1]. However, the Mexico City Diabetes Study demonstrated that the progression from normoglycemia to diabetes ranges over three years with a probable phase of impaired glucose tolerance^[11], which suggests that three years between screens is insufficient for high-risk individuals. In the United Kingdom, the National Institute for Health and Clinical Excellence guidelines recommend glucose estimation prior to discharge, at 6 wk postpartum, and annually thereafter using fasting plasma glucose (FPG)^[12]. In

2010, however, Kakad *et al*^[13] used retrospective data of 470 women to show that diabetes was missed in 26% of women when only the FPG was used for screening. Furthermore, unlike OGTT, FPG does not allow for detection of impaired glucose tolerance. Hemoglobin A1c, an additional parameter introduced to the diagnostic criteria of pre-diabetes and diabetes in 2009^[14], is also considered unsuitable for use in postpartum women due to its low sensitivity on its own^[15] or in combination with FPG^[16]. Thus, OGTT with 75 g fasting glucose challenge and two-hour glucose measurements is the preferred screening method for women with previous GDM^[17]. The interpretations should be based on diagnostic cutoffs for pre-diabetes and diabetes for non-pregnant adults^[1].

Tabák *et al*^[18] used serial measurements of yearly glucose levels over 13 years to evaluate glycemic parameters in normoglycemics and diabetics. They found that during the transformation from normoglycemia to diabetes, FPG and post-load glucose gradually increased, followed by an abrupt increase approximately two years before a diagnosis of DM. This indicates that continual glycemic measurements during screening can be even more informative and predictive, despite being within the normal range. Therefore, it is suggested that rather than looking solely at isolated values at any given time, changes in glycemic measures should be observed.

With the global increase in the prevalence of DM^[19], the current recommendations for screening women with GDM for the development of T2DM should be revised. The present guidelines detect problems only when they reach the end point (diabetes), or a landmark very close to the end point (pre-diabetes). Can we use knowledge of the underlying pathophysiology to identify these cases earlier, before they reach the end point? The transition from normoglycemia to diabetes is a continuous process^[11,18,20]. Although the glycemic profile assessed by FPG or post-load glucose should return to normal after delivery in a woman with a diagnosis of GDM, these parameters are not indicators of the ongoing pathophysiologic process. An analysis of the British Whitehall II study showed a steep decline in insulin sensitivity, along with a marked increase followed by a steep decrease in insulin secretion, approximately 3-5 years before the onset of diabetes^[18]. These parameters can be estimated by the Homeostatic Model Assessment^[21]. However, this assessment by itself is inappropriate for evaluation of beta-cell function, and serial measurements are required in order to observe the longitudinal changes in insulin secretion^[22]. Repetitive monitoring of insulin sensitivity and secretion may be confined to the initial postpartum years due to increased cost, as Kim *et al*^[3] showed that T2DM appears rapidly within the first five years and plateaus after ten years. Furthermore, these measurements can be limited to women with a higher predictive risk of developing diabetes, such

as those who are overweight^[23], have a higher pre-pregnancy body mass index^[24,25], were diagnosed with GDM before the 24th week of gestation^[25], and who needed insulin for glycemic control during pregnancy^[23]. Finally, the recent call for developing standardized screening protocols for Indian women with GDM^[26] is worth considering for all Asian women, as they show a greater risk than Caucasian women^[23].

Nonetheless, the risk of developing T2DM can persist for more than 25 years in women with a history of GDM^[8,18,27]. Therefore, continued life-long follow-up of these women is justified, particularly with recognition of the fact that ageing is an independent risk factor for T2DM. In addition, women who are not diagnosed with GDM but have mild glucose abnormalities^[28] or a single abnormal value in the OGTT^[29] should be screened because of the increased risk for developing T2DM. However, as revised recommendations stipulate that only one abnormal value, not two, is sufficient to for a diagnosis of GDM^[1], more women may be recommended for T2DM screening.

LIFESTYLE INTERVENTIONS

Lifestyle interventions are the most appropriate initial approach to mitigate the development of diabetes in high risk individuals, such as those with a history of GDM^[30], and can reduce the incidence of DM by at least 50%^[27,31]. Such interventions may slow down or arrest the pathophysiologic processes, such as the beta-cell exhaustion that occurs in response to chronic insulin resistance^[32,33].

Lifestyle intervention programs designed for high-risk individuals generally propose a low-calorie, low-fat diet with moderate intensity physical activity (e.g., brisk walking) for 150-180 min per week to achieve a weight reduction of 5%-7% of the initial body weight^[31,34-36]. The recommended calorie limit varies between 1000-1200 kcal/d^[35] and 1200-1800 kcal/d^[34]. Although it is advised that no more than 30% of energy should come from fats^[36], a recent study found adequate glycemic control with a very low-carbohydrate, high-fat, non-calorie-restricted diet^[37]. Other simple measures include increasing the amount of fiber in the diet^[36], decreasing the amount of energy-dense foods, such as fast foods, increasing the amount of fruit and vegetable intake^[38], and controlling portion size^[35]. Although it is important to combine physical activity with dietary support to enhance the efficacy of an intervention program^[39], results of a small study showed that women perceived diet as more important for the prevention of T2DM than physical activity^[40], emphasizing the importance of effective counseling to reinforce the value of both aspects for weight reduction and maintenance^[35].

Although almost all published protocols are based on similar principles of intervention, a thorough investigation of these illustrates minor but important differences between them, especially when it comes

to the stage of implementation. To augment dietary and exercise interventions, Gabbe *et al*^[35] suggested incorporation of behavioral therapy, which includes stress management, stimulus control, problem solving, and goal setting. The Mothers After Gestational Diabetes in Australia Diabetes Prevention Program offers an intervention program handbook, six face-to-face sessions, and two follow-up telephone calls within the 12-mo follow-up period to ensure that participants achieve the program goals^[36]. Substantial decreases in glycemic and anthropometric parameters after one year of intervention^[41] is strong evidence for implementation of an effective lifestyle intervention program by community health workers^[34]. A randomized control study for high-risk Hispanic women initiated interventions during late pregnancy, and continued for 12 mo postpartum^[38]. Further support for prenatal implementation was provided by greater weight loss and improved health behaviors in the postpartum period in mothers who underwent a low glycemic index dietary intervention during pregnancy^[42]. It is the responsibility of the researchers and health care personnel planning the interventions to utilize such reported evidence when designing implementation strategies for a particular population.

Although almost all programs aimed at preventing T2DM promote increased physical activity, healthy eating, and weight loss, "out of the box" thinking is necessary in order to increase participant acceptance of, and thus adherence to, a given intervention. A high level of acceptance was reported in a novel intervention in England that used group leisure activities for adults at risk for DM^[43], though the recruitment procedure may have contributed to these results. Another interesting study protocol published in 2013 used motivational interviews to influence lifestyle changes in individuals with impaired fasting glucose^[44], a method based on the transtheoretical model of health behavior change^[45].

Although pharmacologic interventions are also beneficial in attenuating the onset of T2DM in women with a history of GDM^[27,46], a discussion of these is beyond the scope of this review.

Barriers to effective screening and lifestyle interventions and strategies to overcome them

Despite the importance of clear understandings of the nature of the disease, the risk for developing DM, and measures to prevent or delay its onset, the knowledge itself may not be enough. A recent qualitative study exploring factors that influence postnatal health behaviors in women with GDM showed that, although nearly all participants were aware of the increased risk for diabetes, this knowledge did not motivate them for action^[47]. However, a low level of awareness remains, even among college-educated affluent women^[48], which justifies the need for intensive awareness programs to counsel these women.

The health care team has an enormous respon-

sibility to educate these patients about the diabetes risk and the importance of regular screening, to motivate them to adapt to healthy lifestyles, and to support them to adhere to these changes. Although an OGTT is mandatory for women with prior GDM, a population-based cohort study in Canada found that women who chose an obstetrician for follow-up as opposed to a family physician were more likely to undergo a postpartum OGTT^[49], which highlights the importance of educating all levels of health personnel on current recommendations. However, there are conflicting results concerning the efficiency of obstetricians for enforcing postpartum T2DM screening of GDM women^[50,51]. It is the responsibility of the health care personnel to maintain records of these women and routinely remind them^[52], preferably through some form of written information^[53], as postal reminders^[54] or laboratory slips^[48] greatly increase the screening rates. Text message-reminder systems for screening^[55] and internet-based programs for lifestyle intervention^[56] are novel approaches worth trying in this era of technological dependence.

Postpartum women are a special group with a unique set of problems. The most common barriers to lifestyle interventions reported by these women were insufficient time^[40,57,58], lack of support for child care^[40,47,57], and other family commitments^[40,47]. As the amount of available social support is associated with adherence to lifestyle interventions^[57], educational and counseling sessions should be extended to the spouse and the immediate family of these women.

CONCLUSION

This review highlights important aspects concerning the screening of women with GDM, during the prenatal and postpartum periods, and thereafter. Women with GDM are a unique group for whom diabetes prevention strategies can be applied. In addition to being familiar with the general recommendations for screening and managing these patients, health care personnel should be able to appropriately support their patients to ensure greater acceptance of these valuable screening tests and interventional programs. The real challenge is not the planning of a lifestyle intervention, but implementing it effectively within the target population.

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Role of peroxisome proliferator-activated receptors gene polymorphisms in type 2 diabetes and metabolic syndrome

Chen Dong, Hui Zhou, Chong Shen, Lu-Gang Yu, Yi Ding, Yong-Hong Zhang, Zhi-Rong Guo

Chen Dong, Hui Zhou, Yong-Hong Zhang, Zhi-Rong Guo, Department of Epidemiology, School of Public Health, Soochow University, Suzhou 215123, Jiangsu Province, China

Hui Zhou, Lu-Gang Yu, Yi Ding, Suzhou Industrial Park Centers for Disease Control and Prevention, Suzhou 215123, Jiangsu Province, China

Chong Shen, Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, Nanjing 210000, Jiangsu Province, China

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Correspondence to: Zhi-Rong Guo, MD, Department of Epidemiology, School of Public Health, Soochow University, 199 Ren'ai Road, Industrial Park District, Suzhou 215123, Jiangsu Province, China. guozhirong28@163.com

Telephone: +86-512-65880079

Fax: +86-512-65884830

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MetS patients have about five-fold greater risk of the T2DM development compared with people without the syndrome. Peroxisome proliferator-activated receptors are a subgroup of the nuclear hormone receptor superfamily of ligand-activated transcription factors which play an important role in the pathogenesis of MetS and T2DM. All three members of the peroxisome proliferator-activated receptor (PPAR) nuclear receptor subfamily, PPAR α , PPAR β/δ and PPAR γ are critical in regulating insulin sensitivity, adipogenesis, lipid metabolism, and blood pressure. Recently, more and more studies indicated that the gene polymorphism of PPARs, such as Leu¹⁶²Val and Val²²⁷Ala of PPAR α , +294T > C of PPAR β/δ , Pro¹²Ala and C1431T of PPAR γ , are significantly associated with the onset and progressing of MetS and T2DM in different population worldwide. Furthermore, a large body of evidence demonstrated that the glucose metabolism and lipid metabolism were influenced by gene-gene interaction among PPARs genes. However, given the complexity pathogenesis of metabolic disease, it is unlikely that genetic variation of a single locus would provide an adequate explanation of inter-individual differences which results in diverse clinical syndromes. Thus, gene-gene interactions and gene-environment interactions associated with T2DM and MetS need future comprehensive studies.

Key words: Polymorphisms; Metabolic syndrome; Type 2 diabetes mellitus; Peroxisome proliferator-activated receptors

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Core tip: Recently, more and more studies indicated that the gene polymorphism influence of peroxisome proliferator-activated receptors (PPARs), including PPAR α , PPAR β/δ and PPAR γ , acted as a pivotal role in the onset and progressing of metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM). We reviewed the recent advances in the relationships between PPARs polymorphisms and MetS and T2DM. Also, we discussed

Abstract

Metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) are the serious public health problems worldwide. Moreover, it is estimated that

the effects of gene-gene interaction among *PPARs* genes on the MetS and T2DM herein.

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INTRODUCTION

Globally, about 25% and 5.4% of adult population have been estimated to have metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM), respectively^[1]. MetS is defined as a constellation of metabolic disorders including insulin resistance, central obesity, dyslipidemia and hypertension. The underlying cause of the MetS has been linked to the disorders of glucose metabolism including insulin resistance and glucose intolerance^[2,3]. One study in Nigeria reported that the prevalence of the MetS in T2DM patients is up to 86%^[4]. The study in Cameroon indicated that 71.7% T2DM patients diagnosed with the MetS^[5]. Thus, it is estimated that MetS patients have about five-fold greater risk of the T2DM development compared with people without the syndrome^[6].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are part of the superfamily includes receptors for steroid hormones, thyroid hormones, retinoic acid and fat-soluble vitamin A and D. The primary role of PPARs is to regulate glucose, fatty acid and lipoprotein metabolism, energy balance, cell proliferation and differentiation, inflammation and atherosclerosis^[7]. PPAR α , the first member of the PPAR family identified in 1990, is mainly expressed in tissues in which fatty acid catabolism is important^[8,9]. Since that time, two additional members of the family, PPAR β/δ and PPAR γ , have been identified^[10,11]. Recently, more and more studies on the associations of PPARs polymorphisms and disorders of glucose metabolism and abnormal lipid metabolism have been published, indicating that the gene polymorphism influence of PPARs acted as a pivotal role in the development of MetS and T2DM^[12-15]. This review is aimed to summarize the recent advances in the relationships between PPARs polymorphisms and the metabolic disorders that related with MetS and T2DM. Moreover, the effects of gene-gene interaction among *PPARs* genes on the MetS and T2DM also will be discussed.

PPAR α

PPAR α gene is located on chromosome 22q12.2-13.1, and it is the first member of the *PPAR* isotypes to be cloned and was named based on its ability to be

activated by peroxisome proliferator chemicals. PPAR α is robustly expressed in tissues with elevated fatty acid catabolism and regulates transcription of multiple genes involved in glucose metabolism, such as the liver, heart and skeletal muscle, where it functions as a major regulator of fatty acid homeostasis^[8,9]. Along with regulation of lipid and glucose metabolism, PPAR α is as an attractive candidate gene for the risk of MetS and T2DM^[7].

Role of *PPAR α* gene polymorphisms in T2DM

Until now, more than 20 different base substitutions have been identified in the *PPAR α* gene. Among of them, Leu¹⁶²Val (rs1800206) has been shown to be significantly related with the risk of T2DM in different population^[16-20]. Flavell *et al.*^[17] reported that the variant of Leu¹⁶²Val variant was associated with increased plasma levels of total-cholesterol, HDL-cholesterol, and apoA-I, indicating that PPAR α gene variation influences the onset and progression of T2DM. Furthermore, the PPAR α haplotype significantly influenced age at diagnosis, with the C-L-C and C-V-C haplotypes [rs135539 (intron 1)-Leu¹⁶²Val (rs1800206)-rs4253778 (intron 7)] accelerating onset of diabetes by 5.9 and 10 years, respectively, as compared with the common A-L-G haplotype, and was associated with an odds ratio for early-onset diabetes (age at diagnosis \leq 45 years) of 3.75. Intron 1 C-allele (rs135539) carriers also progressed more rapidly to insulin monotherapy (AA 9.4 ± 1.5 and AC + CC 5.3 ± 1.1 years). In another study, Andrulionyte *et al.*^[19] reported that the presence of the G (162V) allele of rs1800206 in *PPAR α* gene increased the risk of developing diabetes. Moreover, haplotypes C-G-C and A-G-C, based on SNPs rs135539, rs1800206, and rs4253778, increased the risk of developing diabetes by 4.58-fold and 3.18-fold, respectively, compared with the C-C-C haplotype. Additionally, it should be noted that the Leu¹⁶²Val polymorphism has different effects on gene transcription. Evans *et al.*^[20] demonstrated that the Leu¹⁶²Val polymorphism was associated with a lower body mass index (BMI) in two independently recruited groups of patients with T2DM, suggesting that Leu¹⁶²Val polymorphism in PPAR α protects T2DM patients from the overweight which is frequently associated with their condition.

Role of *PPAR α* gene polymorphisms in MetS

Leu¹⁶²Val polymorphism not only plays a pivotal role in the T2DM development, but also significantly associated with the risk of MetS. In young Caucasians males, Uthurralt *et al.*^[21] found Leu¹⁶²Val polymorphism of PPAR α to be a strong determinant of serum triglyceride levels, where carriers of the V allele showed 78% increase in triglycerides relative to L homozygotes. Moreover, men with the V allele showed lower HDL, but women did not. Recently, Smalinskiene *et al.*^[22] reported that males with the G (162V) allele of rs1800206 in *PPAR α* gene had higher OR of elevated

triglyceride levels vs carriers of *PPARα* genotype CC, indicating that *PPARα* Leu¹⁶²Val polymorphism gene influences the onset and development of MetS.

Val²²⁷Ala, a non-synonymous variant at the *PPARα* locus encoding a substitution of valine for alanine at amino acid residue 227, is another important *PPARα* polymorphism reported that associated with MetS development^[23-28]. In Japanese population, significant interactions between *PPARα* Val²²⁷Ala polymorphism and triglyceride levels and AST/ALT ratios were found in drinkers^[23,24]. Chan *et al*^[26] reported that the level of weight, BMI, hip circumference, waist circumference, waist-hip ratio, percentage of body fat, abdominal wall fat thickness in Chinese subjects with Val²²⁷Ala variant were significantly lower than that in Val²²⁷Val type. Additionally, in Chinese females, the presence of the A227 allele was significantly associated with lower serum concentrations of total cholesterol and triglycerides^[26,27]. Moreover, Chan's results also showed that the Val²²⁷Ala polymorphism modulates the association between dietary polyunsaturated fatty acid intake and serum high density lipoprotein concentration^[26].

In addition, the other variants of *PPARα* gene associated with MetS were also demonstrated in previous studies^[29-33]. A Rotterdam study observed that the minor alleles of the *PPARα* rs4253728 and rs4823613 polymorphisms are associated with a better total and LDL-cholesterol-lowering response to simvastatin, possibly through influence on CYP3A4^[33]. Therefore, better understanding the associations between *PPARα* polymorphisms and lipo-protein metabolism would be helpful for the prevention and treatment of MetS.

PPARα

PPARδ, also known as *PPARβ*, has 441 amino acid residues. Its coding gene is located in 6p 21.1-21.2, which includes 11 exons. *PPARδ* is widely expressed in the liver, kidneys, cardiac and skeletal muscle, adipose tissue, brain, colon and vasculature^[34,35]. Animal studies found that *PPARδ* knockout mice showed glucose intolerance on normal chow, and were prone to obesity on high-fat diet^[36,37]. *PPARδ* activation in the liver also appears to decrease hepatic glucose output, thereby contributing to improved glucose tolerance and insulin sensitivity^[36,37]. Meanwhile, treatment with *PPARδ*-specific agonist enhanced β-oxidation, decreased free fatty acid, and improved insulin sensitivity in mice and moderately obese men^[38,39]. Hence, *PPARδ* has emerged as a key role for the development of MetS and T2DM in recent years.

Role of *PPARδ* gene polymorphisms in T2DM

PPARδ is an important candidate gene for T2DM. About ten years ago, Vanttinen *et al*^[40] reported that a statistically significant increase in insulin-stimulated whole-body and skeletal muscle glucose uptake

in carriers of the alleles of three variants in *PPARδ* (rs6902123, rs2076167 and rs1053049), and the association was strongest for the rs6902123 variant. After that, the results from "The STOP-NIDDM Trial" demonstrated an increased risk of conversion to overt T2DM in carriers of the rs6902123 variant^[41]. Similar to these findings, Lu *et al*^[42] observed that rs6902123 was significantly associated with risk of T2DM and impaired fasting glucose in Chinese Han population. The minor C allele of rs6902123 was associated with increased levels of fasting glucose and HbA1c. In addition, a previous study revealed that the haplotype, composed of -13454G>T, -87T>C, 2022+12G>A, 2629T>C, and 2806C>G, is closely related to fasting plasma glucose and BMI of normal people in Korea^[43]. Also, Hu *et al*^[44] and Yu *et al*^[45] reported that gene polymorphism of *PPARδ*, -87T>C, is significantly associated with higher fasting plasma glucose concentrations in both normal glucose tolerant and diabetic subjects.

However, with 886 middle age Chinese female T2DM patients, Villegas *et al*^[46] did not find a main gene effect of *PPARδ* on T2DM or an interaction between the genes with BMI or exercise participation and the risk of T2DM. The similar result was also observed in another study of 7495 middle age white people that sequenced the *PPARδ* gene and found no association between variants and T2DM^[47]. The reason for this disparity is not clear. It should be considered that both genetic and environmental heterogeneity, including differences in their interaction, could give rise to population-specific discrepancies in the association of allelic variants and insulin resistance and thereby account for the inconsistent findings.

Role of *PPARδ* gene polymorphisms in MetS

Based on the analysis of a *PPARδ* null mouse model, it was demonstrated that *PPARδ* gene-deficient mice who bypassed the lethal placental defect displayed a lean phenotype, with a significantly smaller amount of fat mass. In addition, the muscle-specific *PPARδ* transgenic mice displayed increased mitochondrial-rich, oxidative type-1 myofibers with enhanced oxidative enzymatic activities^[36,37,48,49]. Skogsberg *et al*^[50] screened the 5'-untranslated region of the human *PPARδ* gene and found that a +294T > C (also named -87T > C, rs2016520) polymorphism in nucleotide 15 of exon 4 (located 87 nucleotides upstream of the start codon), was significantly associated with plasma levels of LDL and cholesterol in two cohorts of healthy men. In a Canada study, Robitaille *et al*^[51] reported that *PPARδ* +294T > C polymorphism may be associated with a lower risk to exhibit the MetS and this association is influenced by dietary fat intake. Also, Aberle *et al*^[52] showed that a highly significant association between the +294T > C and lower HDL-cholesterol levels in dyslipidemic female subjects. Moreover, MetS patients with CC genotype had significantly higher total and LDL-cholesterol

levels than those with TT and TC genotypes. The risk variant of *PPARδ* +294T >C marker was associated with higher LDL-cholesterol and increased serum total cholesterol^[53]. Additionally, several other studies demonstrated that the *PPARδ* +294T >C polymorphism was associated with modifications of serum lipid concentrations in healthy subjects and the risk of CAD in dyslipidemic women and hypercholesterolemic men and cholesterol metabolites in Alzheimer's disease patients^[54,55].

However, previous studies of *PPARδ* +294T >C polymorphism provided conflicting results regarding association with MetS. In another study in Scottish males, Skogsberg *et al.*^[56] reported that the +294C allele did not influence LDL-cholesterol concentrations. Gouni-Berthold *et al.*^[57] demonstrated that the presence of the C allele had no effect on triglyceride, HDL-cholesterol, and LDL-cholesterol levels, both in diabetic and non-diabetic German controls, or both in men and in women. In a Chinese study, Wei *et al.*^[58] showed that serum total cholesterol, HDL-cholesterol, LDL-cholesterol, ApoA1, and ApoB levels were not correlated with +294T >C polymorphism in nondrinkers. In addition, Grarup *et al.*^[47] also did not replicate the associations of +294T >C polymorphism with metabolic traits in 7495 middle-aged white people. Therefore, more studies focused on the impact of *PPARδ* gene polymorphism on the MetS development should be performed in different populations in future.

PPAR γ

The gene of *PPAR γ* (isoforms *PPAR γ 1*, *PPAR γ 2* and *PPAR γ 3*) is located on chromosome 3p25 encodes a nuclear transcription factor involved in the expression of hundreds of genes. *PPAR γ* gene contains 9 exons, spans more than 100 kb. Since 1997, more and more evidences indicated that both common and rare polymorphisms of the genes of *PPAR γ* acted as key roles in the regulation of lipid and glucose metabolism^[59-62]. Rare mutations of *PPAR γ* (loss-of-function mutations) exhibit a limited impact due to their low frequency but are associated with severe phenotypes such as hypertension, T2DM and MetS^[63]. Conversely, common polymorphisms of *PPAR γ* significantly associated with the risk of T2DM development, obesity and cardiovascular diseases in the general population due to their relatively high frequency^[64].

Role of *PPAR γ* gene polymorphisms in T2DM

PPAR γ was the first gene reproducibly associated with T2DM. The association between the substitution of alanine by proline at codon 12 of *PPAR γ 2* (Ala12 allele) and the risk for T2DM has been widely studied since Yen *et al.*^[65], first reported this polymorphism. In a recent study on the association between Pro¹²Ala polymorphism with both T2DM and the development of diabetic nephropathy, the results demonstrated

that the Pro/Pro genotype was the most common in diabetic patients as well as in controls followed by Pro/Ala genotype and Ala/Ala genotypes was the least common one. The allelic frequency of Pro was significantly higher in diabetic patients than controls and also significantly higher in diabetics with nephropathy than without nephropathy^[66]. In South Africa population, Vergotine *et al.*^[67] reported that the Pro¹²Ala of *PPAR γ 2* is significantly associated with insulin resistance and this polymorphism interacts with IRS1 Gly⁹⁷²Arg, to increase the risk of T2DM. In addition, Wang *et al.*^[68] demonstrated that the presence of the Ala allele may contribute to improved insulin secretory capacity and may confer protection from T2DM and obesity in the Chinese population. Moreover, a meta-analysis confirmed the association between the *PPAR γ 2* Pro12 allele and T2DM, and suggested that patients who carry the Pro12 allele have a 1.27-fold higher risk for developing T2DM than Ala12 carriers. This seemingly modest effect translates into a staggering 25% population-attributable risk because of the higher frequency of the Pro12 allele, especially in Japanese and European populations^[69].

Compared to the effects of the common Pro¹²Ala variant, rare mutations of *PPAR γ* gene affecting the ligand-binding domain of *PPAR γ* , such as 185Stop, Arg⁴²⁵Cys, and Pro⁴⁶⁷Leu, also associated with decreased transcriptional activity, improves glucose homeostasis and insulin sensitivity^[70-72]. Additionally, the other *PPAR γ* polymorphisms such as Cys¹¹⁴Arg, Cys¹³¹Tyr and Cys¹⁶²Trp could restrict wild-type *PPAR γ* action *via* a non-DNA binding, transcriptional interference mechanism. Heterozygous carriers of these new mutations are severely insulin resistant also been reported in the previous studies^[73,74].

Role of *PPAR γ* gene polymorphisms in MetS

The functional mutation Pro¹²Ala has also been reported to be associated with MetS in several populations^[75,76]. Tellechea *et al.*^[75] reported that individuals carrying the Ala12 allele of *PPAR γ* have a high risk for MetS and IR, especially among nonsmokers from Buenos Aires, Argentina. Also, The Québec Family Study observed that Ala12 carriers had a higher BMI, WC, fat mass than Pro/Pro homozygotes, suggesting that this polymorphism can modulate the association between dietary fat intake and components of the MetS^[76]. However, studies investigating the association between Pro¹²Ala polymorphisms and the risk of MetS in different populations have been inconsistent. In a large French population-based study, Meirhaeghe *et al.*^[77] found no association between Pro¹²Ala polymorphism of *PPAR γ* and MetS. Based on the analysis of 423 subjects with MetS and families without MetS, Yang *et al.*^[78] reported that Pro¹²Ala polymorphism was not associated directly with MetS, although MetS patients with Ala allele have higher fasting blood sugar (FBS) and higher left ventricular voltage. Similar to these findings, Ala carriers of middle-aged Swedish individuals did

not show statistically significantly different levels of fasting blood glucose, triglycerides, HDL-cholesterol, waist circumference or BP when compared with Pro¹²Pro homozygotes, suggesting that Pro¹²Ala polymorphism in *PPAR γ* gene does not have a major role in determining MetS prevalence^[79]. More recently, a meta-analysis included 4456 cases and 10343 controls from 10 case-control studies, indicated that no significant statistical difference was observed between the variant and metabolic syndrome, even if stratified by ethnicity, definition of metabolic syndrome, source of control groups, and quality score of selected studies^[80].

Another polymorphism, the C1431T silent substitution (rs3856806) in the 6th exon of *PPAR γ* , has also been shown to be associated with MetS in the different populations^[78,81]. In Iranian population, a significant difference in the frequencies of the C1431T genotypes was observed between MetS and control subjects. The T allele carriers had a significantly increased risk of MetS compared to the CC genotype even after correction for multiple-testing and adjustment for age, sex and genotype^[81]. In Chinese population, the association of C1431T polymorphism with MetS has also been observed. There were significant differences in terms of gender, FBS, LDL-cholesterol levels, triglyceride between CC genotype and CT +TT genotype groups in patients with MetS^[78]. However, not all studies had similar results. In Meirhaeghe's French population study, polymorphisms of C1431T were not individually associated with MetS. However connected with the other three polymorphisms, -681C>G, P2-689C>T, Pro¹²Ala, haplotypes are significantly associated with the risk for MetS^[82].

GENE-GENE INTERACTION AMONG *PPAR α* , *PPAR δ* AND *PPAR γ*

Until now, increasing evidences suggested that gene-gene interaction among *PPAR α* , *PPAR δ* and *PPAR γ* influenced the onset and progressing of T2DM and MetS^[41,83-88]. Andrulionyte *et al.*^[41] reported that SNP rs6902123 of *PPAR δ* alone and in combination with the Pro¹²Ala polymorphism of *PPAR γ* 2 predicted the conversion from impaired glucose tolerance (IGT) to T2DM. More recently, our results indicated that there was a significant association between plasma Lp(a) level and gene-gene interaction among the polymorphisms rs1800206, rs135539 in *PPAR α* and rs10865710, rs1805192, and rs4684847 in *PPAR γ* , suggesting that *PPAR α / γ* gene may influence the risk of T2DM and MetS by regulating Lp(a) level^[83,84]. In addition, the results from our another study demonstrated that gene-gene interaction among *PPAR α / δ / γ* polymorphisms contribute to the risk of hypertriglyceridemia independently or in an interactive manner^[86,87]. Thus, gene-gene interactions among SNPs in *PPAR α* , *PPAR δ* and *PPAR γ* should be further investigated in future in order to better understand the small single gene effects that

cannot be detected by single-locus studies.

CONCLUSION

Although the molecular mechanisms are still uncovered, more and more studies indicated that the gene polymorphism influence of PPARs acted as a pivotal role in the development of MetS and T2DM. Therefore, identification of polymorphic variants of PPARs in different populations and the genotypic associations between SNPs and gene-gene interactions would be helpful for the prevention and treatment of T2DM and MetS. However, given the complexity pathogenesis of metabolic disease, it is unlikely that genetic variation of a single locus would provide an adequate explanation of inter-individual differences which results in diverse clinical syndromes. To this end, gene-gene interactions and gene-environment interactions associated with T2DM and MetS needs future comprehensive studies.

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Basic Study

Advanced glycation end-product expression is upregulated in the gastrointestinal tract of type 2 diabetic rats

Peng-Min Chen, Hans Gregersen, Jing-Bo Zhao

Peng-Min Chen, Department of Molecular Biology, Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing 100029, China

Hans Gregersen, GIOME Center, College of Bioengineering, Chongqing University, Chongqing 400045, China

Jing-Bo Zhao, Institute of Clinical Medicine, Aarhus University, Aarhus N 8200, Denmark

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Institutional animal care and use committee: All procedures involving animals were reviewed and approved by the Danish Committee for Animal Experimentation. The license number is 2008/561-1530. Animals in poor clinical condition were euthanized and excluded from the study. The rats were euthanized with CO₂ inspiration during the anesthesia. The animals were acclimatized to laboratory conditions (22 °C, 12 h/12 h light/dark, 50% humidity, ad libitum access to food and water) for 2 wk prior to experimentation. The animal protocol was designed to minimize pain or discomfort to the animals.

Conflict-of-interest: We declare that we have no proprietary, financial, professional or other personal interest of any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled "Advanced Glycation End-Product expression is upregulated in the Gastrointestinal Tract of Type 2 Diabetic Rats".

Data sharing: No additional data are available.

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Correspondence to: Hans Gregersen, Professor, GIOME Center, College of Bioengineering, Chongqing University, 83 Shabei Road, Chongqing 400045, China. hag@giome.org
Telephone: +86-186-00556254

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Abstract

AIM: To investigate changes in advanced glycation end products (AGEs) and their receptor (RAGE) expression in the gastrointestinal (GI) tract in type 2 diabetic rats.

METHODS: Eight inherited type 2 diabetic rats Goto-Kakizaki (GK) and ten age-matched normal rats were used in the study. From 18 wk of age, the body weight and blood glucose were measured every week and 2 wk respectively. When the rats reached 32 wk, two-centimeter segments of esophagus, duodenum, jejunum, ileum, and colon were excised and the wet weight was measured. The segments were fixed in 10% formalin, embedded in paraffin and five micron sections were cut. The layer thickness was measured in Hematoxylin and Eosin-stained slides. AGE [N epsilon-(carboxymethyl) lysine and N epsilon-(carboxyethyl)lysine] and RAGE were detected by immunohistochemistry staining and image analysis was done using Sigmascan Pro 4.0 image analysis software.

RESULTS: The blood glucose concentration (mmol/L) at 18 wk age was highest in the GK group (8.88 ± 1.87 vs 6.90 ± 0.43 , $P < 0.001$), a difference that continued to exist until the end of the experiment. The wet weight per unit length (mg/cm) increased in esophagus, jejunum and colon from the normal to the GK group (60.64 ± 9.96 vs 68.56 ± 11.69 , $P < 0.05$ for esophagus; 87.01 ± 9.35 vs 105.29 ± 15.45 , $P < 0.01$ for jejunum; 91.37 ± 7.25 vs 97.28 ± 10.90 , $P < 0.05$ for colon). Histologically, the layer thickness of the GI

tract was higher for esophagus, jejunum and colon in the GK group [full thickness (μm): 575.37 ± 69.22 vs 753.20 ± 150.41 , $P < 0.01$ for esophagus; 813.51 ± 44.44 vs 884.81 ± 45.31 , $P < 0.05$ for jejunum; 467.12 ± 65.92 vs 572.26 ± 93.60 , $P < 0.05$ for colon]. In esophagus, the AGE and RAGE mainly distributed in striated muscle cells and squamous epithelial cells. The AGE distribution was much stronger in the GK group compared to the normal group both in the striated muscle layer and mucosa layer (immuno-positive area/total measuring area %: 4.52 ± 0.89 vs 10.96 ± 1.34 , $P < 0.01$ for muscle; 8.90 ± 2.62 vs 22.45 ± 1.26 , $P < 0.01$ for mucosa). No visible difference was found for RAGE distribution between the two groups. In the intestine AGE and RAGE distributed in epithelial cells of villi and crypt. RAGE was also found in neurons in the myenteric and submucosal plexus. The intensity of AGE staining in mucosa of all segments and RAGE staining in neurons in all segments were strongest in the diabetes group. Significant difference for AGE was found in the epithelial cells of villi and crypt in duodenum (immuno-positive area/total measuring area %: 13.37 ± 3.51 vs 37.48 ± 8.43 , $P < 0.05$ for villi; 0.38 ± 0.12 vs 1.87 ± 0.53 , $P < 0.05$ for crypt) and for RAGE in neurons of all segments (*e.g.*, for jejunum: no staining neurons% 0 vs 0 , mild 36.0 ± 5.2 vs 28.7 ± 3.5 , moderate 53.2 ± 4.8 vs 55.8 ± 5.4 , strong 10.7 ± 1.1 vs 15.4 ± 2.0 , $P < 0.05$). In the colon, RAGE was primarily found in neurons in the myenteric and submucosal plexus. It was stronger in the diabetes group than in the normal group (no staining neurons% 6.2 ± 0.2 vs 0.3 ± 0.04 , mild 14.9 ± 2.1 vs 17.6 ± 1.5 , moderate 53.1 ± 4.6 vs 44.7 ± 4.4 , strong 25.6 ± 18 vs 43.6 ± 4.0 , $P < 0.05$). In the rectum, RAGE was primarily found in the mucosa epithelial cells.

CONCLUSION: The AGE and RAGE expression was up-regulated in the GI tract of GK diabetic rats and may contribute to GI dysfunction in type 2 diabetic patients.

Key words: Diabetes mellitus; Gastrointestinal complications; Advanced glycation end products; Receptor of advanced glycation end products

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Core tip: Changes in advanced glycation end products (AGEs) and their receptor (RAGE) expression in the gastrointestinal (GI) tract in type 2 diabetic rats were studied. The AGE and RAGE were widely distributed in epithelial cells of all segments as well as in striated muscle cells in the esophagus. RAGE also distributed in neurons in all segments. Up-regulated AGE and RAGE expression was found in the GI tract of GK diabetic rats. The altered AGE and RAGE may be a contributing factor for GI dysfunction in type 2 diabetic patients.

Chen PM, Gregersen H, Zhao JB. Advanced glycation end-product expression is upregulated in the gastrointestinal tract of type 2 diabetic rats. *World J Diabetes* 2015; 6(4): 662-672

INTRODUCTION

Sensory-motor abnormalities are common in the gastrointestinal (GI) tract in diabetes mellitus patients. Symptoms may arise from the entire GI tract. Common complaints are dysphagia, heartburn, abdominal pain, early satiety, nausea, vomiting, constipation, and diarrhea^[1-3]. The pathogenesis of such symptoms in diabetes mellitus is complex, multi-factorial with motor dysfunction, glycemic control, autonomic neuropathy, and psychological factors, and is not well understood^[4].

Previous studies demonstrated changes in the morphological and biomechanical properties of the GI tract during diabetes, *e.g.*, the wall thickness and stiffness of GI tract increased^[5-7]. The structure or deformation changes may alter the relative positions of the mechanosensitive afferents (zero setting of the mechanosensitive afferents). The changes in stress distribution and wall stiffness likely alter the stress in the vicinity of the mechanosensitive afferents. Consequently, the perception and motility of the intestinal tract will change as well. Therefore, the morphological changes and biomechanical remodeling are likely to affect the function of mechanosensitive afferents in the GI wall and further affect the motor and sensory function. Only sparse information, however, is available about the mechanisms for these changes.

Advanced glycation end products (AGEs) are formed in physiological states and gradually increases with age but AGEs formation is accelerated in diabetes^[8]. AGEs can lead to changes in structure and function directly in the target protein. They also can bind to their receptor (RAGE), leading to activation of signaling pathways resulting in serial changes^[9-11]. AGEs and RAGE play important roles for diabetic complications in the cardiovascular system^[12-14] and for retinopathy^[15] and nephropathy^[16,17]. It was also demonstrated that AGEs and RAGE were associated with diabetic-induced arterial wall stiffening^[18-20]. Therefore, they may also play an important role in the diabetic GI tract. In our previous study we demonstrated that AGE [N epsilon-(carboxymethyl)lysine, CML and N epsilon-(carboxyethyl)lysine, CEL] and RAGE were up-regulated in the small intestine and colon of streptozotocin (STZ)-induced diabetic rats^[21]. However, to the best of our knowledge, data on the distribution of AGE and RAGE in the GI tract of type-2 diabetes have never been described.

The aims of this study were to investigate the AGE and RAGE distribution in the GI tract in type-2 diabetic rats and to compare those with normal rats. The data obtained may serve as the basis for further studying AGE and RAGE effects on type 2 GI diabetic dysfunctions.

MATERIALS AND METHODS

Reagents

Anti-AGEs mouse monoclonal antibody (6D12), against N(epsilon)-(carboxymethyl)lysine (CML, a major immunological epitope in AGEs) and N epsilon-(carboxyethyl)lysine (CEL) was purchased from COSMO BIO CO.,LTD. Japan. Other substances were rabbit polyclonal antibody against the N-terminal of human RAGE from Cell Applications, INC, United States; LSAB2 System-HRP for rat specimens, proteinase K, citrate buffer (pH = 6.0, 10xconcentrated), bovine serum albumin (BSA) and Mayer haematoxylin from Dako A/S, Denmark; soluble RAGE from Shanghai Yanji Bio; STZ, ethanol, methanol and xylene from Sigma-Aldrich Denmark A/S, Vallensbæk Strand, Denmark. Blood glucose analyzer and test strips were supplied by Hemocue Corporation, Sweden. The slides and cover glasses we used for immunohistochemical staining were Menzel-Glaser products, Germany.

Animal

Approval of the protocol was obtained from the Danish Committee for Animal Experimentation. Eight inherited type 2 diabetic Goto-Kakizak rats (GK group), 12 wk old and weighting about 330 g, were purchased from Taconic Europe DK-8680 Ry, Denmark. Ten age-matched normal rats (same strain as GK rats) served as controls (Normal group). During the breeding, the rats freely drank tap water and ate food except fasting over night before measuring body weight and blood glucose, which were done every week for body weight and every 2 wk for blood glucose from week 18. The rats survived until 32 wk of age.

Sampling

At the termination of the experiments, the rats were anaesthetized with Hypnorm 0.5 mg and Dormicum 0.25 mg per 100 g body weight (Hypnorm: Dormicum: sterile water = 1:1:2; subcutaneous injection). Two-centimeter segments of esophagus, duodenum, jejunum, ileum, and colon were excized. The esophageal segment was taken from the distal end of esophagus; the duodenal segment from 5 cm distal to pylorus sphincter; the jejunal segment from 5 cm distal to the ligament of Treitz; the ileal segment from 5 cm proximal to the ileo-cecal valve; and the colon from the middle part. Residual contents in the lumen were gently cleared using Krebs solution of the following composition (mmol/L): NaCl, 118; KCl, 4.7; NaHCO₃, 25; NaH₂PO₄, 1.0; MgCl₂, 1.2; CaCl₂-H₂O, 2.5; Glucose, 11; ascorbic acid, 0.11 and the wet weight was measured. Thereafter the rats were killed by injecting an overdose anesthetics.

General histological staining

All samples were fixed in 10% phosphate-buffered formalin about 24 h. The specimen were dehydrated in a series of graded ethanol (70%, 96% and 99%) and

embedded in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and the paraffin was cleared from the slides with coconut oil (over 15 min, 60 °C). The sections were rehydrated in 99%, 96% and 70% ethanol followed by a 10 min wash in water and stained with hematoxylin and eosin (HE). The layer thickness was measured by the same pathologist in a blinded review and sixteen determinations were made on each specimen and averaged.

Immunohistochemical staining

Tissue pretreatment: The tissue samples for immunohistochemistry were also fixed in 10% phosphate-buffered formalin about 24 h, embedded in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and placed in a water bath at 40 °C. Thereafter, sections were transferred onto pretreated microscopic slides, which electrostatically attracted formalin fixed tissue and binding them to the slides. After drying the slides completely at room temperature, they were treated in an oven at 37 °C overnight to enhance the attachment of tissue to the slides. The sections were deparaffinized two times in xylene, 15 min per time, and rehydrated in 100%, 95%, 90%, 80%, 70%, 60% and 50% ethanol two times respectively, 3 s per time, followed by rinsing for 10 min and washing in 0.01M PBS (pH 7.4).

AGE: After treatment with H₂O₂ (3% in ethanol, room temperature, 15 min) and proteinase K (100 µg/mL, 100 µL, 37 °C, 20 min), the sections were incubated with 5% BSA-PBS buffer (room temperature, 30 min) for blocking non-specific staining. Afterwards, the sections were incubated with the primary antibody 6D12 [1:100, diluted in 1% bovine serum albumin-Phosphate buffered saline (BSA-PBS)], which has been thoroughly characterized by Ikeda group^[22], or normal mouse IgG (250 µg/mL) pre-treated with excessive CML (1:250, diluted in 1% BSA-PBS, negative control) overnight at 4 °C. The sections were then washed and incubated with LINK (biotinylated anti-rabbit and anti-mouse immunoglobulins) and afterwards with STREPTAVIDIN PEROXIDASE (streptavidin conjugated with horseradish peroxidase) at room temperature for 10 min (both are part of reagents of LSAB2 System-HRP, products of Dako Company, Denmark). Then the peroxidase activity was visualized by incubating the sections in substrate working solution containing hydrogen peroxide and 3,3'-diaminobenzidine tetrahydrochloride at room temperature for 5 min. The sections were rinsed for 10 min, counterstained with Mayer Haematoxylin for 1 min, treated in HCl-ethanol for 3 s, dehydrated in 80%, 90%, 95%, 100% ethanol for 3 s, respectively. Then the slides were immersed in xylene for 15 min two times and mounted.

RAGE: The primary antibody against RAGE was produced in rabbits immunized with a synthetic peptide corresponding to a sequence at the N-terminal of

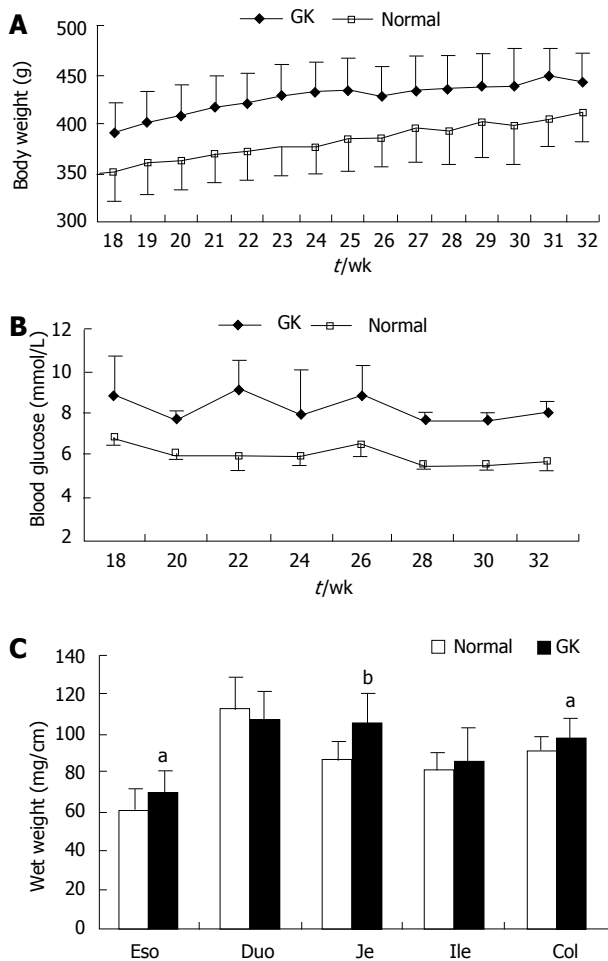


Figure 1 Body weight (A) and the blood glucose level (B) were higher in Goto-Kakizaki group than in the normal group ($P < 0.001$ and $P < 0.01$). The wet weight per unit length of intestinal and colon segments is shown in Figure 1C (compared with normal group: ^a $P < 0.05$, ^b $P < 0.01$). Values are mean \pm SD, $n = 8$ for each group. Eso: Esophagus; Duo: Duodenum; Je: Jejunum; Ile: Ileum; Col: Colon; GK: Inherited type 2 diabetic Goto-Kakizaki rats.

human RAGE. Only two amino acids are different from the related rat sequence. For RAGE immunostaining, instead of treating sections with proteinase K, the sections were boiled in 10 mmol/L citrate buffer (pH = 6.0) 18 min for retrieving antigen and using normal rat lung as positive control as the RAGE is highly expressed in the lung^[23]. The primary antibody was diluted (1:60) with 1% BSA-PBS and normal rabbit serum (diluted 1:60) pre-treated with excessive soluble RAGE was used as negative control. Other processes were similar to the AGE immunostaining.

Image analysis

To minimize errors, 6 to 10 photographs were randomly taken of different locations of same layer in each slide. After that, images of the different parts such as villus and crypt were saved as individual image files. The region of interest (ROI) was defined using Sigmascan Pro 4.0 image analysis software (Jandel Scientific, Germany). The color due to 3,3'-diaminobenzidine staining was distinguished in the ROI using intensity

thresholds. Finally the images were exported as binary images and the area fraction of AGE or RAGE positive staining was calculated by a MATLAB program (MATLAB 6.5, The MathWorks Inc. United States).

Data analysis

According to the image analysis above, the fraction of AGE in mucosa (villi and crypt were analyzed separately in the intestinal segments), muscle layers, and the fraction of RAGE in the mucosa and muscle layer were computed as: Fraction of AGE or RAGE = immunopositive area/total measuring area. It was difficult to calculate the fraction of RAGE in neurons in the same way. Therefore, the immunoreactivity of RAGE in each neuron was categorized by the stained intensity, *i.e.*, negative, mild, moderate and strong^[24].

Statistical analysis

The results were expressed as mean \pm SD unless indicated in the text. The differences between the diabetes and normal groups were tested using Student's *t* test and Anova. The results were regarded as significant when $P < 0.05$.

RESULTS

General information

The body weight and blood glucose level of GK group were significantly higher than those of the Normal group during the whole experimental period (Figure 1A and 1B, $P < 0.001$ and $P < 0.01$, respectively).

The wet weights per unit length of esophagus, jejunum and colon segments were highest in the GK group (Figure 1C, $P < 0.05$ and $P < 0.01$, respectively). No significant difference were found for duodenum and ileum between the two groups (Figure 1C, $P > 0.05$).

General histological changes

Compared with the Normal group, the full wall thickness of esophagus, jejunum and colon remarkably increased in the GK group (Figure 2A, $P < 0.05$ and $P < 0.01$, respectively). No significant difference was found in duodenum and ileum between two groups. The smooth muscle thickness of esophagus and colon (both circumferential and longitudinal smooth muscle) increased remarkably in GK group. The villous height of jejunum increased in the GK group (Figure 2B-D, $P < 0.05$ and $P < 0.01$). No significant difference was found for other layers.

Distribution of AGE

The immune-positive area of AGE was yellow-brown (Figure 3A and B). These colors were not found in the negative control slides (without primary antibody), demonstrating that the stained color was specific for AGE.

In the esophagus, AGE distribution was inhomogeneous and mainly distributed in striated muscle

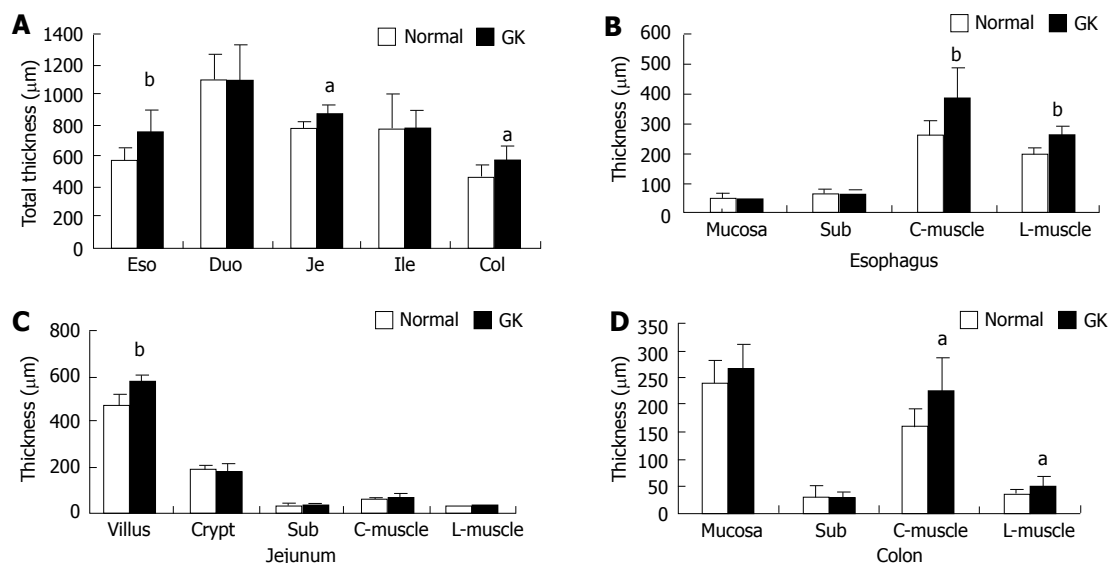


Figure 2 The wall and layer thickness. A: Total wall thickness; B: Layer thickness of esophagus; C: Layer thickness of jejunum; D: Layer thickness of colon. Values are mean \pm SD, $n = 8$ for each group (compared with normal group: ^a $P < 0.05$, ^b $P < 0.01$). Eso: Esophagus; Duo: Duodenum; Je: Jejunum; Ile: Ileum; Col: Colon; Sub: Submucosa; C-muscle: Circumferential smooth muscle; L-muscle: Longitudinal smooth muscle; GK: Inherited type 2 diabetic Goto-Kakizaki rats.

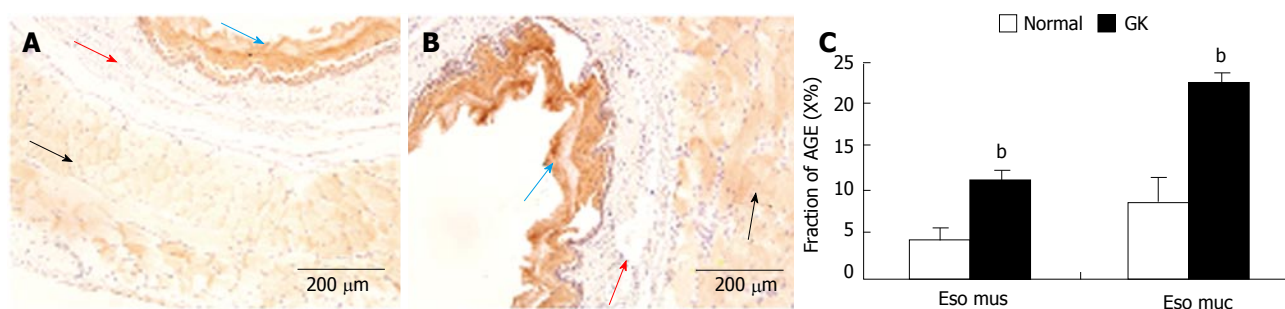


Figure 3 Example of advanced glycation end products immune-staining in esophagus (A, normal; B, diabetic); C: Shows the statistic result of immune-staining intensity in esophagus muscle and mucosa. Values are mean \pm SD, $n = 8$ for each group (compared with normal group: ^b $P < 0.01$). The immune-positive area of AGE showed yellow-brown color. Eso mus: Esophageal muscle; Eso muc: Esophageal mucosa; GK: Inherited type 2 diabetic Goto-Kakizaki rats; AGE: Advanced glycation end product. Blue arrow: mucosa; Red arrows: Submucosa; Black arrows: Muscle.

and squamous epithelial cells. Compared with normal group, the intensity of immune-staining for AGE was much stronger in the GK group (Figure 3C, $P < 0.01$). No visible stained color was found in submucosa layer (Figure 3A and B).

In the intestine, AGE was mainly distributed in the mucosa layer, especially in epithelial cells of villi (Figure 4A-4E) and crypt. No visible stained color was found in submucosa, smooth muscle and ganglia. The crypt epithelial cells in ileum and colon were slightly stained. The distribution of AGE in the epithelial cells was inhomogeneous, the surface part was much stronger than the bottom part in villous epithelial cells but it showed an opposite pattern in the crypt epithelial cells. The intensity of AGE staining of the epithelial cells in villi was stronger than that in crypts ($P < 0.01$). In the mucosa, the intensity of AGE staining was similar between duodenal and jejunal segments (Figure 4A-C, F, G) but they were stronger than those in colon (Figure 4A, 4C, 4E and 4F). The mucosa of ileum showed the weakest intensity of AGE staining among different

intestinal segments (Figure 4D). Compared with the Normal group, the intensity of AGE staining in mucosa of all segments were stronger in the GK group (Figure 4F and G). Significant difference was found in the epithelial cells of villi and crypt in duodenum (Figure 4F and G, $P < 0.05$).

Distribution of RAGE

The immune-positive area of RAGE also showed yellow-brown color (Figure 5) that was not found in the negative control slides (without primary antibody). Therefore, the stained color was specific for RAGE.

In esophagus, the immune-positive staining for RAGE was mainly observed in the striated muscle cells and mucosa squamous epithelial cells. The RAGE distribution was inhomogeneous in the striated muscle layer and graduated decreased from bottom to surface. No visible stained color was found in the submucosa layer (Figure 5). The intensity of RAGE staining did not differ between Normal and GK group both in the striated muscle cells and mucosa squamous epithelial

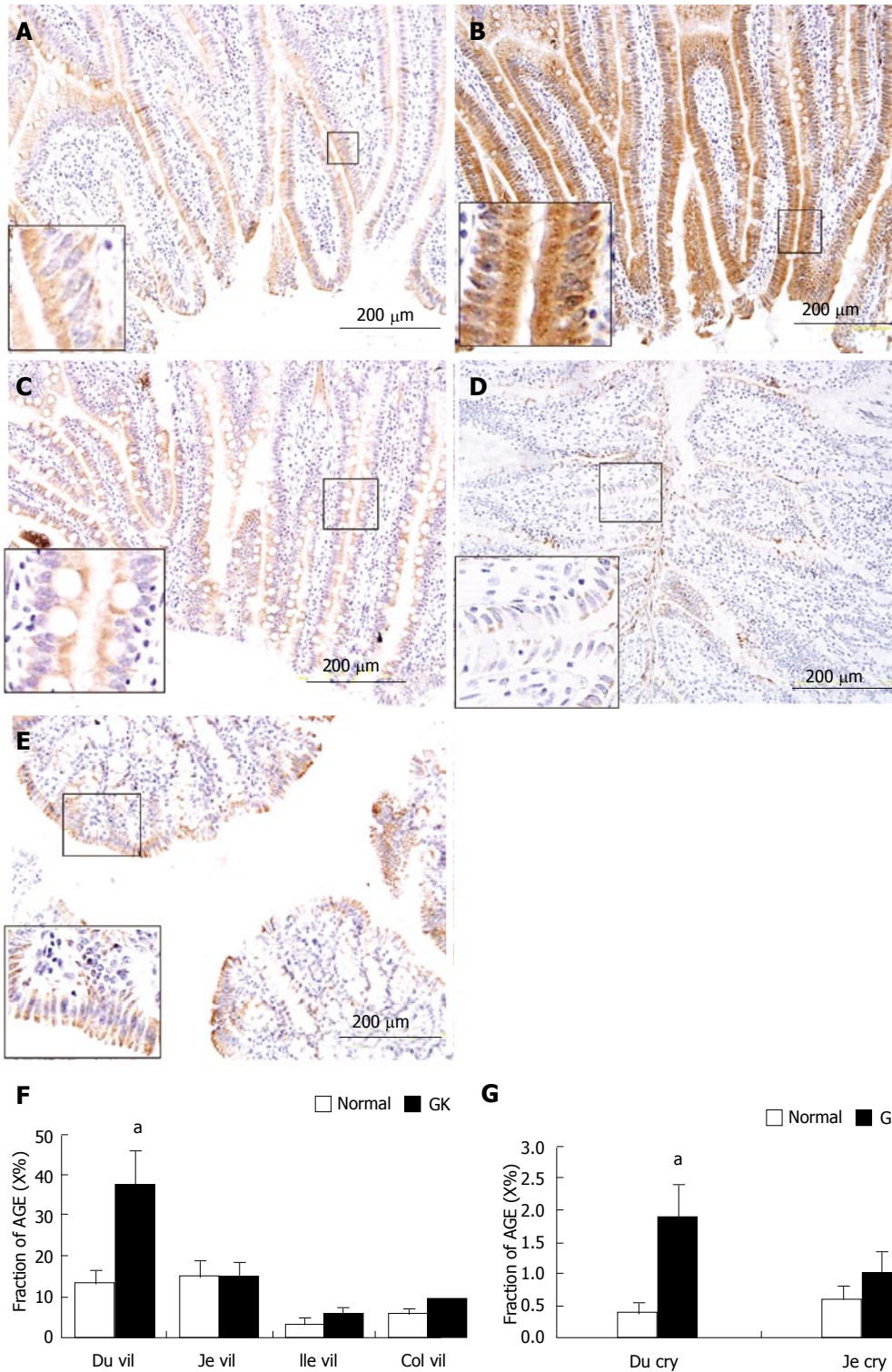


Figure 4 Example of advanced glycation end products immune-staining in villi of duodenum (A, normal; B, diabetic), jejunum (C), ileum (D) and mucosa in colon (E). The immune-positive area of AGE showed yellow-brown color; 4F and 4G: Show the statistical result of immune-staining intensity in villous epithelial cells of duodenum, jejunum, ileum and colon as well as in crypt epithelial cells of duodenum and jejunum. As shown in the magnification area (big frame vs small frame), the AGE distribution in epithelial cells was inhomogeneous, the surface part was much stronger than bottom part. Values are mean \pm SD, $n =$ for each group (compared with normal group: ^a $P < 0.05$). Du vil: Duodenum villi; Je vil: Jejunum villi; Ile vil: Ileum villi; Col mu: Colon mucosa; Du cry: Duodenum crypt; GK: Inherited type 2 diabetic Goto-Kakizaki rats; AGE: Advanced glycation end products.

cells ($P > 0.05$).

In the small intestine, the immune-positive staining for RAGE was observed in the epithelial cells of villi (Figure 6A, C, E) and crypts (Figure 6B, D, F), and

in neurons in the myenteric and submucosal plexus (Figure 6G). The RAGE was homogeneously distributed in the cells, as shown in villus and crypt epithelial cells, but the intensity of immune-staining was

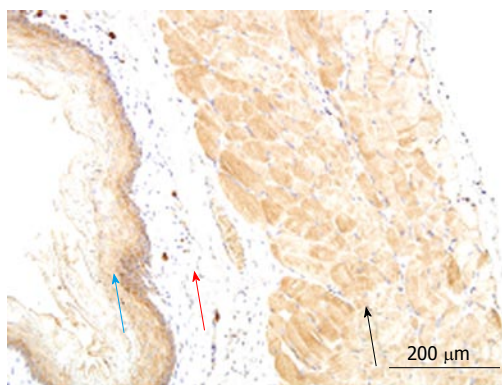


Figure 5 Example of receptor of advanced glycation end products immune-staining in normal esophagus. The immune-positive area of RAGE showed yellow-brown color. RAGE: Receptor of advanced glycation end products. Blue arrow: mucosa; Red arrows: Submucosa; Black arrows: Muscle.

much stronger in villous epithelial cells than in crypt epithelial cells. The strongest staining color occurred in duodenum and the weakest in ileum among the three segments (duodenum > jejunum > ileum, Figure 6A-F). In neurons, RAGE distributed both in cytoplasm and cell membrane (Figure 6G). Compared with the Normal group, the intensity of immune-staining for RAGE increased in the neurons for the three segments (Figure 6G and H) in the GK group ($P < 0.05$), but no significant difference was found in other cells between two groups.

In the colon, the immune-positive staining for RAGE was observed in neurons of the myenteric (Figure 7A) and submucosal plexus. It was stronger in the GK group than in Normal group ($P < 0.05$) (Figure 7A and B). Furthermore, a mild positive staining was also observed in mucosa epithelial cells both in the GK and Normal groups (Figure 7C).

DISCUSSION

Our previous study showed that the expression of AGE and RAGE was up-regulated in the small intestine and colon of STZ-induced type 1 diabetic rats^[21]. STZ rats have high blood glucose but formation of free radicals and STZ cytotoxicity plus its direct effects on AGE formation and RAGE expression in GI tract may be questioned. Using the present model, the confounding effect of STZ can be avoided. The major discovery was that the intensity of AGE immune-staining was significantly increased in striated muscle and mucosa layer of esophagus, and in epithelial cells located in intestinal villi and crypts in the GK group compared to normal rats. RAGE was significantly increased in myenteric and submucosa plexus neurons of all intestinal segments in the GK group.

The distribution of AGE and RAGE in normal GI tract

Ling *et al.*^[25] reported the existence of four kinds of AGEs in stomach and small intestinal epithelial cells in normal rats. Our previous study^[21] showed

homogenous AGE distribution in the cytoplasm of smooth muscle cells, epithelial cells, and neurons of the myenteric and submucosal plexus in the layers of colon and small intestine. Furthermore, homogeneous distribution of RAGE was found in epithelial cells and neurons. The present study confirmed the distribution of both compounds in the colon and small intestine as reported from our previous study. Furthermore, we also found that the AGE and RAGE distributed in the striated muscle and squamous epithelial cells of esophagus and also in the stomach (unpublished data). The present study together with our previous study^[21] is the first reports of the localization of AGE and RAGE in the whole rat GI tract. This provides a basis for further comparison study of the distribution of AGE and RAGE on GI tract with diseases, such as diabetes.

AGE and RAGE changes in GI tract of GK diabetic rats

RAGE and AGE distribution in the GI tract of GK rats was similar to that in normal controls. However, compared with the normal controls, the level of AGE and RAGE at some GI locations was increased in GK rats. However, compared with our previous study^[21], the intensity of AGE and RAGE immune-staining were not so strong in the present study. It is well known that the accumulation and production of AGEs and expression of RAGE are associated with blood glucose level^[26]. The blood glucose level is much lower in GK diabetic rats compared to STZ-induced diabetic rats. This is one plausible explanation for the weaker increasing AGE and RAGE in the GK type-2 diabetic rat model.

Histomorphological and biomechanical GI remodeling occurred during the development of diabetes^[27]. For example the esophagus and colon were morphologically and biomechanically remodeled during the development of diabetes^[6,7]. Abnormal levels of AGE and RAGE found in the present study may be associated with the GI remodeling in the GK diabetic rats. In the present study we found that the mucosa of small intestine and muscle layer of all segments proliferated, accordingly the AGE expression is up-regulated in the mucosa in all segments and esophageal muscle layer in the GK diabetic rats. However, the intensity of immune-staining in muscle had no apparent increase in muscle layer of intestine in GK group despite the fact that the muscle layer showed hyperplasia. Therefore it is speculated that in addition to the affection of AGE, other factors may affect the hyperplasia of GI muscle in GK diabetic rats, such as glucagon-like peptide 2^[28].

In the present experiment no direct evidence showed how tissue growth was affected by AGEs and RAGE. From studies in other organs, it is known that AGE through AGE-RAGE-mediated ROS generation activating angiotensin II-tissue growth factor beta (TGF- β)-S-mad signaling can increase renal interstitial fibroblasts mitogenesis and type I collagen production^[29], mesangial cell hypertrophy and fibronectin synthesis^[9], through AGE-RAGE interaction can cause epithelial

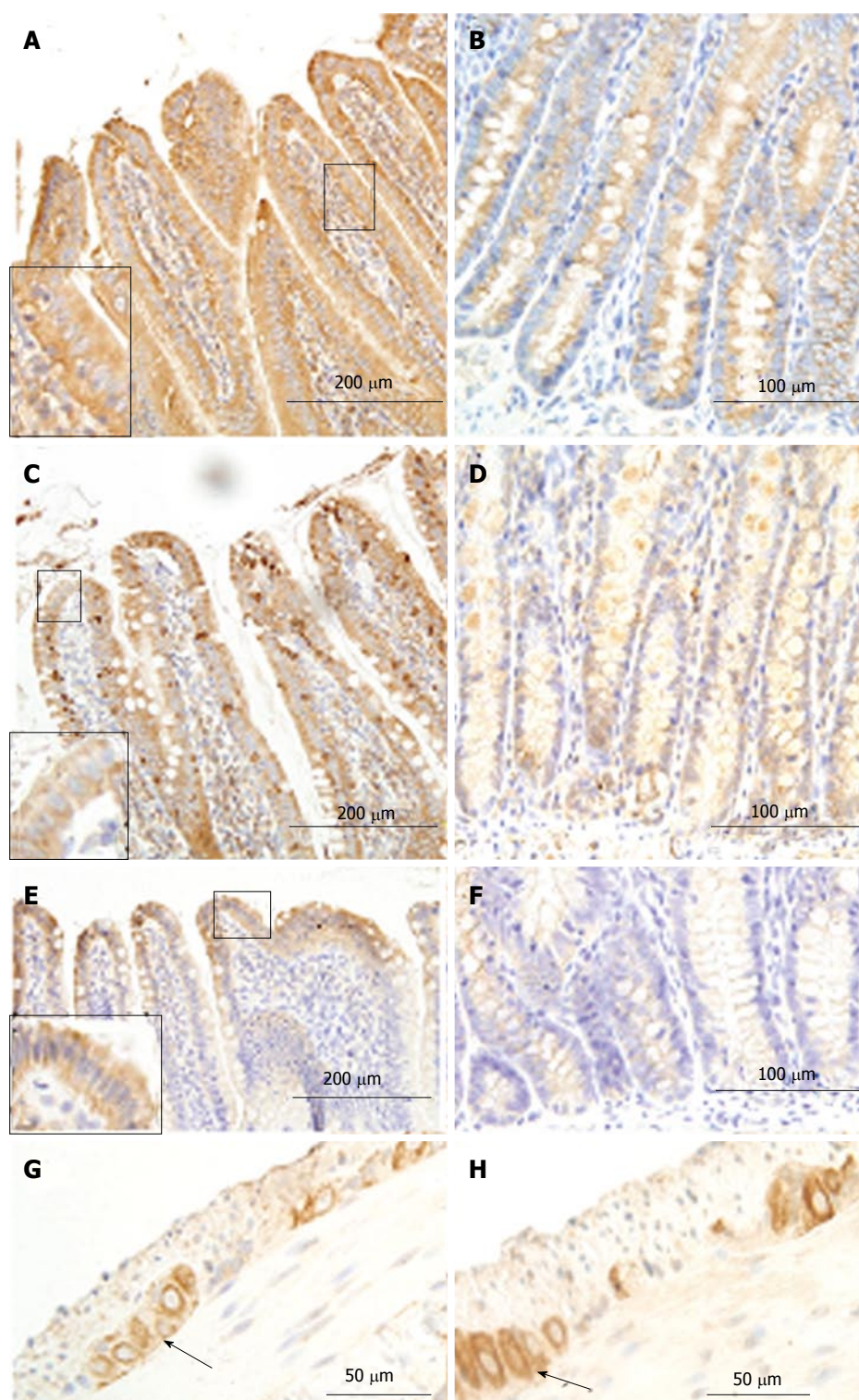


Figure 6 Receptor of advanced glycation end products immune-staining in villi (A, C, E) and crypt (B, D, F) of duodenum (A, B), jejunum (C, D) and ileum (E, F) as well as in ileum ganglia (arrowhead; G: Normal group; H: GK group). As shown in the magnification area (big frame vs small frame), the RAGE homogeneously distributed in the epithelia cells. The intensity of immune-staining in ganglia was stronger in the diabetic group (H) than in the normal group (G) (arrowhead). RAGE: Receptor of advanced glycation end products; GK: Inherited type 2 diabetic Goto-Kakizaki rats.

myofibroblast transdifferentiation^[30] and vascular smooth muscle proliferation^[31], and through galectin-3 induce smooth muscle proliferation^[32]. Therefore, it is feasible that GI tissue proliferation at least in part may be induced by AGE accumulation through the same pathways. Further studies must explore mechanisms

for AGE- and RAGE-induced GI tissue growth and the association with the biomechanical remodelling in diabetes.

AGE and RAGE accumulation impact on GI dysfunction

It is well documented that small intestinal epithelial

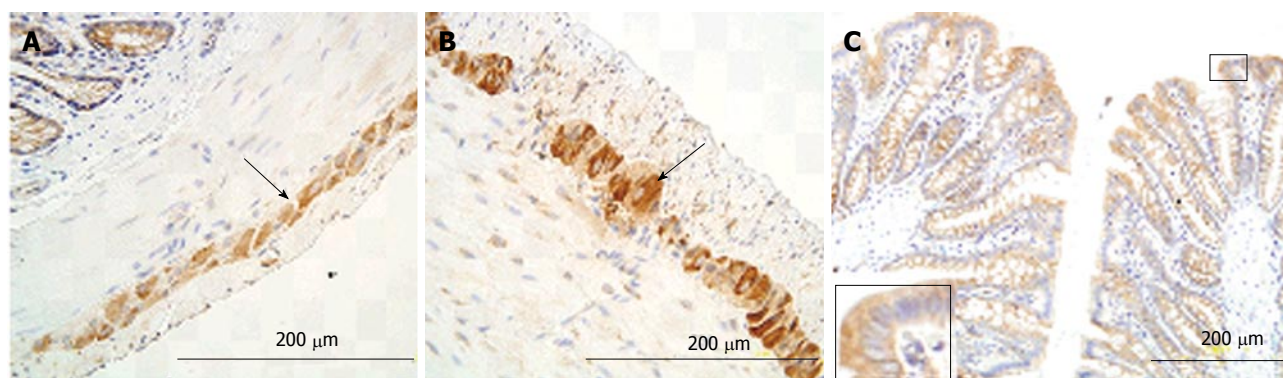


Figure 7 Receptor of advanced glycation end products immune-staining in colon ganglia (arrow; A: Normal; B: Diabetic) and mucosa (C). The intensity of immune-staining in ganglia was stronger in the GK group (B) than in the normal group (A) (arrow). Big frame area vs small frame area. RAGE: Receptor of advanced glycation end products; GK: Inherited type 2 diabetic Goto-Kakizaki rats.

cells are important for digestion and absorption. Many kinds of enzymes located in the enterocytes lining the intestinal villi brush border are involved in digestion^[33]. Furthermore, the small intestinal mucosa is important for absorptive function^[33]. We demonstrated a stronger intensity of AGE immune-staining in the epithelial cells of intestinal crypts and villi in the diabetes group compared to the normal group. Digestive enzyme activity and cell membrane properties may potentially be affected by AGE accumulation. It is also known that non-enzymatic glycation and oxidative stress are important for changes of brush border membrane fluidity^[34]. Digestion and intestinal transport processes occur at the brush border membrane. Changes in fluidity as well as in the membrane composition can alter the enzyme activity in the villi brush border membrane^[35,36]. Multiple cellular signaling cascades can be activated by binding of AGEs to RAGE^[37]. The increased AGE linking with RAGE may change epithelial cell function. Mechanisms linking AGEs/RAGE compounds to intestinal mucosa function in diabetics need more work.

Numerous studies demonstrated abnormal GI motility in diabetics^[27,38]. Diabetic autonomic neuropathy is considered important in the pathogenesis of sensory-motor disordered function in diabetic patients^[39-41]. AGEs and RAGE likely are key players in development of diabetic neuropathy^[23]. Synergistic action of AGEs and endogenous nitric oxide can lead to neuronal apoptosis *in vitro*^[42]. The neuronal AGE formation and accumulation may account for the development of GI neuropathy, primarily as a direct effect on structural and functional proteins, alternatively by activating RAGE indirectly^[43]. GI nerves express nitric oxide synthase (nNOS), which generates a key transmitter nitric oxide in the regulation of GI motility^[44]. Korenagas group demonstrated that AGEs inhibit *via* RAGE nNOS expression *in vitro*^[45]. The expression and function of neuronal nitric oxide synthase decreased in the stomach of spontaneously diabetic BB-rats^[41] and also decreased in duodenum of STZ-induced diabetic rats, which can be prevented by aminoguanidine (a drug

that prevents AGE formation) and ALT-711 (AGE cross-link breaker)^[8]. In the present study direct evidence was provided that RAGE is localized in myenteric and submucosal plexus neurons in the esophagus and intestine. In addition, DM enhanced RAGE intensity and therefore, AGE-RAGE interaction is likely of importance for GI diabetic autonomic neuropathy. We also demonstrated that the intensity muscle tissue AGE immune-staining was strongest in the diabetic esophagus. The accumulation of AGEs in muscle may alter the architecture and contractile proteins of smooth muscle^[46], resulting in the alteration of muscle contraction properties.

AGE was mainly distributed in striated muscle and squamous epithelial cells in esophagus; in small intestinal epithelial cells of crypt and villi and in epithelial cells in colon and rectum. RAGE was mainly distributed in striated muscle and squamous epithelial in esophagus; in epithelial cells in intestine mucosa and neurons in ganglia. High AGE density was found in striated muscle and mucosa layers in esophagus, and villus, crypt in the GK rat small intestine, and the expression of RAGE in the intestine increased in ganglia of GK rats. Increased expression of AGE and RAGE likely contributes to GI disorders associated with DM.

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COMMENTS

Background

In this previous study the authors demonstrated that advanced glycation end products (AGEs) and their receptor (RAGE) were up-regulated in the small intestine and colon of streptozotocin-induced diabetic rats. However, to the best of our knowledge, data on the distribution of AGE and RAGE in the gastrointestinal (GI) tract of type 2 diabetes have never been described.

Research frontiers

Previous studies demonstrated the morphological and biomechanical properties of the GI tract were remodeled during diabetes. However the mechanisms for these changes are not well understood. Therefore, investigation on the

distribution of AGE and RAGE in the GI tract of type-2 diabetes is important for understanding the mechanism of GI remodeling in diabetes.

Innovations and breakthroughs

At present study the authors demonstrated that the AGE and RAGE expression was up-regulated in the GI tract of GK diabetic rats. The increased AGE and RAGE levels may contribute to diabetic GI dysfunction in type 2 diabetic patients.

Applications

The most common type diabetes is type 2 diabetes; therefore it is important to understand the expression of AGE and RAGE in the GI tissues in type 2 diabetes. Knowing the over-expression of AGE and RAGE in the diabetic GI tract may in somehow direct the treatment in the patients suffering from type 2 diabetes.

Terminology

AGEs are formed by non-enzymatic attachment of sugars to the amino groups of various proteins through a series of complex intermediary reactions. Diabetic hyperglycemia accelerates the accumulation of AGEs in the tissues. RAGE is a 55kD transmembrane receptor of the immunoglobulin super family, which binds AGEs. AGEs contribute to diabetic complications through receptor-dependent and -independent pathways.

Peer-review

This is an interesting study. In future, more data should provide to further demonstrate the relationship between increasing AGE and morphological or functional changes in diabetes.

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

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Update on type 2 diabetes-related osteoporosis

Kannikar Wongdee, Narattaphol Charoenphandhu

Kannikar Wongdee, Narattaphol Charoenphandhu, Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Bangkok 10400, Thailand
 Kannikar Wongdee, Office of Academic Management, Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand
 Narattaphol Charoenphandhu, Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Author contributions: Wongdee K and Charoenphandhu N contributed equally for literature review, data analysis and preparation of the manuscript.

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Correspondence to: Narattaphol Charoenphandhu, MD, PhD, Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand. naratt@narattsys.com
 Telephone: +66-2-3547154
 Fax: +66-2-3547154

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Abstract

It was previously understood that body weight gain and obesity observed in type 2 diabetes mellitus (T2DM) could be beneficial since body weight increase elevated bone mineral density and thus helped maintain the skeletal framework. However, a number of recent findings in humans and rodents have revealed that T2DM is not only associated with trabecular defects but also increases cortical porosity, and compromised bone cell function and bone mechanical properties. Hyperglycemia and insulin resistance in T2DM may further induce osteoblast apoptosis and uncoupling bone turnover. Prolonged accumulation of advanced glycation end products and diminished activity of lysyl oxidase, an essential enzyme for collagen cross-link, can lead to structural abnormalities of bone collagen fibrils, brittle matrix, and fragility fractures. Our studies in T2DM rats showed that dyslipidemia, which often occurs in T2DM, could obscure the T2DM-associated changes in bone microstructure and osteopenia. Longitudinal bone growth regulated by the growth plate chondrocytes is also impaired by T2DM since differentiation of growth plate chondrocytes is arrested and retained in the resting state while only a small number of cells undergo hypertrophic differentiation. Such a delayed chondrocyte differentiation may have also resulted from premature apoptosis of the growth plate chondrocytes. Nevertheless, the underlying cellular and molecular mechanisms of insulin resistance in osteoblasts, osteoclasts, osteocytes, and growth plate chondrocytes remain to be investigated.

Key words: Advanced glycation end products; Chondrocyte apoptosis; Collagen; Dyslipidemia; Fracture; Growth plate; Type 2 diabetes mellitus; Osteoporosis

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Core tip: Type 2 diabetes mellitus (T2DM) negatively affects bone density and strength by inducing cellular and extracellular matrix failures. Insulin resistance in T2DM deteriorates osteoblast proliferation and activity, but enhances osteoclast activity, leading to uncoupled bone remodeling. Hyperglycemia also aggravates osteoblast dysfunction, thus contributing to cellular failure. Extracellular matrix failure is caused by abnormal collagen synthesis and aberrant collagen structure and alignment, the latter of which results, in part, from advanced glycation end products (AGEs). With hyperglycemia and AGEs, impaired bone strength may occur despite high bone mineral density. It is, therefore, concluded that T2DM can be considered a cause of osteoporosis and/or poor bone mechanical properties.

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INTRODUCTION

Although type 1 diabetes mellitus (T1DM) is known to compromise bone microstructure^[1,2], how type 2 diabetes mellitus (T2DM) affects bone metabolism has long been debate for decades. It was previously believed that T2DM could be protective against osteoporosis since a number of clinical studies and meta-analyses revealed an increase in bone mineral density (BMD) in T2DM patients^[1,2]. However, several recent lines of evidence in both humans and rodents have corroborated that T2DM is indeed detrimental to bone, leading to impaired osteoblast-mediated bone formation, accelerated bone resorption, microstructural defect, and poor bone quality. The previous controversial data stem from the use of low-resolution X-ray-based techniques, including measurement of areal BMD by dual energy X-ray absorptiometry (DXA), rather than evaluation of high-resolution bone microstructure or bone mechanical properties. This article has updated the recent findings of T2DM-related osteopenia and osteoporosis, as summarized in Table 1.

T2DM INCREASES BONE POROSITY AND DECREASES MECHANICAL BONE STRENGTH

Previous investigations in human mostly focus on trabecular bone changes in T2DM, but recent investigations have moved to the study of cortical changes and mechanical properties. By using more advanced

techniques, such as high-resolution 3-dimensional computed tomography and microindentation, it was found that T2DM negatively affected bone strength despite the presence of relatively high BMD. Several cross-sectional studies in T2DM patients using high-resolution peripheral quantitative computed tomography (HR-pQCT) and magnetic resonance imaging (MRI) consistently revealed quality defects in both cortical and trabecular networks that would increase fracture risk^[3-6]. For instance, Farr *et al.*^[3] by assessing bone quality with HR-pQCT in 30 postmenopausal T2DM patients at distal radius and distal tibia, found lower cortical thickness in T2DM was lower than normal non-diabetic controls, while bone microindentation testing showed lower bone material strength (BMS) in T2DM patients. Moreover, the radius quality evaluated by MRI, showed trabecular network holes being approximately 10% larger in postmenopausal T2DM patients than normal controls^[5]. The cortical part was similarly affected by T2DM^[4]. Patsch *et al.*^[4] investigated changes in bone microarchitecture in postmenopausal T2DM patients with or without fractures at radius and tibia by using DXA and HR-pQCT. Interestingly, they found that T2DM patients with fractures had higher pore-related deficits, *i.e.*, greater cortical pore volume, cortical porosity, and endocortical bone surface, than diabetic patients without fractures^[4], consistent with the previous report in the radii of T2DM patients that having greater cortical pore volume (approximately 150%) and cortical porosity (approximately 125%) than normal individuals^[6]. These cortical defects were often accompanied by impaired mechanical properties, such as increased failure load and low bone bending strength, that led to reduction in overall bone strength and increase in fracture risk^[4,7].

DYSLIPIDEMIA MIGHT OBSCURE T2DM-INDUCED OSTEOPOROSIS

Previously it was believed that greater body weight or obesity associated with T2DM could be beneficial to the skeletal system through increasing BMD and bone mass^[7,8]. However, our group recently reported the possible masking effects of dyslipidemia on diabetic bone in rats^[9]. In our study, we determined the effects of dyslipidemia on bone microstructure were determined in Goto-Kakizaki (GK) diabetic rats treated with high cholesterol diet compared those fed with normal diet. The GK rats-a non-obese T2DM rat model without obesity-induced bone gain-were found to manifest stable fasting hyperglycemia and insulin resistance, while cholesterol-fed GK rats exhibited hypercholesterolemia, hypertriglyceridemia and hyperglycemia without significant weight gain^[10]. Bone histomorphometry revealed that GK rats with T2DM manifested several signs of suppressed osteoblast function, such as decreases in osteoblast surface and bone formation rate, whereas the osteoclast-mediated

Table 1 Recent studies on the type 2 diabetes mellitus-related osteoporosis in humans (2010-2014)

Site of bone	Technique	Subjects	Main findings in T2DM group	Ref.
Distal radius	HR-pQCT	60 postmenopausal women	Low cortical thickness and low trabecular number	[3]
Distal tibia	HR-pQCT		Lower cortical thickness	
Mid-shaft tibia	Microindentation		Poor bone quality (low BMS)	
Distal radius	DXA, HR-pQCT		Higher cortical porosity in T2DM with fractures	
Ultradistal radius	DXA, HR-pQCT	80 postmenopausal women with or without fractures	Higher cortical porosity in T2DM with fractures	[4]
Distal tibia	DXA, HR-pQCT		Lower cortical porosity in T2DM with fractures	
Ultradistal tibia	DXA, HR-pQCT		Lower BMD and cortical porosity in T2DM with fractures	
Distal radius	MRI		Greater trabecular network holes	
Distal radius	HR-pQCT	38 postmenopausal women	Higher cortical porosity	[5]
Tibia	HR-pQCT	1171 men (≥ 65 yr)	Higher volumetric BMD and trabecular thickness	[6]
Mid-shaft radius	pQCT		Lower bone bending strength	
Mid-shaft tibia	pQCT		Lower bone bending strength	

BMD: Bone mineral density; BMS: Bone material strength; DXA: Dual energy X-ray absorptiometry; HR-pQCT: High resolution-peripheral quantitative computed tomography; MRI: Magnetic resonance imaging; T2DM: Type 2 diabetes mellitus.

bone resorption was markedly enhanced. It was noted that, these microstructural changes disappeared after 16-wk of high cholesterol consumption, suggesting that high cholesterol diet and perhaps the resultant dyslipidemia could obscure the T2DM-associated osteopenia and changes in bone microstructural defect^[9]. Thus, the difficulty in detecting bone deterioration in T2DM rats with dyslipidemia could explain, in part, why osteopenia was not observed in some T2DM studies.

T2DM AND LONGITUDINAL BONE GROWTH

Up until now, few studies have investigated relationship between T2DM and longitudinal bone growth. Generally, longitudinal bone growth is controlled by proliferation and differentiation of chondrocytes in the growth plate, which is histologically divided into 3 zones, *i.e.*, resting zone (RZ), proliferative zone (PZ) and hypertrophic zone (HZ). The RZ consists of low mitotic activity stem-like cells that gradually migrate to the PZ where chondrocytes proliferate and align into vertical columns and eventually reach the mature state in HZ. Thereafter, the hypertrophic chondrocytes in HZ undergo apoptosis and are replaced by capillaries and osteoblasts, which later use cartilaginous scaffold as a template for bone formation and bone elongation^[11,12]. Since several investigations reported reduced bone length in diabetic rats compared with normal rats^[9,13], T2DM may be a cause of aberrant growth plate function. Lapmanee *et al.*^[9] examined changes in the growth plate of diabetic GK rats and found impairment of chondrocyte differentiation as indicated by increased RZ height and decreased HZ height. It was possible that differentiation of chondrocyte precursors in T2DM rats were arrested and cells remained in the resting state, with only a small number of proliferating cells undergoing differentiation into hypertrophic chondrocytes^[9].

Aiemlapa *et al.*^[14] further demonstrated the underlying mechanism of delayed growth plate chondrocyte differentiation. By using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay for apoptosis, they found premature apoptosis of chondrocytes in the HZ and chondro-osseous junction of GK rats. The massive loss of growth plate chondrocytes was accompanied by an increase in serum IGF-1 level, and overexpression of parathyroid hormone related protein (PTHrP), *runx*-related transcription factor (Runx2) and vascular endothelial growth factor (VEGF) in the growth plate, all of which might have been compensatory responses to mitigate excessive loss of chondrocytes due to premature apoptosis^[14]. However, it was possible that the effects of T2DM on the growth plate might be dependent on animal strain and model of DM induction. For instance, Wu *et al.*^[15] found acceleration of longitudinal bone growth as indicated by bone elongation and increased heights of PZ and HZ in insulin resistant mice induced by high fat diet. In this model, insulin might remain to have a stimulatory effect on bone growth *in vivo* similar to its reported stimulatory effect on metatarsal linear growth *in vitro*^[15]. Furthermore, high fat diet-induced dyslipidemia could complicate the matter since 7 α -hydroxycholesterol and oxidized low-density lipoprotein (LDL) have been shown to modulate osteoblast and osteoclast functions, which, in turn, could have effects on bone elongation^[16,17].

POSSIBLE CELLULAR MECHANISMS OF T2DM-RELATED FRAGILITY FRACTURES

The pathogenesis of T2DM-related fragility fracture can be looked upon from 2 aspects, *i.e.*, cellular failure and extracellular matrix failure. At cellular level, T2DM was associated with diminished activities of osteoblasts, osteoclasts and osteocytes, and increased apoptosis of bone cells^[9,18-21]. A decrease in osteocyte density (number of osteocyte-occupied lacunae per unit

area) was conspicuously observed in streptozotocin-induced diabetic rats^[21]. Hyperglycemia-induced insulin resistance is another important factor that cause both osteoblast and osteoclast malfunctions^[22]. Since insulin is a suppressor of osteoclast-mediated bone resorption^[23], T2DM-associated insulin resistance could enhance bone resorption. Moreover, high plasma glucose concentration can induce glucotoxicity in cells including osteoblasts, leading to osteoblast apoptosis^[24]. *In vivo* experiment in transgenic liver-specific S503A CEACAM1 mutant (L-SACC1) mice, a model of impaired insulin clearance in the liver causing hyperinsulinemia and insulin resistance, suggested that the abnormally high bone mass in these mice might have resulted from low bone turnover as indicated by decreases in double-labeled surface (as determined by bone histomorphometry) and TRAP-positive osteoclasts, which represent activities of osteoblast-mediated bone formation and osteoclast-mediated bone resorption, respectively^[22]. In other words, insulin resistance in this model was associated with a slowdown in bone turnover, which could eventually result in inadequate healing of microcracks, poor bone quality and increased fracture risk^[22]. In addition, the experiment in high fat diet-fed Zucker diabetic fatty (ZDF) rats also showed impaired osteoblast function as indicated by downregulation of the expression of osteoblast-specific genes, *e.g.*, bone morphogenetic protein-2 (BMP-2), Runx2, osteocalcin and osteopontin. Suppression of osteoblastogenesis in these ZDF rats possibly compromised bone regeneration capacity. Subcritical bone defect regeneration study further showed that nondiabetic rats filled the defect by 57%, whereas diabetic rats could fill only 21% of bone defect in 12 wk^[18].

T2DM not only caused deterioration of bone cell functions (cellular failure), but it also damaged bone extracellular matrix. Most studies suggested that T2DM caused abnormality in the structure of collagen, which is the most abundant protein in organic bone matrix. García-Hernández *et al.*^[25] reported that high glucose concentration indeed increased biomineralization in human alveolar bone-derived osteoblasts, but the mineral quality was lower than that in low glucose-exposed group. Determination of mineral quality in term of calcium/phosphate (Ca/Pi) ratio in the mineralized extracellular matrix nodules by energy-dispersive X-ray microanalysis (EDX) showed that high concentration of glucose significantly decreased Ca/Pi ratio on day 7 and 14 of treatment. Hammond *et al.*^[26] further studied nanoscale morphology of type I collagen in tibiae of ZDF rats by Raman spectroscopy and reference point indentation (RPI), the latter of which applied a force to determine bone mechanical properties by measuring the relative displacement reference position^[26,27]. RPI analysis revealed that bone matrix of ZDF diabetic rats was more resistant to plastic deformation, which might have resulted from abnormal formation of non-

enzymatic collagen cross-link, toughening of the matrix, or the presence of advanced glycation end products (AGEs).

AGEs are non-enzymatic carbohydrate modifications of extracellular and intracellular proteins accumulated in long-lived tissues, such as skin and bone, and are often present in the plasma proteins of patients with DM and renal failure^[28,29]. A number of investigations have revealed that AGEs are considered a factor that provokes fragility fractures in T2DM by inducing abnormal arrangement of collagen^[26,28,30]. By using scanning electron microscope (SEM) and transmission electron microscope (TEM), Aoki *et al.*^[28] provided evidence that the rats subjected to adenine-induced renal failure exhibited AGEs accumulation and suppression of osteoblast function, similar to that observed in T2DM. SEM showed irregularity in collagen fibril alignment, while TEM revealed a wider diameter of collagen fibril in adenine-treated rats with renal osteodystrophy^[28]. Immunohistochemistry also showed greater accumulation of AGEs in peritrabecular osteoblasts of adenine-treated rats than control rats. Further *in vitro* study in AGEs-treated MC3T3-E1 osteoblast-like cells showed a decrease in protein expression of secreted phosphoprotein 1 and lysyl oxidase, a mature osteoblast marker and essential enzyme for collagen cross-link, respectively. It was thus suggested that suppressed osteoblast differentiation and decreased lysyl oxidase production caused structural abnormalities of bone collagen fibrils leading to bone fragility^[28].

Collagen is the most abundant protein in bone organic matrix, and it undergoes intra- and extracellular post-translational modifications^[31]. To stabilize collagen fibrils, lysyl oxidase catalyzes intra- and intermolecular cross-link between collagen molecules essential for bone strength^[31]. It was reported that glycation of collagen caused abnormal arrangement of collagen leading to brittle matrix and fragile bone^[26,28,30], but little is known whether a decrease in lysyl oxidase-dependent collagen cross-link contributes to diabetic bone fragility and osteoporosis. The underlying mechanism of AGEs-attenuated lysyl oxidase activity was explored in mouse and rat primary osteoblasts and it was found that the carboxymethylated collagen, a form of AGEs, was not able to promote lysyl oxidase-mediated cross-linking due to failure of binding between abnormal collagen and discoidin domain receptor-2^[30].

CONCLUSION

Currently, it can be concluded that T2DM compromises bone microstructure by inducing aberrant bone cell function (cellular failure) and abnormal matrix structure (matrix failure). Regarding the cellular effect, T2DM is associated with increased osteoblast apoptosis, diminished osteoblast differentiation, and enhanced osteoclast-mediated bone resorption, which, in part,

resulted from hyperglycemia and insulin resistance. Prolonged accumulation of AGEs coexisting with a decrease in lysyl oxidase activity causes abnormal structure and alignment of collagen, leading to bone fragility. Several confounding factors in T2DM, particularly body weight gain, obesity, and dyslipidemia, are able to mask the detrimental effects of T2DM, and may delay diagnosis of diabetic osteoporosis. In other words, bone is already damaged in T2DM despite a relatively high BMD. Although deleterious effects of T2DM on bone have been elucidated, the underlying cellular and molecular mechanisms remain unclear. For example, how does insulin resistance occur in osteoblasts and how do phosphorylation of insulin-receptor substrate isoforms (IRSs) and resultant insulin resistance in osteoblasts, osteoclasts and perhaps osteocytes contribute to diabetic bone loss? Indeed, osteocytes residing inside lacunae play an important role in bone remodeling in health and disease since they are responsible for inducing bone loss under certain conditions, such as during lactation^[32,33]. Further investigation is required to demonstrate whether osteocytic dysfunction does exist in T2DM.

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Endothelial dysfunction as a predictor of cardiovascular disease in type 1 diabetes

Marcello C Bertoluci, Gislaine V Cé, Antônio MV da Silva, Marco V Wainstein, Winston Boff, Marcia Puñales

Marcello C Bertoluci, Marco V Wainstein, Departamento de Medicina Interna, Universidade Federal do Rio Grande do Sul, Porto Alegre RS 90035-003, Brazil

Marcello C Bertoluci, Serviço de Medicina Interna do Hospital de Clínicas de Porto Alegre, Porto Alegre RS 90035-903, Brazil

Marcello C Bertoluci, Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul, Porto Alegre RS 90035-003, Brazil

Gislaine V Cé, Winston Boff, Marcia Puñales, Instituto da Criança com Diabetes - Grupo Hospitalar Conceição, Porto Alegre RS 91350-250, Brasil

Antônio MV da Silva, Departamento de Fisioterapia e Reabilitação, Universidade Federal de Santa Maria, Santa Maria RS 97105-900, Brasil

Marco V Wainstein, Serviço de Cardiologia do Hospital de Clínicas de Porto Alegre, Porto Alegre RS 90035-903, Brazil

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Correspondence to: Marcello C Bertoluci, MD, DMSc, Serviço de Medicina Interna do Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcellos 2350- Sala 700, Santa Cecília, Porto Alegre 90035-003, Brazil. mbertoluci@uol.com.br
Telephone: +55-51-33598000
Fax: +55-51-33598000

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Abstract

Macro and microvascular disease are the main cause of morbi-mortality in type 1 diabetes (T1DM). Although there is a clear association between endothelial dysfunction and atherosclerosis in type 2 diabetes, a cause-effect relationship is less clear in T1DM. Although endothelial dysfunction (ED) precedes atherosclerosis, it is not clear whether, in recent onset T1DM, it may progress to clinical macrovascular disease. Moreover, endothelial dysfunction may either be reversed spontaneously or in response to intensive glycemic control, long-term exercise training and use of statins. Acute, long-term and post-prandial hyperglycemia as well as duration of diabetes and microalbuminuria are all conditions associated with ED in T1DM. The pathogenesis of endothelial dysfunction is closely related to oxidative-stress. NAD(P)H oxidase over activity induces excessive superoxide production inside the mitochondrial oxidative chain of endothelial cells, thus reducing nitric oxide bioavailability and resulting in peroxynitrite formation, a potent oxidant agent. Moreover, oxidative stress also uncouples endothelial nitric oxide synthase, which becomes dysfunctional, inducing formation of superoxide. Other important mechanisms are the activation of both the polyol and protein kinase C pathways as well as the presence of advanced glycation end-products. Future studies are needed to evaluate the potential clinical applicability of endothelial dysfunction as a marker for early vascular complications in T1DM.

Key words: Endothelial dysfunction; Type 1 diabetes; Cardiovascular disease

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Core tip: Endothelial dysfunction is an early finding in the natural history of type 1 diabetes and is predictive for microvascular disease and premature atherosclerosis. Decreased nitric oxide due oxidative stress is the central pathogenetic mechanism. Polyol pathway activation, protein kinase C (PKC) activation and advanced glycation product formation are also important. Long-term hyperglycemia, repeated hypoglycemia and microalbuminuria are factors associated. Intensive glycemic control and exercise training ameliorate endothelial dysfunction. Statins and renin-angiotensin system blockers are partially effective and may be influenced by hyperglycemia. There is a possible clinical benefit for the use of vitamin E and vitamin C that are still to be confirmed. PKC inhibitors are still investigative.

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INTRODUCTION

Micro and macrovascular complications are leading causes of morbidity and mortality in patients with type 1 diabetes mellitus (T1DM)^[1,2]. Subjects with T1DM are prone to accelerated atherosclerosis^[3] and have 3 to 6 times more risk of cardiovascular death than individuals without diabetes. Endothelial dysfunction (ED) is an early event along the natural history of T1DM, indicating a phenotype in risk for accelerated atherosclerosis, that may be independent of the classical cardiovascular risk factors^[4,5]. Interestingly, traditional cardiovascular risk factors altogether can not explain the totality of the cardiovascular risk in T1DM^[6,7]. Chronic hyperglycemia *per se*, although an important predictor of microvascular disease, is a weak predictor of macrovascular complications in both T1DM and T2DM^[8,9]. Thus, much of the residual cardiovascular risk still remains unexplained. In this context, ED becomes a new important risk factor that should be debated in the cardiovascular scenario. In the present review, we discuss recent evidences in the pathogenesis of endothelial dysfunction, its role as a risk factor for cardiovascular disease and the potential interventions for reducing endothelial dysfunction in T1DM.

THE NORMAL ENDOTHELIUM

The vascular endothelium forms the cell layer that is

directly in contact with the vascular lumen, separated from the smooth muscle layer of the basement membrane. Its role is to maintain the homeostasis between the blood and the arterial wall through the synthesis of substances that modulate vascular tone, inhibit platelet aggregation and control the proliferation of vascular smooth muscle cells^[10].

In endothelial cells, nitric oxide (NO) is essential for the maintenance of integrity and homeostasis of endothelium^[11]. NO is synthesized from L-arginine by the action of endothelial nitric oxide synthase (eNOS) in the presence of oxygen, NADP(H) and the NOS co-factor, tetrahydrobiopterin (BH4)^[12]. The synthesized NO diffuses itself quickly into the smooth muscle cell layer and into platelets where it activates guanylate cyclase (GCA), with consequent production of cyclic GMP (cGMP). The presence of cGMP, in turn, promotes vascular relaxation and inhibition of platelet aggregation, keeping the equilibrium between pro and anti-thrombotic factors in the blood and arterial wall. However, as the half-life of NO is very brief, rapidly oxidizing into nitrate, the continuous activation of eNOS becomes the key determinant of NO synthesis and tissue bioavailability^[11]. Normally, eNOS is activated by the turbulent blood flow against the luminal endothelial wall (shear-stress) as well as by the stretch of vascular wall cells and changes in the oxygen tension, promoting vascular muscle relaxation, an effect known as "endothelial-dependent vasodilation"^[13,14].

The stability of the endothelium is also dependent on endothelial repair and regeneration, which are determined by migration and proliferation of surrounding mature endothelial cell resident the vascular wall^[15]. Recently it has been demonstrated that circulating endothelial progenitor cells (EPCs) are important in the endothelial regeneration. EPCs are circulating bone-marrow-derived cells characterized by the expression of varying surface markers that adhere to the damaged endothelium promoting tissue repair^[15]. Circulating EPCs are considered biomarkers of endothelial function and prognostic indicators of cardiovascular morbi-mortality. Endothelial dysfunction represents the breakdown of this endothelium homeostasis, leading to a pro-thrombotic and pro-inflammatory that may lead to progressive atherosclerosis.

METHODS FOR ASSESSMENT OF ENDOTHELIAL FUNCTION

Endothelial function can be investigated through invasively and non-invasively techniques. Coronary arteries can be evaluated invasively through angiography with quantitative measurement of changes in the vascular diameter in response to infusion of acetylcholine^[16] and also through invasive venous occlusion plethysmography^[17], which measures forearm blood flow in response to acetylcholine infusion in the brachial artery. The invasive nature of

these techniques, involving artery cannulation and infusion of vasoactive drugs^[14], make them unfeasible to widespread use in clinical practice.

Non-invasive techniques, on the other hand, are being increasingly used in clinical settings. The flow mediated dilation (FMD) is the most popular technique currently used^[18-20]. The rationale in FMD is based on reactive hyperemia responsive to shear-stress caused by turbulent blood flow, causing NO to be released and promoting endothelium-dependent vasodilation^[14,21]. The measurement of vascular dilation can be done by capturing images of the brachial artery using high-resolution ultrasound^[18]. Reactive hyperemia occurs after a period of ischemia, induced by occlusion of the brachial artery, with a sphygmomanometer cuff inflated with progressive release of vasodilator mediators such as adenosine and ⁺H ions from ischemic tissue. When the release of blood flow occurs, sudden shear-stress is produced in the brachial vein endothelium, which is a strong stimulus for releasing NO^[19]. This mechanism depends on the integrity of eNOs. The lesser the dilation the more severe the dysfunction. Definition of ED is than considered arbitrarily when the increase in dilation is less than 8%^[20].

Other non-invasive techniques are used less frequently. Peripheral artery tonometry induced by reactive hyperemia^[22] assesses endothelial function by a combination of flow-mediated dilation and measurement of the amplitude of the arterial pulse wave expansion through a pneumatic sensor placed on the index finger. The microvascular reactivity on the forearm skin is evaluated with laser Doppler flowmetry, being the the iontophoresis of acetylcholine the endothelium-dependent vasodilator stimulus. The determination of the complacency of the dorsal hand vein is a minimally invasive method described by Aellig^[23] in 1981 which has been used by our group^[24]. It consists in a infusion of vasoactive drugs into the vein surface of the dorsum of the hand to measure endothelium-dependent vasodilation in response to acetylcholine, bradykinin or isoproterenol. The venous occlusion plethysmography can also be used to measure changes in forearm blood flow in response to reactive hyperemia. Finally, the measurement of the thickness of the intima-media layer (IMT) of the common carotid by ultrasound is a structural marker of atherosclerosis and correlates inversely with FMD in the brachial artery^[25]. Increases in the IMT are indicative of early atherosclerosis.

ENDOTHELIAL CHANGES IN DIABETES

Chronic sustained hyperglycemia in diabetes promotes important structural and functional modifications in the endothelium, as reported in both experimental and clinical studies^[26-29]. In the aorta of rabbits with alloxan-induced diabetes, endothelial changes are visible after 2 wk from the onset of hyperglycemia

and become more severe after 6 wk of the diabetes onset^[26]. The findings include adhesion of leukocytes, platelets and fibrin material on the endothelial surface. In mice, 6 wk after the onset of diabetes induced by streptozotocin (STZ), it is possible to observe increased endothelial permeability and endothelial cell apoptosis^[27].

In a classic study using samples of human skin and subcutaneous tissue obtained from autopsies and biopsies of 24 patients with T1DM, which were compared to 9 non-diabetic controls, the most important finding was the increase in the thickness of the basement membrane in T1DM patients compared with non-diabetic subjects^[28]. In another study^[29], the increased thickness of the capillary basement membrane of skeletal muscle from patients with 12 years of T1DM, could be reversed by intensive glycemic control during one year^[29]. In studies using electron microscopy, endothelial cells obtained from umbilical cord blood from pregnant women with T1DM show increased mitochondrial area when compared to pregnant women without diabetes^[28]. The clinical significance of these structural changes, however, is still not clear to predict future atherosclerosis.

Important functional changes occur in the endothelium of T1DM. Hyperglycemia induces excess of electrons that leak from the oxidative chain and are captured by oxygen, generating superoxide excess and oxidative stress. Excessive superoxide production uncouples eNOS, impairing NO production^[29]. The net effect is a reduction of NO production in response to shear stress in the inner vascular wall. By this way, the main determinant of ED is the preponderance of vasoconstrictor factors released by the endothelium in detriment of vasodilators factors due to the decreased availability of NO^[30]. The dysfunctional endothelium leads to a migration of blood cells into the arterial wall, inducing proliferation of smooth muscle cells, platelet aggregation, LDLc oxidation, monocyte adhesion and synthesis of inflammatory cytokines, all factors contributing to atherogenesis^[31].

In patients with T1DM, functional changes in endothelium occurs very early in the natural history of diabetes^[32]. The duration of diabetes is a major determinant for the presence of endothelial dysfunction in T1DM, being inversely correlated with the endothelium-dependent dilation. ED generally occurs in the first decade of T1DM, earlier than increases in the carotid intima-media layer thickness (Table 1).

ED is a common finding in T1DM, generally seen after 4 years of disease. In the study by Singh *et al*^[33], 31 adolescents with 6.8 years of T1DM and poor glycemic control presented both ED and increased intima-media layer thickness of carotid artery, compared with individuals without diabetes. The duration of diabetes was inversely correlated with the endothelium-dependent dilation^[33]. These results were confirmed by other authors^[34-38] and are in accordance

Table 1 Studies on Flow-Mediated Dilatation and Nitroglycerin Mediated Dilatation in patients with type 1 diabetes

Ref.	n	Age	Time	Micro-albuminuria	HbA1c	FMD		NTG	
						Type 1 diabetes	Non-diabetes	Type 1 diabetes	Non-diabetes
Clarkson <i>et al</i> ^[36]	80	15-40	13	6%	9.5	5.8 ± 3.7 ^a	9.3 ± 3.8	15.6 ± 5.6 ^a	19.7 ± 6.6
Lambert <i>et al</i> ^[37]	52	32	15	No	7.9	12.9 ± 9.8	17.3 ± 9.9	14.3 ± 8.0	17.7 ± 8.7
Enderle <i>et al</i> ^[38]	17	41.5	21	No	8.0	8.2 ± 4.6	7.6 ± 4.2	16.3 ± 4.9	18.4 ± 6.4
Lekakis <i>et al</i> ^[35]	31	32	NO: 13 MA: 20	Yes	N: 6.5 M: 7.1	NO: 5.8 ± 7 ^a MA: 0.7 ± 2.5 ^a	11.0 ± 7 -	NO: 19.0 ± 6.9 ^a MA: 15.0 ± 2.9 ^a	24.0 ± 9.0
Dogra <i>et al</i> ^[34]	34	NO: 44 MA: 48	NO: 20 MA: 25	Yes	8.5-8.7	NO: 5.4 ± 0.6 ^a MA: 3.2 ± 0.3 ^a	7.9 ± 0.6 -	MA: 11.9 ± 1.1 ^a	20.0 ± 1.2
Singh <i>et al</i> ^[33]	31	15	7	No	8.6	4.2 ± 3.8 ^a	8.2 ± 4.2	17.0 ± 6.0	18.0 ± 6.0
Järvisalo <i>et al</i> ^[4]	45	11	4	No	8.9	4.4 ± 3.4 ^a	8.7 ± 3.3	-	-
Ladeia <i>et al</i> ^[39]	18	13	3	80%	9.3	10.9 ± 2.0	11.2 ± 2.4	-	-
Cé <i>et al</i> ^[5]	57	17	7	No	8.6	9.5 ± 6.5 ^a	14.6 ± 5.6	22.3 ± 9.8 ^a	29.3 ± 4.2

Age: Mean age in years; Time: Time of T1DM (in years); HbA1c: Hemoglobin A1c (%); NO: Normoalbuminuria; MA: Microalbuminuria; FMD: Flow-Mediated Dilatation; NTG: Nitroglycerin Mediated Dilatation. ^a*P* < 0.05 *vs* controls.

to the concept that endothelial dysfunction is predictive of early atherosclerosis in T1DM.

More recent data indicate that ED can occur even before 4 years of onset of T1DM^[4,39], preceding the onset of microalbuminuria. Järvisalo *et al*^[4] compared non-obese, poor-controlled, recent onset T1DM children with age-matched children without diabetes, with respect to FMD and the thickness of intima-media carotid. They observed the presence of endothelial dysfunction in 36% of cases, a lower peak of flow mediated dilation response and increased intima-media thickness compared with controls. The authors concluded that ED is a common finding in children in the early years of T1DM and may be a predictor for the development of premature atherosclerosis.

The presence of ED, however, is not uncommon before 4 years of T1DM^[32]. We found a prevalence of 35.7% of ED in a sub-group of T1DM patients with less than 5 years of diabetes^[5]. The data from the above studies indicates that it ED may begin to occur 3 to 5 years from the onset of T1DM.

FACTORS ASSOCIATED WITH ED IN T1DM

Gender

The impact of gender in ED is still undefined, but, in one study, boys with T1DM seemed to be at increased risk. Bruzzi *et al*^[40] studied 39 children with T1DM and 45 healthy age-matched controls, evaluated longitudinally with FMD at baseline and 1 year of follow-up^[40]. At baseline, T1DM boys and girls had similar FMD values, however, after 1 year, boys had more endothelial dysfunction than girls. The rationale of this difference is still unknown since multivariate analysis did not identify important predictors of endothelial dysfunction^[40].

Acute hyperglycemia

Acute hyperglycemia is capable to induce reversible

endothelial dysfunction in normal individuals. When non-diabetic subjects are acutely exposed to high concentrations of glucose during dextrose infusion for 6 h, there is an attenuation of the arterial endothelium-dependent vasodilation induced by methacholine (endothelium-dependent vasodilation) while preserving the vasodilator response to nitroprusside (non-endothelium dependent vasodilation)^[41]. This indicates that acute rises in blood glucose in contact to a previous normal endothelium can cause acute endothelial dysfunction, but it is not sufficient to promote vascular smooth muscle dysfunction. In another study in normal subjects^[42], it was also demonstrated that acute hyperglycemia can cause significant hemodynamic and rheological changes such as increases in systolic and diastolic blood pressure, heart rate and plasma catecholamines, while decreasing arterial blood flow to the leg. Platelet aggregation to ADP and blood viscosity also showed increments. When the authors infused the natural precursor of NO formation, L-arginine, blood pressure and artery flow changes were reversed. When they infused the inhibitor of endogenous NO synthesis, N^G-monomethyl-L-arginine (L-NMMA), hemodynamic effects of hyperglycemia were reproduced, indicating that acute hyperglycemia reduces NO availability even in normal subjects^[42].

The effect of acute high glucose in normal endothelium, however, is not observed in all studies. Houben *et al*^[43], studied the effect of acute glucose infusion for 24 h in normal individuals and did not observed changes in vascular dilatation of skin microcirculation induced by acetylcholine (Ach), nitroprusside, norepinephrine or nitric oxide synthase antagonist (L-NMA). The differences between studies may be due to methodological differences. It is accepted, however, that insulin can attenuate acute endothelial dysfunction, promoting compensatory vasodilation which may have biased the studies. In other studies, where the action of insulin was blocked

by octreotide, the effect of acute hyperglycemia alone was evident^[41].

Long-term hyperglycemia

The association between HbA1c and flow-mediated dilation (FMD) is seen in some cross-sectional studies with patients with T1DM. In the study of Ladeia *et al.*^[39], with 19 normo and microalbuminuric T1DM patients, there was a moderate positive correlation between FMD and HbA1c. In another study^[44], patients with T1DM with HbA1c above 6.0% had significant impairment of endothelial function compared to patients with HbA1c below 6%, indicating that chronic mild increases in mean hyperglycemia are also associated to ED in T1DM.

We studied the impact of chronic glycemic control in endothelial function of T1DM in a historical cohort study^[5]. T1DM adolescents under 5 year of disease were evaluated for ED and had their mean HbA1c obtained from medical records in the same institution since their diagnosis. Considering as a whole, the mean historical HbA1c was clearly higher in T1DM patients with endothelial dysfunction compared with T1DM without ED. Interestingly, we observed a moderate inverse correlation between FMD and the historical mean of HbA1c in the first 2 years after the diagnosis of T1DM but not with the more recent HbA1c. The plausible explanation was that endothelial function could be more affected by the long-term than by the short-term glycemic control, supporting the concept of metabolic memory^[45]. Glycation of the endothelium in the first years of T1DM seems, by this way, decisive to determine future endothelial dysfunction in T1DM^[32].

Post-prandial hyperglycemia

The effect of postprandial hyperglycemia in endothelial function was studied by Giugliano *et al.*^[42] in individuals with type 2 diabetes. The combined effect of postprandial glucose and hypertriglyceridemia was associated with increased serum concentrations of adhesion molecules such as ICAM-1, E-selectin and VCAM-1. Markers of oxidative stress such as nitro-tyrosine increased sharply after ingestion of 75 g of oral glucose. This effect was greater when an additional lipid overload was used, indicating that both acute hyperglycemia and hyperlipemia can affect endothelial function in diabetes^[46].

In another study, the same authors^[47] observed that, after a glucose overload, ED accentuates until the second hour, but returns to basal level after 4 h from the beginning of the overload. Though, lipid overload did not change FMD until the fourth hour. These data suggests that the effect of postprandial glycaemia in endothelial function is independent of the postprandial lipemia, although both might be mediated by increased oxidative stress.

Hypoglycemia

Recently, it was demonstrated that repeated episodes of hypoglycemia in subjects with T1DM may be associated with endothelial dysfunction and could be an aggravating factor for preclinical atherosclerosis. In a case-control study^[48], T1DM with repeated hypoglycemia episodes were compared with age and sex-matched T1DM controls who did not have frequent hypoglycemia. Vascular function was assessed by FMD, intimal-media carotid artery thickness (IMT) and endothelial dysfunction markers such as von Willebrand factor (vWf). The group with increased hypoglycemia episodes presented lower percentages of FMD in response to ischemia, increased IMT measures and higher endothelial function markers. In another study^[49], with cross-sectional design, T1DM children and age and gender-matched healthy children were evaluated for vascular function and continuous glucose monitoring system (CGMS) in order to compare the impact of glucose variability and hypoglycemia episodes in vascular function. Subjects with T1DM had significantly lower FMD compared to healthy children. However, when comparing CGMS parameters, the authors found significant inverse relationship between FMD with hypoglycemia indexes but not with variability indexes.

The biological rationale for hypoglycemia inducing endothelial dysfunction in T1DM is that acute hypoglycemia can induce rapid pro-inflammatory, platelet aggregatory, anti-fibrinolytic response, and recurrent hypoglycemia may induce changes in hemostatic factors and viscosity which may decrease perfusion in diabetic microangiopathy^[50].

Glycemic variability

Glycemic variability (GV) is a term exclusively related to blood glucose fluctuations and must be differentiated from post-prandial glucose (PPG). GV is related to glucose variability along the day, while PPG is related to the precise time of glucose rise after a meal. PPG effect in vascular function can also be influenced by other confounders such as hypertriglyceridemia.

Whether GV is an important factor to cause ED is still a matter of debate. The effect of glycemic variability in ED was studied in T1DM with normal urinary albumin excretion^[51]. Patients were exposed to 48 h of good (mean 113 mg/dL) or poor metabolic control (mean 286 mg/dL) and evaluated with FMD and serological markers of endothelial function. Both endothelium-dependent and endothelium-independent vasodilation were significantly impaired after the poor control period in relation to good glycemic control. There was also a significant increase in vWf levels after the deterioration of control. These results indicate that endothelial function may suffer significant impact of acute variability of blood glucose in T1DM, although it

can be reversed with improvement of glycemic control. However this effect was not seen in other studies. In the DCCT study, the GV in glycemic data using 7-point self monitoring blood glucose (SMBG) obtained every 3 mo did not correlated to macrovascular complications^[52]. We were also not able to detect a relationship between FMD and the standard deviation (SD) of glycemia in T1DM, using day 7-point SMBG for 30 d preceding FMD (data unpublished). Finally, in a cohort study of T1DM^[53], the standard deviation of blood glucose, calculated from self-monitoring blood glucose data along 11 years of follow up, was predictive for incident peripheral neuropathy^[53]. The influence of GV in vascular function and future micro or macro vascular complications of T1DM is not yet established.

Microalbuminuria

Microalbuminuria is strongly associated with ED in T1DM. Dogra *et al.*^[34] studied long-term T1DM patients with microalbuminuria with poor glycemic control who were compared to normoalbuminuric T1DM and to non-diabetic individuals. FMD was more severely impaired in T1DM patients with microalbuminuria being albuminuria an independent predictor of ED. In another study, Lekakis *et al.*^[35] observed lower FMD values in microalbuminuric compared with normoalbuminuric patients. In a similar study with children and adolescents with T1DM with less than 5 years of disease^[39], there was a negative correlation between the percent of endothelium-mediated dilation and albuminuria. Mean FMD was also significantly decreased in microalbuminuric. ED is also present in type 2 diabetes patients with normal albuminuria with long duration T2DM during chronic poor glycemic control^[54]. In T1DM patients with normoalbuminuria ED is less common^[40].

MECHANISMS OF ED IN T1DM

Oxidative stress

Children with T1DM have increased oxidative stress and reduction of anti-oxidant defense compared to healthy children and to their parents^[55,56] and these results are similar in adolescents with T1DM^[57]. Moreover, endothelial progenitor cells are also reduced in children with T1DM compared to non-diabetic controls possibly related to oxidative stress^[58].

Hyperglycemia can cause excessive production of superoxide in the mitochondria oxidative chain of endothelial cells. The excess of superoxide reacts rapidly with NO, reducing NO bioactivity and producing peroxynitrite (ONOO⁻). Peroxynitrite is a potent oxidant agent and an activator of the lipid peroxidation which may impair endothelial function by stimulating arachidonic acid metabolism^[59]. The overproduction of superoxide and NO favors the formation of peroxynitrite by interfering with the production of the eNOS cofactor,

tetrahydrobiopterin (BH4)^[60].

NAD(P)H oxidase is a chief determinant enzyme of superoxide production in animal models of vascular diseases, including diabetes^[32]. In arteries of patients with diabetes who were submitted to artery bypass surgery, it was demonstrated that the endothelium can produce superoxide induced by a dysfunctional eNOS. Dysfunctional eNOS is caused by the oxidation of the co-factor BH4 into BH2. The enzymatic uncoupling of eNOS in human endothelium turns eNOS into dysfunctional eNOS which promotes a transition from NO production to superoxide production^[60,61].

Protein kinase C pathway activation

Protein kinase C (PKC) is a cytoplasmic family of enzymes with a wide variety of actions in intracellular signal transduction. The activation of PKC by decreases endothelium derived nitric oxide synthesis, whereas its inhibition increases NO release. The beta isoforms are activated in response to hyperglycemia^[62]. The activation of PKC system is also associated with increased albuminuria in rats^[63].

There are several mechanisms in which PKC may decrease the bioavailability of NO. PKC antagonizes activation of eNOS, decreases NO concentration, and induces NAD(P)H oxidase to produce superoxide, which, in turn, uncouples eNOS, inducing the production of even more superoxide. PKC is associated with various vascular disorders such as a decrease of Na⁺/K⁺ ATPase, increased extracellular matrix, increased vascular permeability, contractility and cell proliferation. The activation of PKC system is also associated with increased albuminuria in rats^[63].

In a randomized double-blind placebo controlled clinical trial, in healthy individuals submitted to acute hyperglycemia with hyperglycemic clamp technique, FMD was attenuated by hyperglycemia and reversed after treatment for 7 d with the PKC beta inhibitor, LY333531, indicating that the PKC-B system is an important regulator of hyperglycemia-induced endothelial dysfunction^[64].

Advanced glycation products

In the presence of sustained hyperglycemia, tissue proteins such as collagen undergo non-enzymatic glycation and formation of cross-links, resulting in advanced glycation end-products (AGEs). AGEs promotes a permanent chemical modification of proteins, stimulating cellular responses through specific anti-proliferative receptors^[65,66]. These receptors were first observed in experiments in mouse peritoneal macrophages^[66], showing ability to remove modified glycated proteins. AGEs may reduce the availability of endothelial NO, and reactive AGE intermediates may compromise their anti-proliferative effect.

Polyol pathway activation

Chronic hyperglycemia increases the activity of aldose reductase enzyme and leads to activation of polyol pathway, transforming glucose into sorbitol and subsequently into fructose. It also induces the consumption of NADP(H), an important cofactor for NO synthesis^[61]. As NADP(H) is an important cofactor for NOS to NO synthesis, its depletion leads to reduction of NO production. It remains uncertain, however, the magnitude of importance in the prevention of human atherosclerosis.

ED AS A MARKER OF CARDIOVASCULAR RISK IN T1DM

Although endothelial dysfunction and chronic low-grade inflammation have been associated with atherothrombotic cardiovascular disease, independently of traditional cardiovascular risk factors in either individuals with or without diabetes, a clear cause-effect relationship with atherosclerosis is not yet established in the natural history of T1DM.

In non-diabetic patients with coronary disease, endothelial dysfunction is predictive for increasing risk of cardiovascular events^[67]. In an observational study, 157 patients with mild coronary disease were classified according to the severity of ED, which was defined by intracoronary ultrasound with vascular reactivity after administration of acetylcholine, adenosine or nitroglycerin^[67]. They were followed by a mean of 28 mo for the assessment of cardiovascular outcomes. At the end of follow up, patients with more severe ED presented 14% of cardiovascular events, while those with mild or no ED had no cardiovascular outcomes ($P < 0.05$)^[67]. This study demonstrated, for the first time, that patients with mild coronary disease but with severe ED were at increased risk for cardiovascular events.

Serum markers of ED

The vWf and C-Reactive protein (CRP) are related to ED and inflammation. In the population-based cohort study, the HOORN study^[68], the predictive value of the serum ED marker, vWf, was evaluated for cardiovascular mortality in T2DM patients. The cohort including 2,484 caucasian individuals with ages between 50-70 years, in which 27% had T2DM and 27% had impaired glucose tolerance, was followed by 5 years^[68]. Patients with vWf levels in the upper tertile had a 3 fold increase in cardiovascular mortality compared to those in the lower tertiles, even after adjustments for age, sex and glucose tolerance status. The relative risk for all-cause mortality associated with vWf was 2.03 (95%CI: 1.19 to 3.47). The predictive value of vWf was not confirmed in ARIC study^[69], however, vWf is also an independent predictor of cardiovascular mortality in specific populations^[70].

CRP is an inflammatory marker and can be increased in T1DM patients without clinical macroangiopathy, compared with healthy subjects^[71]. This increase is greater in the presence of micro or macroalbuminuria compared with normoalbuminuric patients indicating an association between endothelial dysfunction and vascular inflammation^[71].

The mechanisms by which the cardiovascular risk is associated with elevated levels of vWf and CRP are not completely understood. It may reflect generalized endothelial dysfunction, increased prothrombotic state^[71], inflammation and greater risk for developing atherosclerosis^[72]. Von Willebrand factor in combination with *t*-PA measurement may also be an index for endothelial dysfunction^[73].

Markers of endothelial function can also be determinants of inflammation. In the EURODIAB Prospective Complications Study^[74], a nested case-control study of 543 T1DM participants, the levels of serum markers of ED such as E-selectin, vascular adhesion molecule-1 cell (VCAM-1) and inflammatory markers were determined. In this study, endothelial dysfunction was strongly associated with inflammatory activity suggesting that endothelial dysfunction may interact with vascular inflammation in T1DM which potential consequences in accelerating atherosclerosis^[74].

Flow mediated dilation

Impaired flow mediated dilation (FMD) may also predispose to early atherosclerosis in T1DM patients, as seen by the development of increased carotid artery thickness (IMT). In a cross-sectional study, 45 children with T1DM and 30 healthy matched in age, gender and body size were evaluated for FMD and IMT^[75]. Children with diabetes presented lower peak FMD response and increased IMT compared to non-diabetic children. In another cross-sectional study^[75], T1DM adolescents without diabetes complications were compared with healthy age-matched controls in respect to FMD and the presence of diastolic dysfunction with pulse wave Doppler and tissue Doppler echocardiography measurements. ED was associated with segmental diastolic dysfunction. These studies suggest that ED is associated with indirect evidences of premature atherosclerosis, however, long-term prospective studies are still needed to conclude if FMD is predictive for cardiovascular events in early T1DM.

CLINICAL MANAGEMENT OF ED IN T1DM**Intensive insulin therapy and glycemic control**

There are compelling evidences indicating that optimizing glycemic control with intensive insulin therapy reduces the development and progression of microvascular complications^[1]. Although endothelial

dysfunction and oxidative-stress are early changes in T1DM, both conditions are only partially reversed by insulin therapy alone^[76]. In adults with 8 years of T1DM who are in poor glycemic control, acute intravenous insulin infusion can only partially reverse impaired FMD, even after completely normalizing glycaemia. It is likely that a more prolonged treatment is necessary for achieving a reversion to normal functioning of endothelium-dependent vasodilation in T1DM^[76].

In a clinical trial, 92 children and adolescents with T1DM in conventional insulin therapy were randomized for either continuing in conventional insulin therapy or to switch to a more intensive insulin therapy, including insulin infusion pump and multiple insulin injections. After 1 year of intensive insulin therapy, the baseline vascular response to acetylcholine and the levels of E-selectin improved significantly in the intensive group, while no effect was seen in the conventional group. Interestingly, in this study the benefit was independent of HbA1c, suggesting that intensive insulin therapy may confer vascular protection in addition to improving glycemic control^[76].

Exercise

Exercise has a great impact in the mitigation of ED in patients with cardiovascular risk factors both in T1DM and T2DM. In children with T1DM, 30 min of aerobic training, two times a week, for 18 wk can significantly increase flow mediated dilation in around 65%^[77]. In adults with T1DM, 60 min of aerobic training, 2 times a week, significantly improves flow mediated dilation in more than 50% after 24 wk^[78]. In a cross sectional study, T1DM adolescents who did more than 60 min daily of moderate-to-vigorous physical activity have higher flow mediated dilation than inactive patients with diabetes^[79]. Many other studies also show improvement of vasodilator response in T2DM without coronary artery disease, with both aerobic and mixed aerobic/resistance training with 8 to 12 wk of duration^[80-82].

The main mechanism underlying the amelioration of vasodilation in response to exercise is largely related to the increased in nitric oxide (NO) bioavailability, resulted from the increased activity and expression of the eNOS and the diminished degradation of NO due to the action of radical oxygen species (ROS). Cultured cells experiments^[83] indicate that shear-stress can induce eNOS expression and activity due to stabilization eNOS mRNA or increasing its synthesis. In humans with coronary artery disease, it was also demonstrated a two-fold increase in eNOS expression and a 3.2 increase in the phosphorylation of eNOS after 4 wk of regular training^[84]. The increase in antioxidant defenses such as the activity of superoxide dismutase and glutathione peroxidase is another important mechanism underlying the improvement of endothelial function by exercise seen in patients with

heart failure^[85].

Exercise training can increase the number of circulations EPCs in healthy subjects^[86] and coronary artery disease patients^[87] after 4 wk of regular training. EPCs are decreased in patients with T1DM compared to healthy subjects^[83,88]. The effect of exercise impact in EPCs in type 1 patients, however, remains to be clarified.

Anti-oxidants

Experimental studies demonstrated that antioxidants can modulate the response of the endothelium dependent vasodilation, endothelium-leukocyte interactions and the balance of pro and anti-thrombotic factors^[89-91].

In clinical studies, treatment with oral high-dose vitamin E during 6 mo have yielded conflicting results in ED improvement in patients with T1DM. In the first study, Skyrme-Jones *et al.*^[92] compared the effect of vitamin E 1000 UI/d with placebo in a double-blind randomized clinical trial in patients with T1DM in FMD. They found significant increases in FMD after 3 mo in the group receiving vitamin E. They considered that vitamin E decreased the LDLc oxidant capacity, thus reducing ED. In a double-blind randomized clinical trial in adults with both T1DM and T2DM, Beckman *et al.*^[93] compared the use of a combination of vitamin E (800 UI/d) with vitamin C (1000 mg/d) compared to placebo. After 6 mo, FMD increased significantly in the T1DM group but not in T2DM. These data are promising, however, neither all studies confirm these findings. In a randomized clinical trial, Economides *et al.*^[94] studied the effect of high-dose vitamin E (1800 UI) against placebo in T1DM and T2DM along 12 mo but failed to find improvements in ED^[94]. The clinical effectiveness of vitamin E in improving ED and reducing the progression to atherosclerosis remains to be established in larger trials in T1DM.

Ascorbic acid infused together with intravenous insulin with near normalization of glycaemia, can rapidly normalize endothelial dysfunction in T1DM^[75]. This effect is not completely attained, however, when either intensive glycemic control with insulin or ascorbic acid infusions are used alone, indicating that an additive effect of both treatments exist, an effect that may be limited to new-onset type 1 diabetes^[89]. Ascorbic acid can also decrease transcapillary albumin escape^[95] and urinary albumin excretion in T1DM adults^[96]. In children with T1DM, the combined use of ascorbic acid and vitamin E can increase superoxide-dismutase levels^[57].

Low ingestion of antioxidants, especially vitamins, is associated with increased risk of cardiovascular disease and atherosclerosis^[97,98]. The inverse correlation between concentrations of antioxidant agents, vitamins and disease risk could be associated to higher requirement of antioxidant molecules during inflammatory diseases.

Insufficient supply with these compounds may further accelerate disease process^[99]. On the other hand, these data are not yet been convincingly established in clinical trials and are still controversial^[100-102].

Statins

Statins may have benefic effect on endothelial dysfunction in patients with T1DM. In a clinical trial with 204 long-term T1DM randomized to receive atorvastatin 40 mg plus hypolipemic diet or placebo plus hypolipemic diet for 6 mo, FMD increased 44% and PAI-1 was reduced in atorvastatin group compared to placebo^[103]. Similar results were observed in a small cross-over trial including 16 T1DM with microalbuminuria^[104] who received atorvastatin 40 mg or placebo for 6 wk with a 4-wk period of washout. FMD and non-endothelium dependent vasodilation increased significantly while using atorvastatin. In a meta-analysis of 10 studies including 845 patients with both T1DM and T2DM^[105], statin therapy significantly ameliorates FMD in patients with diabetes, although heterogeneity among trials was found. Statins however, improved FMD only in patients with better endothelial function. Factors associated with improvements were: T1DM, younger age, lower baseline lipid levels and blood pressure. Mechanisms enrolled in this effect are not completely known but may be related to reductions in LDLc as well as pleiotropic anti-oxidant effects of statins.

ACE inhibitors

Experimental evidences suggest that ACE inhibition may have benefic effects to the endothelium *in vitro*^[106,107]. In a clinical trial, quinapril showed benefit in coronary endothelial function of non-diabetic patients with CAD^[108]. ACE inhibitors improve FMD in T1DM with microalbuminuria although not in T1DM with normoalbuminuria^[109]. In another small trial with normotensive T1DM patients with microalbuminuria, ACE-I inhibitors improved both FMD and GTN in the femoral artery after 1 wk of treatment^[110].

The direct renin blockade was studied in normo-albuminuric T1DM with the use of aliskiren during 4 wk of monotherapy, followed by 4 wk of a combination of aliskiren and ramipril. In both conditions of hyperglycemia and euglycemia of short duration, obtained through euglycemic clamp and hyperglycemic clamp techniques, ED ameliorated with aliskiren alone and improved further when in combination with ramipril. This effect only occurred in the euglycemic phase of the study. Effects were abolished when patients became hyperglycemic^[111].

PKC inhibitors

Ruboxistaurin (RBX) is an orally administered isoform-selective inhibitor of PKC which was demonstrated to have beneficial effect in experimental models

of diabetic retinopathy^[112] and in hemodynamic retinal abnormalities of patients with diabetes^[113]. The studies PKC-DRS^[114] and PKC DRS2^[115] showed a 50% reduction in vision loss of patients treated with RBX. The effect of RBX was than studied in 2 combined phase 3 trials: MBDL and MBCU. Both were randomized, double-blind, placebo-controlled, clinical trials, including T1DM and T2DM with ages above 18 years. Patients had HbA1c below 11%, blood pressure below 160/90 mmHg and diabetic retinopathy. Patients were submitted to pan-photocoagulation or focal photocoagulation after randomization. Patients were than randomized to RBX 32 mg or placebo and followed by 36 mo (in MBDL) and 48 mo (in MBCU). Altogether, 1040 patients were randomized. Sustained moderate visual loss occurred in 4.4% of placebo vs 2.3% of RBX treated patients ($P = 0.045$). The results were promising, indicating a potential reduction in visual loss of 50% above standard care^[116]. RBX has also been studied in diabetic neuropathy in other smaller studies. In a 6 mo clinical trial, RBX vs placebo in patients with both T1DM and T2DM. Patients who were randomized for RBX presented improvement of neuropathic symptoms and ameliorated decreased skin microvascular blood flow^[117]. Although promising, RBX is not available for clinical use.

CONCLUSION

ED should be a concern for clinicians as an early and common phenomenon in T1DM, which may be predictive for future microvascular disease and early atherosclerosis. The clinical use of endothelial function measurement in clinical practice, specially FMD, is a potential tool to enhance cardiovascular risk prediction. Long-term intensive insulin treatment with optimized glycemic control along with exercise training are essential to prevent ED in these patients. Drugs such as statins and ACE-inhibitors are partially effective and may be influenced by the degree of hyperglycemia, with better response in microalbuminuric patients. There is also a possible benefit in using anti-oxidants such as vitamin E and vitamin C, but there is a clear demand for long-term randomized clinical trials to define their role in ED treatment. New agents such as PKC inhibitors are still investigative, but hold promise for future treatment of ED in T1DM.

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Management of critically ill patients with type 2 diabetes: The need for personalised therapy

Palash Kar, Karen L Jones, Michael Horowitz, Adam M Deane

Palash Kar, Adam M Deane, Discipline of Acute Care Medicine, Level 5, Eleanor Harrauld Building, University of Adelaide, South Australia 5000, Australia

Palash Kar, Adam M Deane, Intensive Care Unit, Level 4, Emergency Services Building, Royal Adelaide Hospital, South Australia 5000, Australia

Karen L Jones, Michael Horowitz, Adam M Deane, Centre for Research Excellence, University of Adelaide, South Australia 5000, Australia

Karen L Jones, Michael Horowitz, Discipline of Medicine, Level 6, Eleanor Harrauld Building, University of Adelaide, South Australia 5000, Australia

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Correspondence to: Dr. Palash Kar, Intensive Care Unit, Level 4, Emergency Services Building, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia. p_kar@hotmail.com
Telephone: +61-8-82224624

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Abstract

Critical illness in patients with pre-existing diabetes frequently causes deterioration in glycaemic control. Despite the prevalence of diabetes in patients admitted to hospital and intensive care units, the ideal management of hyperglycaemia in these groups is uncertain. There are data that suggest that acute hyperglycaemia in critically ill patients without diabetes is associated with increased mortality and morbidity. Exogenous insulin to keep blood glucose concentrations < 10 mmol/L is accepted as standard of care in this group. However, preliminary data have recently been reported that suggest that chronic hyperglycaemia may result in conditioning, which protects these patients against damage mediated by acute hyperglycaemia. Furthermore, acute glucose-lowering to < 10 mmol/L in patients with diabetes with inadequate glycaemic control prior to their critical illness appears to have the capacity to cause harm. This review focuses on glycaemic control in critically ill patients with type 2 diabetes, the potential for harm from glucose-lowering and the rationale for personalised therapy.

Key words: Diabetes; Critically ill; Intensive care; Management; Personalised therapy

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Core tip: With diabetes increasing in prevalence, the optimal management of glycaemia in critically ill

patients with pre-existing diabetes remains unknown. Recent data has highlighted therapeutic uncertainties specific to these patients with suggestions that targeted blood glucose concentrations may benefit from consideration of a patient's premorbid glucose state. In patients with uncontrolled type 2 diabetes, it may be safer to target blood glucose concentrations between 10-14 mmol/L, however definitive studies of critically ill patients with poorly controlled diabetes are required. In contrast, in patients with CIAH, or those with well-controlled diabetes (HbA1c < 7.0) have data supporting a more conservative target (6-10 mmol/L).

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INTRODUCTION

Patients with diabetes mellitus may develop an acute severe illness that necessitates a level of care that can only be provided within an intensive care unit (ICU)^[1]. In the majority of critically ill patients with pre-existing diabetes, the pathophysiological response to the acute illness or injury, and/or the treatments involved, may lead to deterioration in glycaemic control. Despite the high and increasing prevalence of diabetes (both within the community and in the critically ill), the optimal management of glycaemia in critically ill patients with pre-existing diabetes remains unknown. However, recent data has highlighted the therapeutic uncertainties specific to these patients.

The majority of critically ill patients with diabetes have type 2 diabetes^[2]. The limited information relating to patients with type 1 diabetes precludes speculation as to whether management of glycaemia in this group should be different from that in type 2 diabetes. Accordingly, this review focuses on critically ill patients with type 2 diabetes addressing issues including prevalence, potential rationale for harm and evidence for personalised therapy.

PREVALENCE

In the community type 2 diabetes occurs frequently with global health expenditure estimated at US \$376 billion in 2010, which is expected to rise to US \$490 billion by 2030 due to increasing prevalence^[3,4]. In Australia it is estimated over the last 15 years, the prevalence has increased from 8.5% to 12.0%^[5]. There is a substantial variation in the prevalence of diabetes between countries, peaking in Nauru (31%)^[6]. Factors relating to the increase in prevalence include increasing obesity, increasing age and racial region. A limitation in estimating prevalence is that many patients remain unaware of their diagnosis.

For example, the estimated prevalence in the United States is 13% of the population, of which 40% is unrecognised or undiagnosed^[7].

Diagnosis of diabetes

The prevalence of recognised and unrecognised diabetes varies according to the definitions used, as well as the location and the populations studied. The current diagnostic criteria used by the American Diabetes Association (ADA) involves one of the following; an HbA1c \geq 6.5, a fasting glucose \geq 7 mmol/L, a 2 h post glucose tolerance test following a 75 g oral glucose load of \geq 11.1 mmol/L, or a random blood glucose \geq 11.1 mmol/L with symptoms of hyperglycaemia^[8]. These criteria were ratified by the World Health Organization (WHO) in 2011^[9].

Given each test (HbA1c, fasting, postprandial or random blood glucose) reflects different physiological phenomena, different populations may be diagnosed when using each criterion^[10,11]. Each diagnostic test has advantages and disadvantages. Both the fasting glucose and 2 h post glucose tolerance test are established standards, relatively rapid and easy to perform, and predict microvascular complications. However, these tests are subject to day-to-day variability, require patients to fast and only reflect glucose homeostasis at a single point in time^[12]. HbA1c is convenient (with no fasting required), can predict microvascular complications, is a better predictor of macrovascular disease (than fasting glucose or 2 h post glucose tolerance test) and has low day-to-day variability^[8,12]. Additionally, as the physiological responses to acute illness cause deterioration in glycaemia, estimating glucose control prior to the acute illness - using markers such as HbA1c - to accurately determine which patients have unrecognised diabetes and which patients have "stress hyperglycaemia" is possible^[13]. Weaknesses include variations amongst ethnic groups and age, it may be misrepresentative in certain medical conditions (such as certain forms of anaemia and haemoglobinopathies) and the need for a validated, standardised assay^[12].

Prevalence of diabetes in hospitalised patients

Compared to the general population, the prevalence of diabetes in hospitalised adult patients (*i.e.*, admitted to general wards) is considered to be greater. Depending on the population, estimates range from between 11%-35% of all patients (Table 1).

Numerous studies in the critically ill have evaluated the prevalence of glucose intolerance (Table 1). However, a limitation of the studies reported is that investigators were unable to identify those patients who had so-called "stress hyperglycaemia" (or critical illness associated hyperglycaemia (CIAH) - the condition of acute glucose intolerance that is confined to the period of critical illness) and those who have unrecognised diabetes. Several studies use either fasting blood glucose (\geq 7 mmol/L) and/or random

Table 1 Prevalence of diabetes in hospital population (chronological order)

Ref.	Year	R-D	UR-D	Total study patients	Location	Diabetes diagnosed by	Unrecognised diabetes diagnosed by
Umpierrez <i>et al</i> ^[14]	2002	495 (26%)	223 ¹ (12%)	1886	Atlanta, United States	Admission history	Fasting blood glucose ≥ 7 mmol/L Random blood glucose ≥ 11.1 mmol/L $\times 2$
Wallymahmed <i>et al</i> ^[15]	2005	126 (11%)	13 ¹ (1%)	1129	Liverpool, United Kingdom	Admission history Hospital records	Random blood glucose ≥ 11.1 mmol/L
Wexler <i>et al</i> ^[17]	2008	136 (19%)	33 (5%)	695	Boston, United States	Admission history Hospital records	HbA1c > 6.5
Mazurek <i>et al</i> ^[18]	2010	342 (35%)	152 (16%)	971	New York, United States	Admission history Hospital records Medication review	HbA1c ≥ 6.5
Feldman-Billard <i>et al</i> ^[16]	2013	355 (17%)	156 ¹ (7%)	2141	Multicentre (France)	Admission history	Fasting blood glucose ≥ 7 mmol/L

¹May include patients with stress hyperglycaemia/critical illness associated hyperglycaemia. R-D: Recognised diabetes; UR-D: Unrecognised diabetes.

glucose concentrations (≥ 11.1 mmol/L) for diagnosis of diabetes^[14-16].

Investigators have also measured glycated haemoglobin (HbA1c) on admission to identify hospitalised patients with unrecognised diabetes. A prospective observational study of 695 patients in Boston, Massachusetts^[17], selected a cutoff HbA1c of $> 6.5\%$ to diagnose diabetes, with 19% of patients having diabetes previously diagnosed and 5% having undiagnosed diabetes. Another study of 971 patients admitted to the general medical ward of an urban hospital located in the Bronx, New York^[18] - which may be assumed to admit a larger cohort of lower-income patients - 35% were known to have diabetes, and 16% undiagnosed diabetes, using an HbA1c ≥ 6.5 .

In summary, the prevalence of diabetes in hospitalised patients varies according to geography. In the developed world, diabetes is more prevalent amongst lower socioeconomic groups^[19-21]. Furthermore, diabetes is a risk factor for certain diseases (e.g., cardiovascular disease) and prevalence will be greater if a specific population (e.g., patients presenting with myocardial ischaemia) is studied^[22].

Prevalence of diabetes in patients admitted to ICU

The prevalence of diabetes in patients admitted to the ICU is estimated to be between 12%-40% (Table 2). Similar to the prevalence in hospitalised patients, the wide range reflects the definitions used and the population studied. Multiple single centre observational studies from the United States^[23-25] report prevalence between 13% and 21%, therefore it is likely that the true prevalence is close to this range. More recently, Falciglia *et al*^[26] undertook a retrospective cohort study across 173 ICUs in the United States and reported that 30% of the 259040 patients had a history of diabetes according to ICD-9 codes^[26].

A single centre, observational study from London, United Kingdom^[27], found 16% of patients had a history of diabetes. A retrospective observational study of 4946 patients admitted to one of two hospitals in Melbourne and Sydney, Australia^[28], reported 15%

had diabetes. While a single, mixed medical/surgical ICU from Amsterdam, The Netherlands^[29], found 12% of 5961 patients admitted had a history of diabetes. These data indicate that the prevalence in other developed countries may be similar to, or slightly less than, the United States.

Data from international studies are consistent with this concept. Stegenga *et al*^[30] utilised data collected as part of a randomised interventional study^[31] to evaluate whether diabetes affects the outcome of sepsis in patients admitted to one of 164 ICUs across 11 countries and reported that 23% had pre-existing diabetes. In retrospective observational data derived from 44964 patients admitted to one of 23 ICUs worldwide^[32], 29% had a history of diabetes documented in their medical records, but the prevalence varied substantially according to geography. For example, in an ICU from Geelong, Australia, the prevalence was 14%, while in a hospital < 100 km away (Melbourne) it was 24%, whereas patients admitted to Tampa Bay, United States, the prevalence was 39%.

The prevalence of diabetes in the critically ill varies across studies. Multiple observational studies estimate the prevalence at 12%-30%^[23-29,30,32-35]. However, these studies have significant limitations. Most importantly, the prevalence may be under represented due to diabetes that is either unrecognised or not documented.

A number of interventional studies have also reported diabetes prevalence in ICU patients (Table 2). Two prospective, randomised, controlled studies of surgical and medical ICU patients admitted into the ICU in Leuven, Belgium, compared an intensive insulin therapy (ITT, blood glucose level 4.4-6.1 mmol/L) vs conventional treatment (insulin started if the blood glucose was > 12 mmol/L and maintained between 10-11.1 mmol/L)^[36,37]. These studies reported diabetes at 13% and 17% respectively.

Other interventional studies include single centre^[38,39] and multicentre trials^[40-42], with the largest being in 2009, the NICE-SUGAR (Normoglycaemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation) study. This was conducted across 42 ICUs

Table 2 Prevalence of diabetes in the intensive care unit population (chronological order)

Ref.	Year	Study type	R-D	UR-D	Total study patients	Location	Recognised DM diagnosis	Unrecognised diabetes diagnosed by
Van den Berghe <i>et al</i> ^[36]	2001	Interv	204 (13%)	N/A	1548	Leuven, Belgium	Admission history	N/A
Finney <i>et al</i> ^[27]	2003	Observ	86 (16%)	N/A	523	London, United Kingdom	Unknown	N/A
Whitcomb <i>et al</i> ^[23]	2005	Observ	574 (21%)	395 ¹ (15%)	2713	Baltimore, United States	Admission history	Hyperglycaemia without a history of DM
Van den Berghe <i>et al</i> ^[37]	2006	Interv	203 (17%)	N/A	1200	Leuven, Belgium	Admission history	N/A
Krinsely ^[24]	2006	Observ	1110 (21%)	N/A	5365	Stamford, United States	Hospital records (ICD-9 codes) for the first 2 yr then all available info	N/A
Egi <i>et al</i> ^[28]	2008	Observ	728 (15%)	N/A	4946	Multicentre (Australia)	Hospital records	N/A
Treggiari <i>et al</i> ^[25]	2008	Observ	1361 (13%)	N/A	10456	Seattle, United States	Hospital records	N/A
Arabi <i>et al</i> ^[39]	2008	Interv	208 (40%)	N/A	523	Riyadh, Saudi Arabia	Admission history Hospital records	N/A
Bronkhorst <i>et al</i> ^[38]	2008	Interv	163 (30%)	N/A	537	Multicentre (Germany)	Unknown	N/A
Del La Rosa <i>et al</i> ^[42]	2008	Interv	61 (12%)	N/A	504	Medellin, Colombia	Admission history	N/A
Finfer <i>et al</i> ^[41]	2009	Interv	1211 (20%)	N/A	6029	Multicentre (Australia, NZ, Canada)	Admission history	N/A
Preiser <i>et al</i> ^[40]	2009	Interv	203 (19%)	N/A	1078	Multicentre (Europe)	Admission history	N/A
Falciglia <i>et al</i> ^[26]	2009	Observ	77850 (30%)	N/A	259040	Multicentre (United States)	Hospital records (ICD-9 codes)	N/A
Stegenga <i>et al</i> ^[30]	2010	Observ	188 (23%)	N/A	830	Multicentre (Worldwide)	Admission history	N/A
Hermanides <i>et al</i> ^[29]	2010	Observ	699 (12%)	N/A	5961	Amsterdam, Netherlands	Hospital records (computerised system)	N/A
Krinsely <i>et al</i> ^[33]	2011	Observ	669 (21%)	N/A	3263	Multicentre (United States, Europe)	Hospital records (ICU clinical database)	N/A
Krinsley <i>et al</i> ^[32]	2013	Observ	12880 (29%)	N/A	44964	Multicentre (Worldwide)	Admission history	N/A
Plummer <i>et al</i> ^[34]	2014	Observ	220 (22%)	55 (6%)	1000	Adelaide, Australia	Admission history Phone call to GP HbA1c \geq 6.5	HbA1c \geq 6.5 without a history of DM

¹May include patients with stress hyperglycaemia/critical illness associated hyperglycaemia. Interv: Interventional; Observ: Observational; R-D: Recognised diabetes; UR-D: Unrecognised diabetes; NZ: New Zealand; N/A: Not available.

throughout Australia, New Zealand and Canada^[41], and noted 20% of its 6029 patients with a history of diabetes, with the majority (92%) having type 2 diabetes.

It should be recognised that there are limitations to using data from these interventional studies. Inclusion into these studies usually requires hyperglycaemia and therefore leads to selection bias, which artificially increases any estimate of prevalence. The interventional trials estimated ICU prevalence at 13%-40%^[36-42].

Prevalence of unrecognised diabetes

Patients may have diabetes that is unrecognised prior to admission^[2]. This may not represent "stress hyperglycaemia" or CIAH - as the hyperglycaemia is chronic rather than acute. Unrecognised diabetes is important as it not only impacts on estimations for the actual prevalence of the condition, but, as a growing

body of evidence suggests, chronic glucose control may have implications on optimal acute glucose ranges in the critically ill.

Hospital and ICU prevalence of unrecognised diabetes can be estimated from the studies mentioned (Tables 1 and 2) along with other studies cited below (Table 3). Hospital prevalence is estimated to be between 5%-16%^[16-18,43] and ICU prevalence between 6%-14%^[34,44]. The prevalence in patients with ischaemic heart disease (*e.g.*, presenting with acute myocardial infarction) appears to be higher^[45,46].

In two European studies, patients with an acute myocardial infarct and without a history of diabetes subsequently underwent an oral glucose tolerance test (OGTT) to diagnose diabetes^[45,46]. The prevalence of diabetes was found to be over 30% at discharge, and between 25%-31% at 3 mo. In London (United Kingdom), Emergency Department patients were

Table 3 Prevalence of undiagnosed diabetes in the hospital population (chronological order)

Ref.	Year	Diagnosis	UR-D	Total study patients	Location	Patient population
Norhammer <i>et al</i> ^[45]	2002	OGTT	51 (31%) at discharge 36 (25%) at 3 mo	164 144	Multicentre (Sweden)	Post AMI, Hospital/ICU
George <i>et al</i> ^[47]	2005	Fasting blood glucose \geq 7 mmol/L	13 (3%)	427	London, United Kingdom	Emergency Department
Wexler <i>et al</i> ^[17]	2008	HbA1c > 6.5	33 (5%)	695	Boston, United States	Hospital
Lankisch <i>et al</i> ^[46]	2008	OGTT	31 (32%) at discharge 19 (31%) at 3 mo	96 62	Wuppertal, Germany	Post AMI, Hospital/ICU
Mazurek <i>et al</i> ^[18]	2010	HbA1c \geq 6.5	152 (16%)	971	New York, United States	Hospital
Feldman-Billard <i>et al</i> ^[16]	2013	Fasting blood glucose \geq 7 mmol/L	156 (7%)	2141	Multicentre (France)	Hospital
Plummer <i>et al</i> ^[34]	2014	HbA1c \geq 6.5	55 (6%)	1000	Adelaide, Australia	ICU
Hoang <i>et al</i> ^[44]	2014	HbA1c \geq 6.5	14 (14%)	102	New Haven, United States	Medical ICU
Ochoa <i>et al</i> ^[43]	2014	HbA1c \geq 6.5	8 (9%)	92	Abilene, United States	Hospital

UR-D: Unrecognised diabetes; OGTT: Oral Glucose Tolerance Test; AMI: Acute myocardial infarction.

screened for diabetes *via* fasting blood glucose^[47] and it was reported that 3% patients had unrecognised diabetes.

We recently performed a single centre observational study in a mixed medical/surgical ICU in Adelaide, Australia, and separated patients with diabetes (either known or unrecognised) and CIAH using HbA1c to accurately estimate the prevalence of each condition^[34]. Of 1000 consecutively admitted ICU patients, 22% had known diabetes (5% were type 1) and 6% had unrecognised diabetes (HbA1c \geq 6.5%). The absence of previously diagnosed diabetes was confirmed by a phone call to the patient's usual local medical officer (general practitioner).

Subsequently, Hoang *et al*^[44] also estimated the prevalence of undiagnosed diabetes in a prospective, observational study in a single medical ICU^[44]. All patients with hyperglycaemia and those with known diabetes underwent measurement of HbA1c with diabetes defined as an HbA1c \geq 6.5%. Sixty-six percent of the 299 patients enrolled into the study had a history of diabetes. Of the remaining 102 hyperglycaemic patients without diabetes, 14% had an HbA1c \geq 6.5%.

In summary the prevalence of undiagnosed diabetes is difficult to determine, and as previously noted, depends on the definitions used and the location of the patient population. Current "best estimate", albeit on limited data from single centres, suggest that the prevalence of undiagnosed diabetes is either similar to, or slightly greater than, the background prevalence in the community.

RATIONALE FOR HARM FROM HYPERGLYCAEMIA, HYPOGLYCAEMIA AND GLYCAEMIC VARIABILITY

Hyperglycaemia

Hyperglycaemia in type 2 diabetes reflects the outcome of factors affecting both insulin secretion,

with β -cell dysfunction resulting in a relative insulin deficiency, and insulin resistance as a result of both environmental and genetic factors^[48,49]. However, the pathogenesis of hyperglycaemia in the critically ill patient, either with CIAH, or in those with pre-existing diabetes and experiencing a deterioration in their glucose control, is complex and poorly understood^[2]. Patient predisposition (including insulin resistance and β -cell function), the underlying illness (which can result in catecholamine release, stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, and the release of inflammatory cytokines) and the management involved (including glucocorticoids, vasopressors and nutrition) appear to be of major relevance^[1].

The activation of the HPA axis and the sympathetic system cause the "stress" response. In the majority of patients "stress" hormones (including cortisol and catecholamines) markedly increase. In addition, the underlying illness may stimulate the production of cytokines (such as TNF- α , IL-1 and IL-6)^[1,50]. These three components (HPA axis, sympathetic system and cytokine release) lead to excessive gluconeogenesis, glycogenolysis and insulin resistance, thereby augmenting stress hyperglycaemia^[50]. Glucagon is the major modulator of gluconeogenesis and may be stimulated by TNF- α , however cortisol and adrenaline (epinephrine) are also likely to contribute^[1,51,52].

Insulin resistance is thought to occur due to a number of pathways. Glucose enters cells *via* plasma membrane glucose transporters (GLUTs), which are down regulated in times of stress, possibly due to the presence of TNF- α and IL-1^[50]. Diminished glucose uptake by peripheral tissue may occur due to high cortisol and adrenaline (epinephrine) concentrations^[1,53]. As discussed, acute illness results in increased level of cytokines, which exacerbates hyperglycaemia and stimulates inflammation and oxidative stress^[1].

It should be considered that acute hyperglycaemia may represent a "protective" physiological response of

Table 4 Observational studies (diabetes as a binary variable) and outcomes related to hyperglycaemia (chronological order)

Ref.	Year	Study pts	Study point	Patients without diabetes	Patients with diabetes	Overall message
Rady <i>et al</i> ^[35]	2005	7285	Glycaemia <i>vs</i> hospital mortality	Inc mortality with blood glucose > 8 mmol/L	Inc mortality with blood glucose > 11.1 mmol/L	Mortality inc in non diabetics (10%) compared to diabetics (6%), (<i>P</i> < 0.01)
Whitcomb <i>et al</i> ^[23]	2005	2713	Admission hyperglycaemia (> 11.1 mmol/L) <i>vs</i> in-hospital mortality	Admission hyperglycaemia associated with inc mortality in CICU, CTICU and NSICU	Admission hyperglycaemia not associated with mortality	Mortality inc in non diabetics (10%) compared to diabetics (5%), (<i>P</i> < 0.05)
Krinsley ^[24]	2006	5365	Pre IIT and post IIT <i>vs</i> hospital mortality	Dec mortality risk with mean blood glucose 3.9-6.7 mmol/L Inc mortality risk with mean blood glucose > 7.8 mmol/L Mortality drop 19% (pre-IIT) to 14% (post-IIT), <i>P</i> < 0.01	Dec mortality risk with mean blood glucose 3.9-5.5 mmol/L Inc mortality risk with mean blood glucose > 10.0 mmol/L No statistically significant change in mortality pre and post IIT	Non-diabetics: 4.5-fold inc in mortality from lowest mean blood glucose, 3.9-5.5 mmol/L (9%) to highest, > 10mmol/L (40%) Diabetics: 2-fold inc in mortality from lowest mean blood glucose, 3.9-5.5 mmol/L (13%) to highest, > 10mmol/L (26%)
Egi <i>et al</i> ^[26]	2008	4896	Glycaemia <i>vs</i> mortality	Inc risk of ICU mortality with hyperglycaemia - with non survivors spending more time with blood glucose > 8.0 mmol/L	No association with hyperglycaemia and ICU mortality Lower OR of death at all levels of hyperglycaemia	Diabetic patients: lower ICU mortality (<i>P</i> = 0.02) No difference in hospital mortality between groups (<i>P</i> = 0.3)
Falciaglia <i>et al</i> ^[26]	2009	259040	Glycaemia <i>vs</i> mortality	5-fold inc in mortality from lowest mean blood glucose, 3.9-6.1 mmol/L (8%) to highest, > 16.7 mmol/L (41%)	2-fold inc in mortality from lowest mean blood glucose, 3.9-6.1 mmol/L (6%) to highest, > 16.7 mmol/L (11%)	Hyperglycaemia associated with inc mortality in diabetics and non diabetics Mortality greater for hyperglycemic non diabetics patients
Stegenga <i>et al</i> ^[30]	2010	830	DM <i>vs</i> outcomes of sepsis	Admission hyperglycaemia (> 11.1 mmol/L) associated with inc 28 and 90 d mortality (<i>P</i> < 0.03)	Admission hyperglycaemia had no effect on diabetic mortality	Diabetes did not influence mortality in sepsis
Krinsley <i>et al</i> ^[32]	2013	44964	Hyperglycaemia, hypoglycaemia, and glycemic variability <i>vs</i> mortality (and how DM effects this)	Inc mortality with higher mean blood glucose (\geq 7.8 mmol/L) Dec mortality with lower blood glucose (4.4-7.8 mmol/L)	Inc mortality with mean blood glucose between 4.4-6.1 mmol/L Dec mortality when blood glucose were higher (6.2-10 mmol/L)	Hyperglycaemia, hypoglycaemia, and increased glycemic variability are independently associated with mortality in ICU patients Diabetic status tempers these relations

Inc: Increased; Dec: Decreased; CICU: Cardiac Intensive Care Unit; CTICU: Cardiothoracic Intensive Care Unit; NSICU: Neurosurgical Intensive Care Unit; IIT: Intensive insulin therapy.

the host during periods of stress^[50]. An acute rise in glycaemia may facilitate glucose delivery at critical times and promote anti-apoptotic pathways, protecting against cell death^[50]. While uncontrolled acute hyperglycaemia is clearly harmful, the threshold at which harm occurs in the critically ill patient remains to be determined^[2]. The majority of studies that have evaluated this issue have enrolled heterogeneous cohorts - and patients with diabetes only comprised a small proportion of the sample evaluated. Based on recent data it is increasingly likely that the glucose threshold in a patient with diabetes, particularly those with chronic hyperglycaemia, will differ from that in a patient who is naive to hyperglycaemia. A patient with poorly controlled diabetes, i.e., with a history of high blood glucose levels and consequently high HbA1c, will be more tolerant of hyperglycaemia but susceptible to the adverse effects of hypoglycaemia (see below), such that the thresholds for both variables are greater than a patient who is naive to hyperglycaemia - either those with well controlled diabetes or those with CIAH.

Multiple studies have examined the effects of hyperglycaemia on morbidity and mortality in the ICU population with inconsistent and controversial outcomes. Moreover, the majority of these studies have not categorised patients into those with chronic hyperglycaemia or acute glucose intolerance.

There are numerous observational studies (Table 4). In 2005, a case controlled study of 7285 ICU patients reported that in individuals without known diabetes, mortality was increased when blood glucose levels were > 8 mmol/L but this signal was absent in patients with diabetes^[35]. Overall, mortality was significantly greater in patients without diabetes when compared to patients with diabetes. A retrospective study of 2713 patients admitted into ICU^[23] reported an association between mortality and hyperglycaemia in patients without a history of diabetes in the cardiac, cardiothoracic, and neurosurgical intensive care units. In an audit of 5365 ICU

patients evaluated before and after implementation of an intensive glucose control policy^[24], mortality was increased in patients with hyperglycaemia who were not known to have diabetes when compared to those with diabetes. In 2008, Egi *et al.*^[28] reported a retrospective study of 4946 patients in which ICU mortality increased with increasing mean blood glucose level in patients without diabetes but this signal of harm was absent in those with pre-existing diabetes^[28].

A retrospective cohort study of 259040 ICU admissions also reported an association between mortality and hyperglycaemia, with the relationship far stronger in patients without a diagnosis of diabetes when compared to those with pre-existing diabetes^[26]. A retrospective analysis of a previous study^[31] included 830 patients admitted with severe sepsis (defined as sepsis associated with acute organ dysfunction)^[30], and reported that hyperglycaemia was predictive of subsequent death in those patients not known to have diabetes. Additionally, a multicentre retrospective study of 44964 patients divided into 2 cohorts (with and without known diabetes)^[32], reported increased mortality with higher mean blood glucose concentrations (≥ 7.8 mmol/L) when compared to blood glucose concentrations 4.4-7.8 mmol/L in patients without diabetes. In contrast, patients with diabetes were more likely to die when mean blood glucose concentrations were between 4.4-6.1 mmol/L when compared to patients with greater blood glucose concentrations (6.2-10 mmol/L).

A number of interventional studies have evaluated the relationship between chronic and acute hyperglycaemia and outcomes (Table 5). In a pooled analysis of studies conducted in a single centre in Leuven, intensive insulin therapy (ITT, aiming for blood glucose concentrations between 4.4-6.1 mmol/L) was reported to reduce mortality and morbidity in patients without a diagnosis of diabetes, but this was not the case in patients with diabetes, if anything, there was a trend for harm with intensive insulin therapy in patients with diabetes such that mortality was non-significantly greater at a lower mean blood glucose range (6.1-8.3 mmol/L, 21.2% vs < 6.1 mmol/L, 26.2%, $P = 0.4$ and > 8.3 mmol/L, 21.6%, $P = 0.9$)^[54].

Subsequently, a number of interventional, randomised, controlled trials, containing patients with diabetes, comparing ITT to more conventional glucose targets have been published^[38-42]. A trial of 523 mixed (medical and surgical) ICU patients^[39] reported no survival benefit in patients with diabetes with ITT, but ITT was associated with an increased prevalence of hypoglycaemia. The Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) study assigned 537 ICU patients with severe sepsis to either ITT or more conventional glucose targets while receiving either 10% pentastarch or a modified Ringers lactate in a two-by-two factorial study^[38]. The study was suspended at interim analysis for safety reasons

with ITT being associated with increases in episodes of severe hypoglycaemia and adverse events. De La Rosa *et al.*^[42] also evaluated ITT in 504 ICU patients (61 with diabetes) and there was no mortality or morbidity benefit observed, but an associated increased risk of hypoglycaemia, when administering ITT.

In 2009, the NICE-SUGAR study compared ITT with conventional glucose control in 6029 ICU patients and established that the observations from the initial Leuven studies regarding ITT were not generalisable outside that specialised institution^[41]. However, amongst the 1211 patients with pre-existing diabetes in the NICE-SUGAR study the administration of ITT did not appear more harmful than in patients without diabetes. The Glucontrol study^[40], an international, multicentre trial involving over 1000 ICU patients—was stopped early due to protocol violations, and it was, accordingly, underpowered. However, there was no evidence to suggest any benefit with ITT and data in patients with diabetes were not specifically described.

Recently a number of studies have attempted to measure chronic glycaemia as a dynamic (HbA1c), rather than a binary, variable (*i.e.*, presence of diabetes - yes/no) (Table 6). Egi *et al.*^[55] performed a retrospective observational study of 415 patients with diabetes (from two Australian ICUs) in whom glycated haemoglobin (HbA1c) had been measured within 3 mo of their critical illness and evaluated how this measure of pre-existing glycaemia impacted on the interaction between acute glycaemia and mortality^[55]. It was reported that in patients with elevated preadmission HbA1c levels ($> 7\%$) the number of deaths were significantly fewer when blood glucose concentrations were > 10 mmol/L.

Consistent with this observation, we recently measured HbA1c on admission and glucose concentrations for the first 48 h of ICU admission^[34] and observed that acute peak glucose concentrations were associated with increased mortality only in patients with adequate premorbid glycaemic control (defined as HbA1c $< 7\%$), but not in patients with chronic hyperglycaemia (defined as an HbA1c $\geq 7\%$). This finding was also supported by Hoang *et al.*^[44] who assessed the prevalence of undiagnosed diabetes (*i.e.*, HbA1c $\geq 6.5\%$) among those with hyperglycaemia in a medical ICU. Patients with an HbA1c $\geq 6.5\%$ were found to have significantly lower mortality compared to those with an HbA1c $< 6.5\%$ (11.7% vs 19.3%, $P = 0.038$), despite having greater glucose concentrations.

In summary the outcomes of the largest and most generalisable randomised study are consistent with the concept that the optimal glucose concentrations in unselected critically ill patients are between 6-10 mmol/L^[41]. However, observational data, post-hoc analysis of interventional studies and studies measuring chronic glycaemia as a dynamic variable suggest that patients with pre-existing diabetes may warrant higher targets. Indeed, there is increasing data suggesting that targets should be personalised depending on both

Table 5 Interventional studies (diabetes as a binary variable) and outcomes related to hyperglycaemia (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Van den Bergh <i>et al</i> ^[64]	2006	2748	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (insulin if blood glucose > 12 then target 10-11.1 mmol/L) on mortality	Reduced mortality and morbidity with ITT	No survival benefit with ITT Higher rates of hypoglycaemia	Hosp mortality 20% (40/200) of the DM patients in conventional arm Hosp mortality 22% (46/207) of the DM patients in the ITT arm
Arabi <i>et al</i> ^[39]	2008	523	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 10-11.1 mmol/L) on ICU mortality	Mortality: ITT (14%) vs CIT (14%) - no significant difference (<i>P</i> = 0.2)	Mortality: ITT (13%) vs CIT (20%) - no significant difference (<i>P</i> = 0.3)	No significant difference in ICU mortality between ITT and CIT (<i>P</i> = 0.3)
Brunkhorst <i>et al</i> ^[38]	2008	537	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 10-11.1 mmol/L) on mortality	28 d mortality: ITT 25% vs CIT 23% (<i>P</i> = 0.8) 90 d mortality: ITT 40% vs CIT 32% (<i>P</i> = 0.2)	28 d mortality: ITT 25% vs CIT 32% (<i>P</i> = 0.3) 90 d mortality: ITT 40% vs CIT 42% (<i>P</i> = 0.9)	No mortality benefit with ITT vs CIT Stopped early due to safety risk
Del La Rosa <i>et al</i> ^[42]	2008	504	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 10-11.1 mmol/L) on morbidity and mortality	ICU mortality ITT 37% vs CIT 32% (no significance) ² In-hospital mortality: ITT 40% vs CIT 39% (no significance) ²	Mortality: ITT (38%) vs CIT (31%) - no significant difference	No difference in ICU mortality, 28 d mortality or ICU infections Increased hypoglycaemia in ITT
Finfer <i>et al</i> ^[41]	2009	6029	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose < 10 mmol/L) on mortality	Mortality: ITT (27%) vs CIT (24%) - no significant difference	Mortality: ITT (32%) vs CIT (28%) - no significant difference	ITT arm - inc 90 d mortality No difference in those with and without DM (<i>P</i> = 0.60)
Preiser <i>et al</i> ^[40]	2009	1078	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 7.8-10 mmol/L) on mortality	ICU mortality ITT 17% vs CIT 15% (<i>P</i> = 0.4) ² Hospital mortality: ITT 23% vs CIT 19% (<i>P</i> = 0.1) ²	Not described	Stopped early due to protocol violations

¹Contains pooled data from the 2001 (surgical) and 2006 (medical) study; ²Mortality of total patients (includes non-diabetic and diabetic patients). ITT: Intensive insulin therapy; CIT: Conventional insulin therapy; Inc: Increased; Dec: Decrease.

diabetic status and recent glycaemic control.

Hypoglycaemia

In most cases, treatment of hyperglycaemia in the critically ill involves the use of insulin, which is associated with increased risks of both hypoglycaemia and glycaemic variability^[56]. The severity of illness may also result in a hypoglycaemia and therefore it is important to be circumspect when attributing mortality to hypoglycaemia^[57]. Additionally, hypoglycaemia may have adverse biological effects including an increase in systemic inflammatory response, impairment of the sympathetic nervous system, inhibition of the biological response to stress, along with cerebral vasodilation and neural damage^[2,58]. Experimentally, the use of insulin and consequent hypoglycaemia may be associated with hypotension, vasodilation, and reduced autonomic responses to subsequent hypoglycaemic episodes^[58]. Furthermore, critically ill patients may be more prone to the effects of hypoglycaemia itself, which may include cardiac arrest, seizure and coma^[59].

Studies examining the effects of hypoglycaemia in critically ill patients with pre-existing diabetes are limited. Interventional studies describing this relationship have been summarised (Table 6). Of note, post hoc analysis of the NICE-SUGAR data indicate that intensive insulin therapy increases episodes of moderate (2.3-3.9 mmol/L) and severe (\leq 2.2 mmol/L) hypoglycaemia, both of which are associated with increased risk of death^[56]. This relationship was similar among patients with and without a diagnosis of diabetes.

In addition to these studies, there are a number of observational studies that have evaluated this association (Table 7). A retrospective database review of 408 ICU patients (102 index cases, 306 controls) published in 2007^[60] reported that a history of diabetes was associated with severe hypoglycaemia and that a single

Table 6 Observational studies that have recorded chronic glycaemia as a dynamic variable (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Egi <i>et al</i> ^[55]	2011	415	Does preexisting hyperglycaemia modulate the association between glycemia and outcome in ICU patients with DM	N/A	Patients with elevated preadmission HbA1c levels (> 7%) showed a mortality benefit when mean ICU glucose concentrations were > 10 mmol/L	Relationship between HbA1c and mortality changed according to the levels of time-weighted average of blood glucose concentrations
Plummer <i>et al</i> ^[34]	2014	1000	Prevalence of CIAH and recognized/unrecognized DM in ICU and to evaluate the premorbid glycaemia on the association between acute hyperglycaemia and mortality	50% had CIAH Risk of death inc by 20% for each increase in acute glycaemia of 1 mmol/L	Well controlled DM (HbA1c < 6%) and adequately controlled (DM 6%-7%) - risk of death as per non diabetic patient HbA1c ≥ 7% (insufficiently controlled DM) had no significance between mortality and acute glycaemia	22% had recognised DM 6% had unrecognised diabetes
Hoang <i>et al</i> ^[44]	2014	299	Prevalance of unrecognized DM amongst those with CIAH and the association between baseline glycaemia and mortality	102 (34%) had no history of DM 14/102 (14%) had unrecognized DM (diagnosed with HbA1c ≥ 6.5)	197 (66%) had a history of DM	Lower HbA1c had inc mortality (in this population of CIAH patients) despite lower median glucose values and less glucose variability Mortality in HbA1c < 6.5 (19%) vs HbA1c ≥ 6.5 (12%), <i>P</i> = 0.04

Inc: Increased; Dec: Decreased; N/A: Not available.

hypoglycaemic episode was associated with an increased risk of mortality (compared with those without an episode of severe hypoglycaemia). Egi *et al*^[61] reported mild or moderate hypoglycaemia was associated with mortality in critically ill patients - with mortality substantially increasing according to severity of hypoglycaemia - and patients with diabetes were more likely to suffer from insulin-associated hypoglycaemia.

The blood glucose threshold that adverse events occur may be greater in patients with pre-existing diabetes. In a retrospective multi-centre observational study^[32] increased mortality was reported in 12880 patients with pre-existing diabetes who had mean glucose concentrations between 4.4-6.2 mmol/L. While the investigators were not able to differentiate between patients with well-controlled or poorly-controlled diabetes, these data support the concept that the threshold for "hypoglycaemia" may be increased in critically ill patients with diabetes when compared to non diabetic patients. For example, if a patient typically has blood glucose concentrations above 10 mmol/L, and, in hospital, insulin is administered to achieve blood glucose concentration of about 6 mmol/L, this may result in a "relative" hypoglycaemia.

Glycaemic variability

Glycaemic variability (GV) describes the fluctuations in blood glucose concentrations, as marked fluctuations may be associated with multiple adverse effects such as apoptosis, cytokine production and increased markers of oxidative stress^[59]. Oxidative stress markers have been shown to increase with glucose fluctuations^[62,63]. GV may be assessed by a number of methods. Techniques to quantify variability are reviewed

elsewhere^[64].

Multiple studies in the critically ill have established as an association with poor outcomes and GV^[44,65-71], however the evidence in patients with pre-existing diabetes is limited and inconsistent (Table 8). In 2006, Egi *et al*^[65] published a retrospective, electronic database analysis of 7049 ICU patients in 4 centres around Australia, using standard deviation as a marker of glucose variability, and focusing on the association of blood glucose variability and mortality^[65]. Both mean and standard deviation of blood glucose were independently associated with mortality.

A retrospective, single center cohort study of patients admitted with sepsis reported that GV was also independently associated with increased mortality and importantly, that this was independent of hypoglycaemia and the presence of diabetes^[66]. Another retrospective study of 3252 patients reported that increased GV was associated with mortality^[67] and diabetes was associated with greater GV. A prospective, observational study of 42 patients used non-linear dynamics to measure glycaemia in time series^[69]. Patients underwent continuous glucose monitoring system measuring interstitial glucose concentrations every 5 min for 48 h. The authors reported greater variability was associated with increasing mortality, even in patients with diabetes. However, given the small cohort, these results must be treated with caution.

Other studies have reported no relationship between mortality and GV in patients with diabetes. A retrospective, observational study of 4084 critically ill patients (942 with known diabetes)^[68] reported that GV was associated with mortality in patients without diabetes, but not in patients with diabetes. More

Table 7 Observational studies and outcomes related to hypoglycaemia (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Krinsley and Grover ^[60]	2007	408	Risk factors for developing hypoglycaemia in ICU and outcomes	Severe hypoglycaemia associated with septic shock. Renal insufficiency, mechanical ventilation, illness severity and use of ITT	Associated with inc risk of severe hypoglycaemia ($P < 0.01$) DM had no association with mortality	Mortality in severe hypoglycaemia cohort 56% <i>vs</i> control cohort 40%, $P < 0.01$
Egi <i>et al</i> ^[61]	2010	4946	Hypoglycaemia <i>vs</i> risk of death in critically ill patients	Mild or moderate hypoglycaemia was associated with mortality in critically ill patients Mortality increases as severity of hypoglycaemia increases	Diabetic patients more likely to suffer from insulin-associated hypoglycaemia	22% of total patients had one episode of hypoglycaemia Hospital mortality: hypoglycaemic cohort 37% <i>vs</i> control cohort 20%, $P < 0.01$
Krinsley <i>et al</i> ^[33]	2011	6240 ¹	Mild hypoglycaemia (blood glucose level < 3.9 mmol/L) <i>vs</i> risk of mortality in critically ill patients.	Mild hypoglycaemia was associated with a significantly increased risk of mortality	The association between hypoglycaemia and mortality was independent of diabetic status	Inc severity of hypoglycaemia was associated with inc risk of mortality Hypoglycemic patients had higher mortality regardless of diagnostic category and ICU LOS
Krinsley <i>et al</i> ^[32]	2013	44964	Hyperglycaemia, hypoglycaemia, and glycemic variability <i>vs</i> mortality (and how DM effects this)	Inc mortality with higher mean blood glucose (≥ 7.8 mmol/L) Dec mortality with lower blood glucose (4.4-7.8 mmol/L)	Inc mortality with mean blood glucose between 4.4-6.1 mmol/L Dec mortality when blood glucose were higher (6.2-10 mmol/L)	Hyperglycaemia, hypoglycaemia, and increased glycemic variability are independently associated with mortality in ICU patients Diabetic status tempers these relations

¹Contains partial data from one prospective RCT (Glucontrol trial) and complete data from two observational cohorts (United States and The Netherlands). Inc: Increased; Dec: Decrease; LOS: Length of stay.

recently in the study by Hoang *et al*^[44] of 299 patients there was no association between GV and mortality in their entire cohort, however the group with diabetes (128 patients) had a lower rate of mortality despite having a higher GV. Additionally, a retrospective analysis of 2782 ICU patients, comparing different GV indices and mean glucose concentrations to predict mortality and ICU acquired infections^[70] reported that while GV was associated with infections and mortality in patients without pre-existing diabetes, in those with diabetes GV was greater but was not associated with either mortality or infection.

In summary, there is a strong relationship between GV and mortality in critically ill patients that has been confirmed in multiple studies. However, with respect to patients with diabetes, data are inconsistent. This may be due a number of factors, including small numbers studied resulting in lack of power, or that patients with chronic hyperglycaemia are protected somewhat by glycaemic excursions during acute illness. Research is warranted to further understand whether GV is harmful in patients with pre-existing diabetes.

RATIONALE FOR PERSONALISED THERAPY AND THAT THE HARM FROM EACH OF THESE DOMAINS MAY VARY ACCORDING TO PRE EXISTING PHYSIOLOGY

Diabetes is known to be associated with a large burden

of illness in the outpatient setting and is associated with increased mortality^[72]. Paradoxically, as discussed, multiple studies exist suggesting that acute hyperglycaemia in critically ill patients without diabetes (*i.e.*, patients with CIAH) is associated with increased mortality and morbidity when compared to those with known diabetes^[73]. There is growing evidence that chronic hyperglycaemia may lead to cellular conditioning, and that in fact, may be protective against acute hyperglycaemia mediated damage during an episode critical illness^[1]. These outcomes suggest that current target glucose levels in patients naïve to hyperglycaemia, or those suffering from CIAH, may be harmful to those with chronic hyperglycaemia or poorly controlled diabetes.

CONCLUSION

This review articulates the need for further research to be done to identify the ideal glucose targets in critically ill patient with pre-existing diabetes. Not only does hyperglycaemia occur frequently in this group, but, recent data suggests that targeted blood glucose concentrations may benefit from consideration of a patient's premorbid glucose state.

Our recommendations are to avoid treating patients with diabetes as a homogenous group. Treatment of the critically ill patient with type 2 diabetes should be personalised to their internal milieu. There is preliminary evidence suggesting that higher blood glucose concentrations (*e.g.*, up to 14 mmol/L) in patients with uncontrolled type 2 diabetes may not be

Table 8 Observational and interventional studies and outcomes related to glycaemic variability (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Egi <i>et al</i> ^[65]	2006	7049	GV (measured by SD and %CV) <i>vs</i> mortality (hospital and ICU)	Both mean and GV of blood glucose were significantly and independently associated with ICU and hospital mortality GV was a stronger predictor of ICU mortality than mean glucose concentration	Inc mortality when comparing highest and lowest glucose SD No other significant relation with blood glucose (SD and mean) and ICU/hospital mortality Logistic regression: DM associated with decrease OR for ICU mortality Mortality rise remained even after adjusting for a diagnosis of diabetes	The mean \pm SD of blood glucose: Survivors 1.7 ± 1.3 mmol/L <i>vs</i> Non survivors 2.3 ± 1.6 mmol/L ($P < 0.001$) Post logistic regression analysis, both mean and SD of blood glucose were significantly associated with ICU and hospital Higher observed mortality with increasing levels of variability Higher odds of hospital mortality with lower mean blood glucose + high GV or higher mean blood glucose + lower GV
Ali <i>et al</i> ^[66]	2008	1246	GV <i>vs</i> hospital mortality in septic ICU patients	GV is independently associated with hospital mortality in sepsis	Mortality rise remained even after adjusting for a diagnosis of diabetes	Amount of GV had a significant effect on mortality - <i>e.g.</i> , patients with mean blood glucose $3.9\text{--}5.5$ mmol/L mortality: Lowest GV 6% while high GV 30% Attempts to minimize GV may have a significant beneficial impact on outcomes of critically ill patients without diabetes
Krinsley ^[67]	2008	3252	GV <i>vs</i> mortality in ICU patients	Inc GV conferred a strong independent risk of mortality	Multivariable regression analysis demonstrated that diabetes had an independent positive correlation to SD Higher measures of GV	
Krinsley ^[68]	2009	4084	Impact of DM or its absence on GV as a risk factor for mortality	Low GV was associated with increased survival High GV was associated with increased mortality	No association between GV and mortality among diabetics	
Lundelin <i>et al</i> ^[69]	2010	42	Glycemic dynamics (measured <i>via</i> non-linear dynamics) <i>vs</i> mortality in ICU patients	Loss of complexity (therefore higher variability) in glycaemia time series is associated with higher mortality	This association persisted in diabetics No difference in DFA (detrended fluctuation analysis a measure of complexity) between DM and nondiabetics	In critically ill patients, there is a difference in the complexity of the glycaemic profile between survivors and nonsurvivors Loss of complexity correlates with higher mortality Increased glucose amplitude variation was associated with mortality, irrespective of blood glucose level Lower HbA1c had inc mortality (in this population of CIAH patients) despite lower median glucose values and less glucose variability Mortality in HbA1c < 6.5 (19%) <i>vs</i> HbA1c ≥ 6.5 (12%), $P = 0.04$
Meyfroidt <i>et al</i> ^[71]	2010	2748	Blood glucose signal characteristics <i>vs</i> hospital mortality	GV was independently associated with hospital mortality	Increased mortality was seen in both diabetics and non diabetic patients.	
Hoang <i>et al</i> ^[44]	2014	299	Prevalence of unrecognized DM amongst those with CIAH and the association between baseline glycaemia and mortality	102 (34%) had no history of DM 14/102 (14%) had unrecognized DM (diagnosed with HbA1c ≥ 6.5)	197 (66%) had a history of DM	
Donati <i>et al</i> ^[70]	2014	2782	GV and mean BGIs <i>vs</i> mortality and intensive care unit-acquired infections	High GV is associated with higher risk of ICU acquired infection and mortality	Diabetic patients had higher mean BGL and GV No change in mortality or infections	Mean BGL was not associated with infections and mortality

[†]Interventional study data - pooled from the Leuven trials. GV: Glycaemic variability; SD: Standard deviation; %CV: Coefficient of variation; Inc: Increased; Dec: Decreased; OR: Odds rat.

harmful. For this reason it may be safer to target blood glucose concentrations between 10–14 mmol/L in this group. However, definitive studies of critically ill patients with poorly controlled diabetes are required before this approach is incorporated into clinical practice. In contrast, in patients with CIAH, or those with well-controlled diabetes (HbA1c < 7.0), a more conservative target (6–10 mmol/L) is supported by considerable data.

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Co-occurrence of type 1 diabetes mellitus and celiac disease

Amit Akirov, Orit Pinhas-Hamiel

Amit Akirov, Beilinson Campus, Rabin Medical Center, Petach-Tikva 49100, Israel

Amit Akirov, Orit Pinhas-Hamiel, Sackler School of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel

Orit Pinhas-Hamiel, Pediatric Endocrine and Diabetes Unit, Safra Children's Hospital, Sheba Medical Center, Ramat-Gan 52621, Israel

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Correspondence to: Amit Akirov, MD, Beilinson Campus, Rabin Medical Center, 1 Kaplan Street, Petach-Tikva 49100, Israel. amit.akirov@gmail.com
 Telephone: +972-3-524650760
 Fax: +972-3-9221605

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Abstract

The co-occurrence of celiac disease (CD) and type 1 diabetes (T1DM) has been reported as 5-7 times more

prevalent than CD alone. The clinical presentation and natural history of CD in patients with T1DM may vary considerably. Less than 10% of patients with T1DM and CD show gastrointestinal symptoms. Therefore, experts support screening for CD in T1DM patients, though there is no consensus as to the recommended frequency of screening. When stratified by time since CD diagnosis, longer follow-up and coexistence of CD are associated with significant increased risk of diabetic associated morbidity and mortality. Early CD diagnosis and treatment with a gluten-free diet are essential.

Key words: Type 1 diabetes mellitus; Celiac disease; Glycemic control; Gluten free diet; Pediatrics

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Core tip: Increased prevalence rates of celiac disease (CD) are described among individuals with type 1 diabetes mellitus (T1DM). Specifically celiac disease is more prevalent in females with T1DM. Less than 10% of patients with T1DM and CD show gastrointestinal symptoms therefore screening is necessary. The significant increase of diabetic associated morbidity and mortality, emphasize the importance of early diagnosis of CD and appropriate treatment with gluten-free diet.

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INTRODUCTION

Celiac disease

Celiac disease (CD), previously known as celiac sprue, affects 0.6%-1.0% of the population worldwide, with wide geographic variation, for unknown reasons^[1,2]. The autoimmune disorder is triggered in genetically

predisposed patients by gluten ingestion^[1-4]. Symptoms of CD include malabsorption and malnutrition, vitamin deficiencies, iron deficiency anemia, failure to thrive, short stature, diarrhea, anorexia, constipation, vomiting, and abdominal distension. Other complications associated with untreated CD include osteoporosis, obstetric complications, and neurologic disorders, as well as enteropathy-associated T-cell lymphoma and adenocarcinoma of the jejunum^[5,6]. However, several reports in the literature state that many cases of CD are asymptomatic or associated with mild symptoms^[7-9]. Diagnosis of CD is based on intestinal biopsy and the presence of specific antibodies; however, most cases of CD remain undiagnosed^[10,11]. Currently, the only effective treatment for CD is a lifelong gluten-free diet (GFD), which results in resolution or improvement for most individuals^[12].

Epidemiology of T1DM and CD

The association between CD and T1DM was first described in the late 1960s^[13]. Studies published during the last few years have demonstrated elevated prevalence rates of CD among individuals with T1DM: 4.4% in the United Kingdom, 3.7% in Israel, 4.8% in Greece, and 6.4% in Germany; and as high as 10.5% in Brazil and 11.1% in India^[14-19].

The incidence of T1DM is rapidly increasing in children and adolescents, with a reported increase of 3% annually^[20,21]. Similarly, a longitudinal study documented an increase in the prevalence of CD in the mid 1990s, from 3.3% to 10.6%, most probably due to changes in environmental factors^[22].

CD is a female predominant disease, and is 2-3 times more common among females^[23]. Although there is no gender difference in the prevalence rates of T1DM, CD is also more prevalent in females than in males with T1DM. The etiological risk factors for developing antibodies against the small bowel are thought to be different from those for T1DM^[24-26].

Genetics

Genetic background plays a key role in the predisposition to CD, as suggested by higher prevalence among family members and higher concordance rates in monozygotic than dizygotic twins (over 80% compared with 11%)^[27].

The human leukocyte antigen (HLA) plays a key role in the genetic predisposition to CD, as there is a strong association between both *HLA-DQ2* and *HLA-DQ8*, and between CD. The negative predictive value of HLA typing is high, as CD is extremely rare in patients carrying neither *DQ2* nor *DQ8* alleles^[28,29].

An overlap in the genetic susceptibility conferred by *HLA-DQ2* is the basis for the increased prevalence of CD in patients with T1DM. Over 90% of those with CD express *HLA-DR3/DQ2* haplotype, as well as 55% of those with T1DM, compared with less than 25% of the general population^[30]. Bakker *et al.*^[31] confirmed

the high prevalence of *HLA-DQ2* haplotypes in patients with both T1DM and CD, and reported that *HLA-DQ2* homozygosity confers the highest risk for CD among patients with CD. *DQ2* has been cited by a number of studies as the major susceptibility factor for CD. *HLA-DQ8*, another important allele for CD, is considered a stronger susceptibility factor for T1DM. *DQ8* heterozygosity is claimed to be the strongest risk factor for the development of T1DM^[32,33]. Trynka *et al.*^[34] reported 57 independent CD association signals from 39 non-HLA genes that confer a predisposition to CD. However, although genetic predisposition is essential, it is not sufficient for the development of CD, as the pathogenesis of CD involves an external trigger, namely gluten.

SCREENING AND DIAGNOSIS

In the general population

According to the modified guidelines of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)^[10], testing for CD is recommended in children and adolescents with otherwise unexplained signs and symptoms suggestive of CD, and among asymptomatic children and adolescents with an increased risk for CD, such as patients with T1DM or first-degree relatives with CD.

Diagnosis of CD is based on the presence of villous atrophy and crypt hyperplasia by intestinal biopsy and the presence of antibodies against tissue transglutaminase (TTG) or endomysium (EMA). The diagnosis is confirmed by an antibody decline to a GFD. According to ESPGHAN guidelines, in patients with suspected CD and certain conditions (typical symptoms, high titer of TTG antibodies and predisposing HLA genotypes), there is no obligation to complete duodenal biopsy and histology^[10].

Finally, potential CD, a term coined by Ferguson in the 1990s, refers to patients with positive CD-associated antibodies, but with normal, or almost normal, jejuna mucosa^[35]. These patients usually have mild symptoms, if any, of CD. The number of patients diagnosed with potential CD is increasing, following the raised attention for CD and screening tests of high-risk populations^[36]. Numerous studies in the general population demonstrate that CD is often only diagnosed after several years of CD related complaints^[37-39]. Some reported that diagnosis of irritable bowel syndrome preceded the correct diagnosis of CD in many patients^[40,41].

CD in T1DM patients

Less than 10% of patients with T1DM and CD show gastrointestinal symptoms^[42]. Therefore, most professional societies recommend screening of patients with T1DM for CD. However, there is no consensus regarding the recommended screening tests and the frequency of screening^[43].

Recommended screening test: Most guidelines support screening based on TTG IgA (confirmed by EMA), or TTG IgG in patients with IgA deficiency, because of its high sensitivity and specificity^[44,45]. Most experts argue that in patients with CD-associated antibodies, it is mandatory to perform esophagoduodenoscopy with small bowel biopsies to confirm the diagnosis^[10].

Timing and frequency of screening: Neither in the guidelines issued by ESPGHAN nor those issued by the National Institute for Health and Clinical Excellence, is the timing of screening specified. As for the frequency of screening, ESPGHAN guidelines recommend retesting at intervals, with no firm evidence, but opinion is every 2-3 years^[10]. NICE guidelines state that the evidence is insufficient to make a recommendation regarding the frequency of screening for CD in patients with T1DM^[10,46]. The International Society for Pediatric and Adolescent Diabetes recommends screening for CD at diagnosis of T1DM, every year in the first five years of follow-up, and less frequently in successive years^[4,47,48].

Bakker *et al.*^[31] reported that almost 50% of T1DM patients diagnosed with CD in adulthood had CD related complaints for over 5 years prior to the diagnosis of CD. Furthermore, their findings demonstrated a bimodal distribution of the age of diagnosis of CD in patients with T1DM, with peak incidence rates at the ages of 10 and 45 years.

CLINICAL PRESENTATION OF CD IN PATIENTS WITH T1DM

CD diagnosis most often follows the diagnosis of T1DM, and only a minority of patients were diagnosed first with CD^[7,31,49].

Age of onset

The mean age at onset of T1DM is younger in those with both T1DM and CD, than in those with only T1DM^[25,50]. In an observational cohort study of 4379 people aged ≤ 18 years from Australia, the mean age at T1DM onset was 6.6 ± 4.0 years in those with T1DM and CD, compared with 8.4 ± 4.1 years in those without CD^[51].

Signs and symptoms

The natural history of CD in patients with T1DM may vary considerably, as the diagnosis of CD can precede the diagnosis of T1DM, or be established at the onset of T1DM, during routine screening tests at follow-up. Accordingly, the presentation of CD varies greatly, from asymptomatic or mild symptoms to poor growth and considerable morbidity^[7-9,44]. In individuals with diabetes, symptoms of CD may be divided into two main categories, those directly associated with CD and those related to the impact of CD on diabetes.

Signs and symptoms directly associated with CD

These include malabsorption and malnutrition, vitamin deficiencies, iron deficiency anemia, failure to thrive, short stature, diarrhea, anorexia, constipation, vomiting, and abdominal distension.

Growth in children with CD and T1DM compared to children with T1DM alone: As stated above, differences between reports may be due to whether CD diagnosis results from routine screening or is prompted from signs and symptoms.

Body weight was found to be significantly lower among children with T1DM with screening-identified CD compared to those with T1DM only; however, there was no difference in height^[52]. Of 41951 children and adolescents surveyed in Germany, only 22273 (53%) had been screened for CD. Those with both T1DM and CD had a significantly lower weight standard deviation and height standard deviation score (SDS)^[50]. In a subgroup of 183 patients, those with both diseases had significantly lower height and weight SDS after 5-year follow-up^[53]. Previously, we demonstrated a higher prevalence of growth impairment among patients with both CD and T1DM, compared to patients with T1DM alone. Patients with CD were, on average, significantly shorter than their genetic target height potential, compared to patients with T1DM alone. Furthermore, poor adherence to GFD resulted in continuous growth impairment, compared to steady improvement among those with good adherence to a GFD^[16]. Of note, patients with CD who do not improve their growth velocity after GFD should be evaluated for growth hormone deficiency secondary to autoimmune hypophysitis^[54].

Signs related to the impact of CD on diabetes at diagnosis of CD

Glycemic control: Data remain inconsistent regarding glycemic control in patients with dual diagnosis of CD and T1DM. Data may differ based on the points of time HbA1c levels were assessed (at diagnosis vs at follow-up), whether diagnosis was based on routine screening or on symptoms, and in longitudinal studies whether adherence to GFD was assessed in parallel.

(1) HbA1c levels at diagnosis of CD. Malabsorption of nutrients may cause a reduction in HbA1c levels. In a controlled study in children mean age 10 years with T1DM duration of about 4 years, HbA1c levels at baseline did not differ significantly between patients with T1DM and CD, and between those with T1DM alone^[55]. Yet, among adult T1DM patients who were newly diagnosed with CD, glycemic control was significantly worse than for those with T1DM alone, 8.2% vs 7.5%, $P = 0.05$ ^[56]. The difference between these studies may reflect the impact of delayed diagnosis of CD.

(2) HbA1c levels at follow-up. In a controlled prospective 2-year follow-up study, mean HbA1c

levels did not differ significantly between patients with both T1DM and CD and between those with T1DM alone^[57]. Similarly, in a large cohort from 297 centers in Germany and Austria, no statistically significant differences were found in mean HbA1c levels, between children with and without CD, mean age of 13.7 after 5 years of follow-up^[50].

Acute events-hypoglycemic and diabetes keto-acidosis: CD is associated with mucosal changes that may interfere with the absorption of carbohydrates, even without leading to true malabsorption. An increased risk for symptomatic hypoglycemia was reported in the 6 mo before and after diagnosis of CD^[58]. However, during long-term follow-up and under GFD, no differences were found in the numbers of severe hypoglycemic episodes^[50]. There are no reports of increased risk of DKA episodes in individuals with both T1DM and CD^[50].

Insulin requirements: One study reported significantly lower insulin requirements among patients with T1DM and CD than among those with T1DM alone^[52]; yet the mean insulin requirement increased significantly from 0.88 to 1.1 units/kg per day after 12 mo GFD. In another study, there was no difference in insulin dosage per kilogram per day between patients with both T1DM and CD, mean CD duration of 3 years, and those without CD^[59].

Other autoimmune diseases: Patients with CD are at increased risk for other autoimmune diseases, such as autoimmune thyroid disorders. Thyroid disorders have been reported to be an important risk factor for the development of CD among patients with T1DM^[49,60].

COMPLICATIONS IN PATIENTS WITH T1DM AND CD

Complications may be divided into two main categories, those directly associated with CD and those related to the impact of CD on diabetes.

The long term complications associated with untreated CD include osteoporosis, obstetric complications, and neurologic disorders, as well as enteropathy-associated T-cell lymphoma and adenocarcinoma of the jejunum^[2,6].

Diabetes associated complications in patients with CD

As for long-term complications among patients with T1DM and CD, the data are conflicting: some report that CD increases rates of complications^[56], some show no difference, and others suggest lower incidence of complications^[61]. These discrepancies may be due to differences in duration of undiagnosed CD.

Prevalence of complications in patients with T1DM and newly diagnosed CD

Among adults with T1DM duration of over 20 years, those with newly diagnosed CD had worse glycemic control and a significantly higher prevalence of retinopathy (58.3% vs 25%), nephropathy (41.6% vs 4.2%), and peripheral neuropathy (41.6% vs 16.6%)^[56]. In contrast, Picarelli *et al*^[61] reported significantly lower prevalence of nephropathy and retinopathy among adult T1DM aged about 50 years, with T1DM duration of about 18 years and newly diagnosed CD. The difference between these studies may be due to the unknown duration of undiagnosed CD, and to the difference in HbA1c levels between studies. In the latter, only those with HbA1c levels < 7.5% were included.

The prevalence of complications in patients with T1DM and CD: Long-term follow-up

A lower prevalence of retinopathy was reported in individuals with median durations of T1DM and CD of 27 and 3 years respectively, compared with controls (38.7% vs 67.4%). However, no difference in the prevalence of nephropathy was found between the groups^[59]. The duration of CD was found to be correlated with the risk of diabetic retinopathy. When stratified by time since CD diagnosis, individuals with T1DM and CD were at a lower risk of retinopathy in the first 5 years after CD diagnosis, followed by a neutral risk in years 5 to 10. However, with longer follow-up, coexisting CD was a 2.83 increased risk factor for diabetic retinopathy at 10 to 15 years of follow-up, and a three-fold risk after 15 years of follow-up^[62].

Patients with both T1DM and CD were reported to have more severe subclinical atherosclerosis than those presenting with only T1DM or CD. Among patients with both T1DM and CD, mean age of 39 years, mean T1DM duration of 18 years and CD duration of 8.5 years, carotid intima-media thickness was significantly greater in those with both T1DM and CD than in those with either T1DM or CD, suggesting that the association of these autoimmune diseases might accelerate the atherosclerotic process^[63].

Finally, mortality in patients with both T1DM and CD was studied in 960 individuals aged 30 years, compared with 4608 with T1DM alone, matched for sex, age and disease duration. CD was not a risk factor for death in patients with T1DM during the first 5 years after CD diagnosis, but thereafter the hazard ratio for mortality increased as a function of follow-up time. Having a CD diagnosis for > 15 years was associated with a 2.80-fold increased risk of death in individuals with T1DM^[64].

TREATMENT

The standard therapy for CD is GFD, which requires

avoiding wheat, rye, barley and oats. Patients with CD must follow this strict diet for their entire life. Delay in starting GFD increases the risk of osteoporosis, gastrointestinal malignancies, iron deficiency anemia, infertility, and other autoimmune disorders. Adherence to GFD augments the restrictions required by a diabetic dietary regimen.

GFD impact on glycemic control

Good glycemic control is essential to reduce the risks of T1DM related complications. However, many specially prepared gluten-free foods have high glycemic indices, and thus affect glucose levels, insulin requirements, lipid profiles and body mass indices (BMI). GFD may worsen glycemic control and can thus increase the difficulties of disease management for patients with T1DM and CD^[42]. Numerous studies have evaluated the effect of CD and GFD on the metabolic control of patients with T1DM. Some reported better metabolic control with GFD among CD patients with T1DM^[65,66]. Others did not show any change in HbA1c levels with GFD^[67-72], and some reported worse glycemic control with GFD^[73].

GFD impact on weight and height

Data on weight gain in patients with CD are inconsistent. Some studies report that treatment with GFD promotes a significant catch-up growth while others show no difference. The time of follow-up, age and stage of puberty of patients in different studies may explain the discrepancies. Twelve months after commencement of GFD, one study showed no statistically significant change in the SDS for height, weight and BMI of the 23 children assessed^[74]. In a separate study, children with T1DM and CD had lower SDS for height and weight at CD diagnosis. After 2 years of follow-up, SDS was significantly increased for weight, and for height in prepubertal children^[57].

Adherence to GFD

The compliance rates to GFD among patients with CD and T1DM is less than 60%, compared with about 80% among those with CD only^[75]. The more severe problems of GFD adherence usually occur during adolescence^[44].

QUALITY OF LIFE IN CHILDREN WITH T1DM

Families of children with both CD and T1DM report a higher burden than those affected by T1DM only. Similarly, health care providers perceived family burden to increase over time^[76]. Yet, among children aged 8-18 years, no significant differences in quality of life were observed. However, parents of children with both CD and T1D did express greater concern about their children's social functioning. Adults (mean age 49 years) with both CD and T1DM scored lower in

general health perception, social functioning and role limitation, as a result of physical health and emotional problems. In addition, concerns about diabetes related and social problems were significantly higher in those with both diagnoses^[77].

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Recent progress in the genetics of diabetic microvascular complications

Yi-Cheng Chang, Emily Yun-Chia Chang, Lee-Ming Chuang

Yi-Cheng Chang, Graduate Institute of Medical Genomics and Proteomics, National Taiwan University, Taipei 100, Taiwan

Yi-Cheng Chang, Department of Medicine, National Taiwan University Hospital, HsinChu branch, HsinChu 300, Taiwan

Yi-Cheng Chang, Lee-Ming Chuang, Department of Medicine, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Emily Yun-Chia Chang, Lee-Ming Chuang, Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan

Lee-Ming Chuang, Institute of Preventive Medicine, College of Public Health, National Taiwan University, Taipei 100, Taiwan

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Correspondence to: Dr. Lee-Ming Chuang, MD, PhD, Department of Internal Medicine, National Taiwan University Hospital, 7, Chung Shan S. Rd, Taipei 100, Taiwan. leeming@ntu.edu.tw
 Telephone: +886-2-23123456
 Fax: +886-2-23938859

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Abstract

Diabetic complications including diabetic nephropathy, retinopathy, and neuropathy are as major causes of morbidity and mortality in diabetes individuals worldwide and current therapies are still unsatisfactory. One of the reasons for failure to develop effective treatment is the lack of fundamental understanding for underlying mechanisms. Genetic studies are powerful tools to dissect disease mechanism. The heritability (h^2) was estimated to be 0.3-0.44 for diabetic nephropathy and 0.25-0.50 for diabetic retinopathy respectively. Previous linkage studies for diabetic nephropathy have identified overlapped linkage regions in 1q43-44, 3q21-23, 3q26, 10p12-15, 18q22-23, 19q13, 22q11-12.3 in multiple ethnic groups. Genome-wide association studies (GWAS) of diabetic nephropathy have been conducted in several populations. However, most of the identified risk loci could not be replicated by independent studies with a few exceptions including those in *ELMO1*, *FRMD3*, *CARS*, *MYO16/IRS2*, and *APOL3-MYH9* genes. Functional studies of these genes revealed the involvement of cytoskeleton reorganization (especially non-muscle type myosin), phagocytosis of apoptotic cells, fibroblast migration, insulin signaling, and epithelial clonal expansion in the pathogenesis of diabetic nephropathy. Linkage analyses of diabetic retinopathy overlapped only in 1q36 region and current results from GWAS for diabetic retinopathy are inconsistent. Conclusive results from genetic studies for diabetic neuropathy are lacking. For now, small sample sizes, confounding by population stratification, different phenotype definitions between studies, ethnic-specific associations, the influence of environmental factors, and the possible contribution of rare variants may explain the inconsistencies between studies.

Key words: Microvascular complications; Nephropathy; Retinopathy; Neuropathy; Diabetes

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Core tip: Most risk genetic loci identified by genome-wide association studies (GWAS) for diabetic nephropathy could not be replicated by independent studies with a few exceptions including those in *ELMO1*, *FRMD3*, *CARS*, *MYO16/IRS2*, and *APOL3-MYH9* genes. These findings highlighted the importance of cytoskeleton reorganization, phagocytosis of apoptotic cells, fibroblast migration, insulin signaling, and epithelial clonal expansion in the pathogenesis of diabetic nephropathy. Conclusive results from GWAS for diabetic retinopathy and diabetic neuropathy are currently lacking.

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INTRODUCTION

The prevalence of diabetes mellitus is increasing globally, especially in developing countries^[1]. This surge of diabetes mellitus prevalence poses a serious threat to the public and diabetic complications are ranked as major causes of morbidity and mortality worldwide. Several common mechanisms underlying these microvascular complications including the polyol pathway, advanced glycation end products pathway, protein kinase C pathway, the hexosamine pathway, and cytokines such as nuclear factor- κ B, tumor growth factor- β , and vascular endothelial growth factor are well described and the unifying mechanism of superoxide production have been proposed^[2]. Nevertheless, therapies targeting these pathways have not been very successful^[3-5]. One of the reasons is the lack of fundamental understanding for underlying mechanisms.

Genetic studies provide a powerful tool to the understanding of disease mechanism. Previous family linkage analyses have successfully identified mutations responsible for high-penetrating monogenetic disease. Some discoveries, for example, the identification of *PCSK9* mutation through linkage analyses in hypercholesterolemic families, have resulted in major breakthroughs in therapy^[6,7]. However, family linkage analysis is generally not adequately powered to detect genetic loci of complex disease. Over the last few years, the advent of genome-wide association studies (GWAS) have launched a great leap toward the genetic basis of complex diseases such type 2 diabetes mellitus, cancers, and psychiatric diseases. Intriguingly, many of the identified genetic loci were not previously considered to be related to these diseases and the discoveries indeed illuminated

important pathophysiological pathways to these complex diseases. Diabetic microvascular complications are complex traits influenced by both environmental and genetic factors, and compelling evidences indicate that diabetic microvascular complications are heritable^[8-12]. Here in this review, we only summarized the progress in the genetics for diabetic microvascular complications.

GENETICS STUDIES OF DIABETIC NEPHROPATHY

Linkage studies of diabetic nephropathy

The heritability (h^2) of diabetic nephropathy (DN) defined by reduced glomerular filtration rate (GFR) or albuminuria was estimated to be 0.3-0.44 in multiple Caucasian diabetic populations^[8-10]. Previous linkage studies have repeatedly identified linkage region in 1q43-44, 3q21-23, 3q26, 10p12-15, 18q22-23, 19q13, 22q11-12.3 in multiple ethnic groups (Figure 1, Table 1)^[13-24]. However, these linkage regions usually spanned over megabases and therefore exact locus or risk gene is unclear. In contrast, the resolution of linkage disequilibrium mapping (also called association mapping) is much higher than linkage studies. The distinction between linkage and association mapping is that family linkage mapping use the small amount of recombination events that occurs in each generation within a pedigree to localize a chromosomal region, which usually contains hundreds of genes; while population-based case-control association mapping uses large amount of recombinations that occurred during the evolutionary history of a population to locate the risk loci, which generally did not extend over a few genes. However, population-based case-control association studies are susceptible to the population stratification and independent replication is essential to confirm the result of association studies.

Association studies of DN in type 2 diabetic patients

Several GWAS of DN have been conducted in several ethnic populations (Table 1, Figure 1). *ELMO1* (the engulfment and cell motility 1 gene) was first found to be associated with diabetic nephropathy in a GWAS in Japanese 2 diabetic patients (546 DN cases and 334 type 2 diabetic controls)^[25]. Replication studies in the GoKinD collection (558 DN cases and 820 type 2 diabetes controls)^[26], two African American cohorts [1136 end-stage renal disease (ESRD) diabetes cases and type 2 diabetic 1160 controls]^[27], a Chinese population (123 DN cases and 77 type 2 diabetic controls)^[28], and a Caucasian GWAS (547 ESRD and 549 type 1 diabetic controls)^[29] confirmed this finding although the risk SNPs are not exactly the same with those reported in the original Japanese population (intron 16-20 in original Japanese GWAS, intron 16-20 in GoKinD, intron 13 in African Americans, intron 18 in Chinese). In a large meta-analysis of the GENIE



consortium (including UK-ROI, FinnDiane, and GoKinD US) involving 2966 DN cases and 3399 type 1 diabetic controls, an expanded investigation of the *ELMO1* locus yielded only nominal associations with DN^[30]. Furthermore, another replication studies in Pima Indians of Arizona involving 248 DN cases and 524 diabetic controls found significant association of SNPs in intron 13 but the associations were in the opposite direction from those observed in African Americans^[31], and another study in 455 Mexican-American patients with DN and 437 controls failed to replicate the association^[32].

A GWAS in Pima Indians comparing 105 diabetic ESRD and 103 controls identified plasmacytoma variant translocation (*PVT1*), an lncRNA gene, was associated with DN in type 2 diabetic patients^[33]. Another large GWAS in an initial set of 965 African American type 2 diabetic patients with ESRD and 1029 controls without type 2 diabetes or kidney disease and further replication studies in 1246 type 2 diabetic patients identified *SASH1* (SH3 Domain Containing 1), *RPS12* (ribosomal protein S12), *AUH* (AU RNA binding protein/enoyl-CoA hydratase), *MSRB3* (methionine sulfoxide reductase B3), *LIMK2* (LIM domain kinase 2), *SFI1* (Sfi1 homolog, spindle assembly associated),

Table 1 Genome-wide linkage studies for diabetic nephropathy and retinopathy

Ethnicity and sample size	Type of diabetes	Phenotype definition	Linkage region (LOD score or <i>P</i> -value or MLS)	Ref.
Diabetic nephropathy				
954 African American, 781 American Indians, 614 European American, 1611 Mexican Americans (FIND)	1 + 2	Estimated GFR	10p12.31 ¹ (LOD: 2.16), 1q43 ¹ (2.26), 2q31.3 (1.91), 3p12.1 (2.19), 7q11.22 (2.19), 10p14 ¹ (2.16), 15q12 (2.84), 20q11.11 ¹ (3.34)	[13]
218 African American, 335 American Indians, 119 European American, 469 Mexican Americans (FIND)	1 + 2	Urine ACR	7q21.3 ($P = 8.6 \times 10^{-5}$), 10p15.3 ¹ (1.29×10^{-5}), 14q23.1 (7.8×10^{-4}), 18q22.3 ¹ (2.17×10^{-3})	[14]
3972 Americans (African American, American Indians, European American, Mexican Americans) (FIND)	1 + 2	DN defined by macroalbuminuria or ESRD, ACR	DN: 1q43 ¹ (LOD: 2.00), 6p24.3 (2.84), 7p21.3 (2.81), 10p15.1 ¹ (2.10), 11p15.3 (2.28), 15q21.1 (2.04) ACR: 2q22.3 (2.04), 3p13 (2.76), 7q21.2 (2.96), 16q13 (2.31), 22q12.3 ¹ (2.29)	[23]
882 American (African American, American Indians, European American, Mexican Americans) (FIND)	1 + 2	eGFR	1q43 ¹ (LOD: 1.87), 7q36.1 (4.23), 8q13.3 (2.75), 15q22.3 (2.08), 18q23.3 (1.40)	[24]
100 United States sibling pairs (Joslin Study on Genetics of Diabetic Nephropathy)	1	Proteinuria or ESRD	1q44 ¹ (MLS: 1.6), 2q14.1 (2.1), 3p13 (0.6), 5q14.2 (2.7), 10q26.1 (2.4), 17p13.1 (1.9), 19q13.43 ¹ (3.1), 20p12.1 (1.8)	[15]
63 extended United States families (Joslin Study on Genetics of Diabetic Nephropathy)	2	GFR	2q33.3 (LOD: 4.1), 10q23.31 (3.1), 18p11.22 (2.2)	[19]
556 Finnish, Danish, and French (FinnDiane)	1	Macroalbuminuria or ESRD	3q21-25 ¹ (LOD: 0.76), 6p21 (2.31), 9p21.2, 16p12, 19q13 ¹ (1.61), 22q11 (2.78)	[16]
83 Finnish sibling pairs	1	Macroalbuminuria or ESRD	3q21.3-23 ¹ (MLS: 2.67)	[21]
18 Turkish family + 101 sibling pairs of Pima India	2	Macroalbuminuria	18q22.3-23 ¹ (max LOD: 6.14)	[17]
201 Pima India sibling pairs	2	Macroalbuminuria or ESRD	3q26.1 ¹ (LOD: 1.48), 7q32.3 (2.04), 20p12.3 (1.83)	[18]
206 African American sibling pairs	2	ESRD	3q13.3 ¹ (LOD: 4.55), 7p21.1 (3.59), 18q22.1 ¹ (3.72)	[22]
691 West African	2	GFR	7p12.2 (LOD: 1.84), 16q24.1 (3.56), 17p13.2 (2.08)	[20]
Diabetic retinopathy				
282 Mexican American sibling pairs	2	Non-proliferative DR and proliferative DR	3q12.3 (LOD: 2.41), 12p13.31 (2.47), 20q13.12 (4.47), 6p24.1 (2.28), 15q26.3 (2.53), 19q13.42 (2.21)	[45]
725 Pima Indian sibling pairs	2	Worse eye score	1p36.13 (LOD: 3.1)	[46]
210 Pima Indian sibling pairs	2	Hemorrhage, microaneurysm, and proliferative DR	3q26.31 (LOD: 1.36), 9q22.33 (1.46)	[18]

¹Overlapped region. MLS: Maximum LOD score; DN: Diabetic nephropathy; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; ACR: Albumin-to-creatinine ratio; DR: Diabetic retinopathy.

APOL3 (apolipoprotein L, 3), and *MYH9* (myosin, heavy chain 9, non-muscle) genes as risk loci^[34]. Among them, the association of *MYH9* risk variants has been replicated in another study involving 1963 European Americans diabetic patients^[35]. Compelling evidence demonstrated that *APOL3-MYH9* gene clusters are also associated with non-diabetic nephropathy including focal segmental glomerulosclerosis and hypertensive nephropathy in African American as well as other ethnic populations^[36-38].

Association studies of DN in type 1 diabetic patients

A large GWAS in a initial set of 820 DN cases and 885 type 1 diabetic controls in the GoKinD study and a replication set of 1304 participants in the Diabetes Control and Complication Trial/Epidemiology of Diabetes Control and Complication (EDIC) identified *FRMD3* (FERM domain containing 3), cysteinyl-tRNA synthase (*CARS*), carboxypeptidase, vitellogenic-like (*CPVL*)/chimerin 2, and intergenic region at 13q33.3 between *MYO16* and insulin receptor substrate 2 (*IRS2*) associated with DN^[39]. Interestingly, another genome-wide linkage analysis and regional association fine

mapping in 1007 general Mongolian also identified SNPs in the *FRMD3*, glycine amidinotransferase, and spermatogenesis associated 5-like 1 genes associated with estimated glomerular filtration rate^[40]. A family-based candidate-gene association study involving 798 type 2 diabetic members in the Joslin Study of Genetics of Nephropathy replicated the association of SNPs in the *FRMD3*, *CARS*, and 13q33.3 between *MYO16* and *IRS2* genes^[41]. Another GWAS in 547 Caucasian ESRD cases and 549 type 1 diabetic controls identified *ZMIZ1* (zinc finger, MIZ-type containing 1) gene is associated with DN^[29]. This study also observed significant association of 13q33 variant near the *MYO16/IRS2* genes^[29]. However, in a large replication study of 1535 Japanese type 1 and 2 diabetic patients, only variants in 13q33.3 between *MYO16/IRS2* gene but not those in *FRMD3*, *CPVL/CHN2*, or *CARS* are significantly associated with DN^[42]. Furthermore, a large meta-analysis of the GENIE consortium (UK-ROI, FinnDiane, and GoKinD US) involving 2966 type 1 diabetic cases with DN and 3399 type 1 diabetes controls failed to replicate the association between SNPs in the *FRMD3*, *CARS*, and 13q33 loci near *MYO16*

Table 2 Genome-wide association studies for diabetic nephropathy, retinopathy, and neuropathy

Patients	Ethnic	Case	Control	Gene	Ref.	Replication studies	Non-replication studies
Diabetic nephropathy							
T2DM	Japanese	459 DN	242	<i>ELMO1</i> ¹	[25]	26, 27, 28, 29, 30	31, 32
T2DM	European	105 ESRD	102	<i>PVT1</i>	[33]		
T2DM	African American	965 ESRD	1029	<i>SASH1, RPS12, AUH, MSRB3, LIMK2-SKI1, APOL3-MYH9</i> ¹	[34]	35	
T1DM	Caucasian (GoKinD, DCCT/EDIC)	820 ESRD	885	<i>FRMD3</i> ¹ , <i>CARS, CPVL/CHN2</i> , 13q3 between <i>MYO16/IRS2</i> ¹	[39]	40, 41, 42	42, 30
T1DM	Caucasian	547 ESRD	549	<i>ZMIZ1</i>	[29]		
T1DM	GENIE (UK-ROI, FinnDiane, GoKinUS) + 9 follow-up studies	Stage 1: 4315 ESRD Stage 2: 1880 ESRD	Stage 1: 8568 Stage 2: 6656	<i>AFF3, RGMA/MCTP2, ERBB4</i>	[43]		
T1DM	Caucasian (FinnDiane + 7 follow-up studies)	5675 T1DM Urine albumin excretion rate		<i>PSD3, SH2D4A</i>	[44]		
Diabetic retinopathy							
T1DM	Caucasian (GoKinD and EDIC)	2829 PDR and macular edema	1856	<i>AKT3/ZNF238, LEKR1/CCNL1, KRT18P34/VEPH1, A2BP1</i>	[47]		
T2DM	Taiwanese	174 NPDR and PDR	575	<i>MYSM1, FSTL5, C5orfF21, PLXD2, ARHGAP22, HS6ST3</i>	[48]		
T2DM	Taiwanese	437 PDR	570	<i>TBC1D4-COMMD6-UCHL3, LRP2-BBS5, and ARL4C-SH3BP4</i>	[49]		
T2DM	Mexican-American	103 severe DR	183	<i>CAMK4, FMN1</i> genes	[50]		
Diabetic neuropathy							
United Kingdom	United Kingdom (GoDART)	572 diabetic neuropathic pain	2491	<i>GFRA2</i>	[51]		

¹Loci that could be replicated in independent studies. T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; DN: Diabetic nephropathy; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; DR: Diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy.

and *IRS2* genes^[30].

A recent huge meta-analysis involving 4315 type 1 diabetic nephropathy and ESRD cases and 8568 type 1 diabetic controls of the GENIE consortium and subsequent replication analyses in 9 independent cohorts (1880 cases and 6656 controls) revealed risk SNPs in the *AFF3* (AF4/FMR2 family, member 3) and *ERBB4* (v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4) genes and an intergenic SNP between *RGMA* (repulsive guidance molecule family member a)/*MCTP2* (multiple C2 domains, transmembrane 2) genes^[43]. Another large GWAS for 24-h urine albumin excretion rare in type 1 diabetic patients including an initial set of 1925 patients (FinnDiane) and 3750 additional patients from 7 follow-up studies (Steno Diabetes Center, Italian individuals from the Milano region, Umea Diabetes Study from Sweden, Scania Diabetes Registry, NFS-ORPQ, UK-ROI) identified the strongest signal from the *PSD3* (pleckstrin and Sec7 domain containing 3)/*SH2D4A* (SH2 domain containing 4A) genes^[44].

Collectively, current data from GWAS are not very consistent and only genetic loci in the *ELMO1*, *FRMD3*, *APOL3-MYH9*, *CARS*, and 13q33 between *MYO16* and *IRS2* genes have been successfully replicated in independent studies.

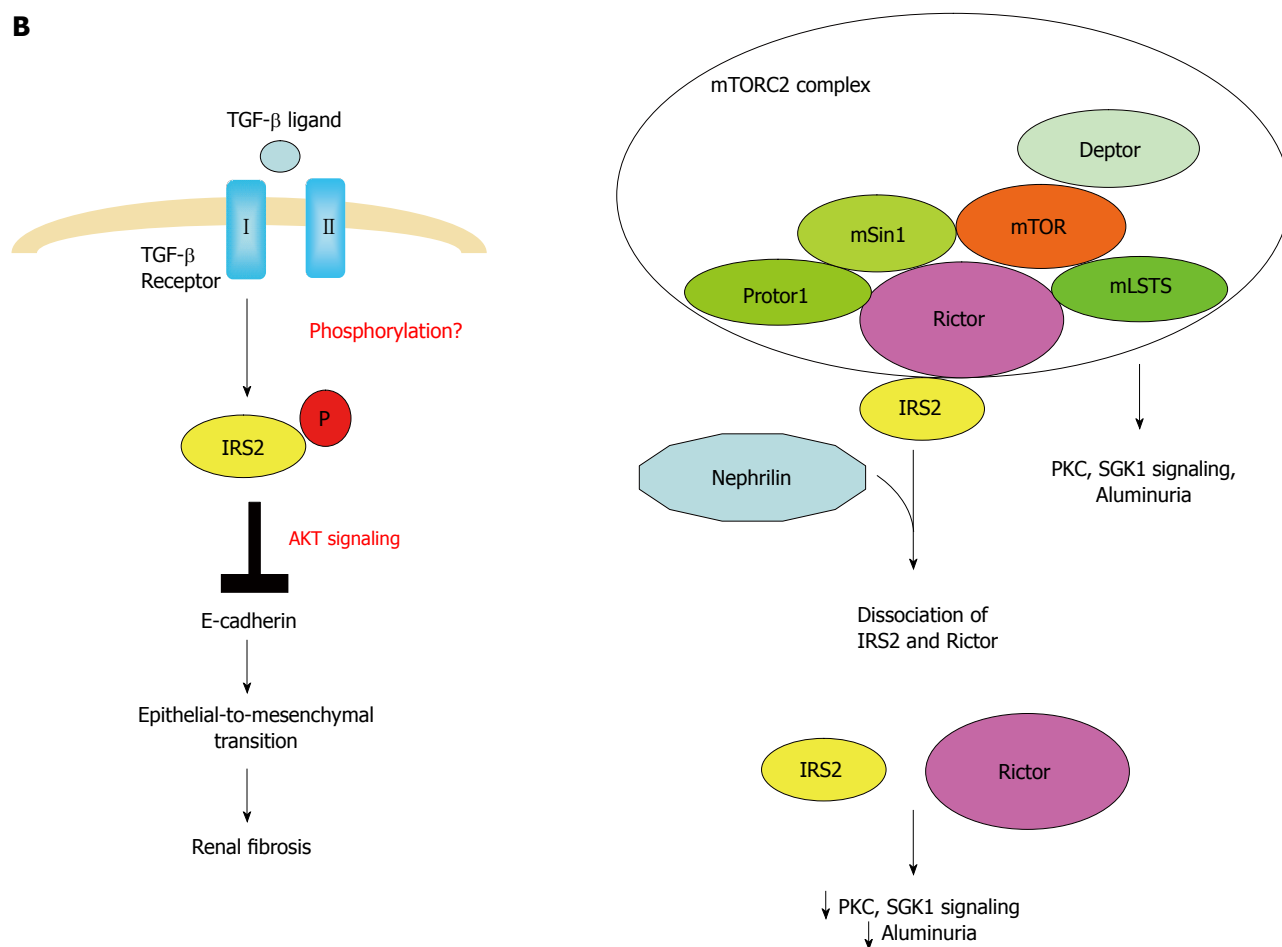
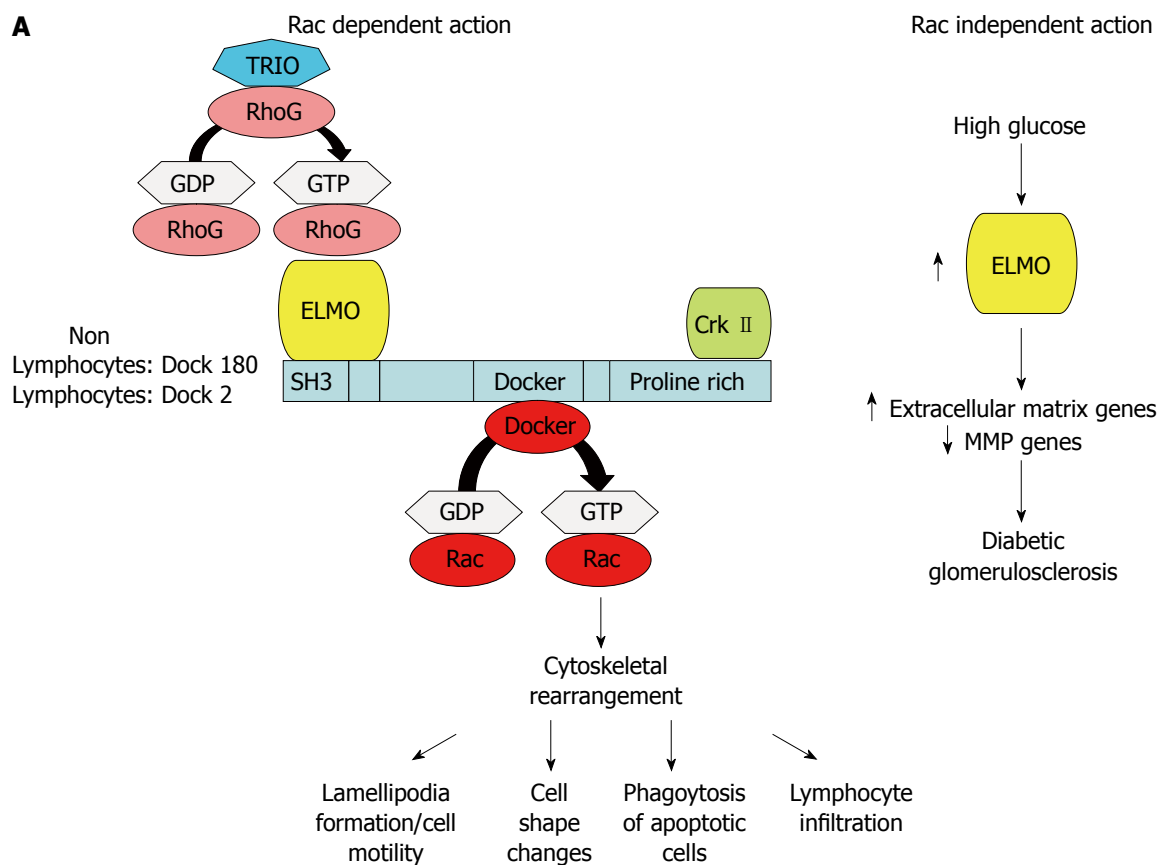
GENETIC STUDIES OF DIABETIC RETINOPATHY

Linkage studies of diabetic retinopathy

The heritability of diabetic proliferative retinopathy is estimated to be 0.25-0.50 in Caucasian populations^[11,12]. Previous results of three family linkage analyses for diabetic retinopathy (DR) are summarized in Table 1 and Figure 1^[18,45,46]. However, the only overlapped region is 1q36 between Pima Indians (LOD: 3.1) and Mexican Americans (LOD: 1.24) studies^[45,46].

Association studies of DR

Four GWAS of DR have been published till now (Table 2, Figure 1). A large meta-analysis of GWAS in the GoKinD and EDIC cohorts involving 2829 cases of severe diabetic retinopathy defined by proliferative retinopathy and macular edema and 1856 type 1 diabetic controls identified several possible loci including intergenic SNPs between *AKT3/ZNF238*, *LEKR1/CCNL1*, *KRT18P34/VEPH1* and SNP in the *A2BP1* genes with *P*-value less than 10⁻⁶^[47]. After excluding cases with concomitant nephropathy to identify DR-specific genes, SNPs in the intergenic region between *LOC728275/LOC728316*, the *CCDC101/NUPR1/SULT1A2/SULT1A1* gene clusters, the *FAM18B*,



C

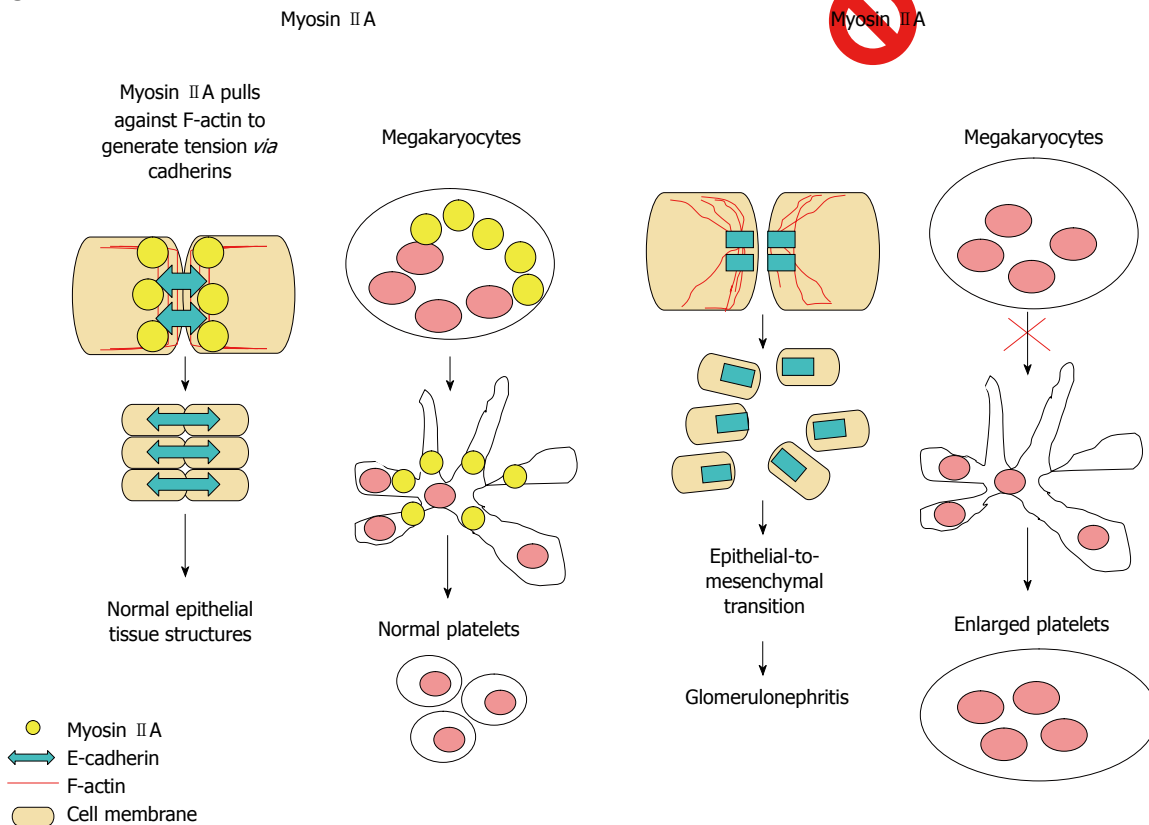


Figure 2 Possible molecular mechanisms. Possible molecular mechanisms by which *ELMO1* (A), *IRS2* (B), and *MYH9* (C) regulate diabetic nephropathy. TRIO: Triple functional domain (PTPRF interacting); RhoG: Ras homolog family member G; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; MMP: Matrix metalloproteinases; Crk II: V-Crk Avian Sarcoma Virus CT10 Oncogene Homolog II; TGF- β : Transforming growth factor beta; AKT: Protein kinase B; mLSTs: Mammalian lethal with SEC13 protein 8; mTOR: Mammalian target of rapamycin; mTORC2: Mammalian target of rapamycin complex 2; mSin1: Mammalian SAPK interacting protein; PKC: Protein kinase C; SGK1: Serum- and glucocorticoid-induced kinase 1.

AKAP11/FABP3P2/TNFSF11 gene cluster, and intergenic region between *COX5BL1/LOC441026*, *ZNRF1*, *PCSK2*, *C10orf112* genes were found to be associated with DR^[47]. A GWAS for DR involving 174 Taiwanese type 2 diabetic non-proliferative and proliferative retinopathy cases and 575 controls identified several genetic loci with *P*-value less than 10^{-6} , including *MYSM1*, *FSTL5*, *C5orf21*, *PLXD2*, *ARHGAP22*, and *HS6ST3*^[48]. Another GWAS in Taiwanese identified three risk loci in *TBC1D4-COMMD6-UCHL3*, *LRP2-BBS5*, and *ARL4C-SH3BP4* genes in the initial set of 437 cases of proliferative retinopathy and 570 type 2 diabetic controls. However, none of them were replicated in another 585 Hispanic diabetics^[49]. A smaller GWAS comparing 103 Mexican-American type 2 diabetics with severe retinopathy and 183 type 2 diabetics identified suggestive signals in the *CAMK4* and *FMN1* genes^[50]. However, the results from these 4 GWAS did not overlap with each other.

GENETIC STUDY OF DIABETIC NEUROPATHY

There was no heritability estimation for diabetic neuropathy in human and no family linkage study for

diabetic neuropathy. Only GWAS comparing 572 diabetic neuropathic pain cases defined by treatment for diabetic neuropathic pain and positive monofilament test and 2491 diabetic controls in the Genetics of Diabetes Audit and Research Tayside (GoDARTS) identified potential signals from *GFRA2* gene^[51] (Table 2, Figure 1).

PHYSIOLOGICAL INSIGHT FROM GENETIC STUDIES

The *ELMO1* gene encode for a signaling molecule involved in phagocytosis of apoptotic cells^[52,53], fibroblast migration^[52,54,55], cytoskeleton reorganization^[56], and lymphocyte infiltration^[57] through interaction with DOCK2 and DOCK180 (Figure 2A). *ELMO1* expression was found to be elevated in cells cultured under high glucose conditions and in the kidney of diabetic mice, but was weakly detectable in tubular and glomerular epithelial cells in normal kidney^[25].

The *FRMD3* gene encodes for a member of the protein 4.1 superfamily. *FRMD3* has been demonstrated to be silenced in lung cancer tissue in genomic screening. *FRMD3* overexpression in different epithelial cell lines decreased clonal expansion, indicating *FRMD3*

as a potential tumor suppressor gene^[58]. The *CARS* encodes for a cysteinyl-tRNA synthetase, which is a frequent gene fusion partner of anaplastic lymphoma kinase found in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor^[59,60]. However, the link between *FRMD3* or *CARS* and diabetic nephropathy is currently poorly understood.

The 13q33 risk loci lie between the *MYO16* and *IRS2* genes. The *MYO16* gene encodes a novel unconventional myosin with divergent tails that is presumed to bind to membranous compartments and interact with actin filaments. *MYO16* has also been shown to be expressed during brain development and regulate neuronal morphogenesis through interaction with protein phosphatase and modulation of phosphoinositide 3-kinase signaling^[61]. A GWAS for autism has identified risk loci within an intergenic region between the *MYO16* and *IRS2* genes^[62]. A genome-wide linkage study and regional fine mapping for schizophrenia^[63] and another GWAS of the Framingham Heart Study for pulse pressure^[64] have identified *MYO16* as risk loci, indicating *MYO16* may play pleiotropic functions.

The *IRS2* gene encodes for an adaptor protein that interacts directly with the insulin receptors and the insulin-like growth factor I receptor and is a key mediator of insulin signaling. *IRS2* was expressed in renal epithelial and tubular cells. Deletion of *Irs2* causes reduced kidney size and reduced glomerular number in mice^[65]. A study of transcriptome and metabolome profiles of the primary cultured inner medullary collecting duct cells grown in hyperosmolar culture medium identified *IRS2* levels to be significantly altered^[66]. *IRS2* expression in kidney tubules has also been shown to be elevated nine fold in human diabetic nephropathy patients^[67]. Transforming growth factor (TGF)- β 1 is the primary cytokine shown to induce fibrosis. *IRS2* has been shown to mediate TGF- β 1 signals in kidney epithelial cells^[68]. *IRS2* has also been shown to interact with nuclear complex of rictor to regulate albuminuria in diabetic mice^[69] (Figure 2B).

Mutations in *MYH9* results in a familial autosomal dominant syndrome characterized by a variety of clinical features, including macrothrombocytopenia, deafness, nephritis, and cataract^[70]. GWAS also identified common *MYH9* polymorphism as risk loci for non-diabetic nephropathy including focal segmental glomerulosclerosis and hypertensive nephropathy^[36,27]. *MYH9* encodes the non-muscle myosin heavy chain 9, which, with other subunits, forms myosin II. Myosin II is a motor protein that binds actin to regulate cellular motility. *MYH9* is expressed in the podocytes, as well as in mesangial cells and arteriolar and peritubular capillaries in kidneys^[71]. Classical deletion of *Myh9* in mice results in embryonic lethality due to loss of cell-cell adhesion and loss of cell movement during gastrulation. Podocyte-specific deletion of *Myh9* in C57BL/6 mice results in susceptibility to experimental doxorubicin hydrochloride glomerulopathy^[71]. Several

strains of *Myh9* knockin mice showed macrothrombocytopenia, premature cataract formation, kidney abnormalities, including albuminuria, focal segmental glomerulosclerosis and progressive kidney disease, and mild hearing loss^[72,73] (Figure 2C).

LIMITATIONS AND PROSPECTIVE

The major limitation of family linkage studies is their low resolution and power to detect variants with small effects, especially for complex genetic diseases. GWAS is a hypothesis-free and unbiased tool with finer resolution and greater power to detect risk loci. However, false positivity often results from population admixture or stratification in GWAS. Therefore, independent replications are essential for genetic association studies. However, current results from GWAS are not consistent since most identified loci are not reproducible except for a few genes such as *ELMO1*, *CARS*, *FRMD3*, *MYO16/IRS2*, and *APOL3/MYH9*. Small sample sizes, different phenotype definitions between studies, population-specific associations, and strong influence of environmental factors (medications, co-morbidities) may explain the failure of GWAS for diabetic complications. While GWAS are usually designed for common variants, rare variants with intermediate effects within should also be pursued with next-generation sequencing. The interaction with environmental factors should also be taken into account.

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Functions of Müller cell-derived vascular endothelial growth factor in diabetic retinopathy

Juan-Juan Wang, Meili Zhu, Yun-Zheng Le

Juan-Juan Wang, Meili Zhu, Yun-Zheng Le, Department of Medicine Endocrinology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States
 Juan-Juan Wang, Department of Ophthalmology, Huizhou Municipal Central Hospital, Huizhou 516000, Guangdong Province, China

Yun-Zheng Le, Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States

Yun-Zheng Le, Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States

Yun-Zheng Le, Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States

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Correspondence to: Yun-Zheng Le, PhD, Department of Medicine Endocrinology, University of Oklahoma Health Sciences Center, 941 S. L. Young Blvd., BSEB 302G, Oklahoma City, OK 73104, United States. yun-le@ouhsc.edu
 Telephone: +1-405-2711087
 Fax: +1-405-2713973

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Abstract

Müller cells are macroglia and play many essential roles as supporting cells in the retina. To respond to pathological changes in diabetic retinopathy (DR), a major complication in the eye of diabetic patients, retinal Müller glia produce a high level of vascular endothelial growth factor (VEGF or VEGF-A). As VEGF is expressed by multiple retinal cell-types and Müller glia comprise only a small portion of cells in the retina, it has been a great challenge to reveal the function of VEGF or other globally expressed proteins produced by Müller cells. With the development of conditional gene targeting tools, it is now possible to dissect the function of Müller cell-derived VEGF *in vivo*. By using conditional gene targeting approach, we demonstrate that Müller glia are a major source of retinal VEGF in diabetic mice and Müller cell-derived VEGF plays a significant role in the alteration of protein expression and peroxynitration, which leads to retinal inflammation, neovascularization, vascular leakage, and vascular lesion, key pathological changes in DR. Therefore, Müller glia are a potential cellular target for the treatment of DR, a leading cause of blindness.

Key words: Müller glia; Vascular endothelial growth factor; Protein modification; Inflammation; Blood-retina barriers; Diabetic retinopathy

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Core tip: Diabetic retinopathy is a disorder of blood-retina barriers (BRBs) and neurons. Anti-vascular endothelial growth factor (VEGF) drugs are explored

for treating BRB breakdown in the disease. As VEGF is also potentially beneficial, it is essential to understand the cellular and molecular mechanisms of VEGF action in the retina. Discussion is centered on the usefulness of conditional gene targeting mice in dissecting the function of globally expressed VEGF and in identifying significant roles for Müller glia-derived VEGF in diabetes-induced changes in protein expression/modification, inflammation, and BRB lesions and leakage.

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INTRODUCTION

Müller cells, the principal macroglia of mammalian retina, span from the vitreal surface to subretinal space and cover all retinal layers. This structural arrangement is ideal for them to serve as major supporting cells in regulating physiological and pathological responses in retinal vasculature and neurons. In the retina, Müller glia play many essential roles in metabolism, functions, maintenance, and protection by providing trophic factors, removing metabolic wastes, controlling extracellular space volumes and ion and water homeostasis, participating visual cycles, releasing neurotransmitters, regulating blood-retina barrier (BRB) function, and modulating innate immunity^[1]. Müller glia is also a major respondent to various stresses by reactive gliosis which involves in morphological, biochemical, and physiological changes. Under some pathological conditions, uncontrollable alteration in growth factor production, such as overexpression of vascular endothelial growth factor (VEGF-A or VEGF) in diabetic retinopathy (DR), results in a detrimental effect and causes vision loss. In this mini-review, we will discuss the general role of VEGF in DR and the function of Müller cell-derived-VEGF (MCD-VEGF) in protein expression/modification, inflammation, BRB function, and vascular and neuronal integrity in diabetic animals.

DIABETIC RETINOPATHY

DR is a leading cause of blindness in working-age people in industrialized countries and is traditionally regarded as a microvascular complication in the eye of diabetic patients due to an apparent breakdown of the endothelial barrier, which is formed by orderly arranged tight junction proteins. The structural interactions between these tight junction proteins control the fluid flow through the barrier^[2]. Patients with endothelial barrier breakdown demonstrate the following key clinical characteristics: retinal

hemorrhages, microaneurysms, cotton-wool spots, lipid exudates, macular edema, capillary occlusion, and retinal neovascularization^[2]. Studies with retinal pigment epithelium (RPE) barrier by others and us suggest that the breakdown of the tight junctions in the RPE barrier may contribute substantially to diabetes-induced blood-content leakage^[3-5], which is responsible for at least some form of macular edema^[6]. Therefore, DR is not just a microvascular disease, but rather a disorder of BRB. Macular edema resulted from BRB breakdown and retinal neovascularization are two most devastating causes of vision loss in diabetic patients. On the other hand, it is increasingly recognized that the loss of retinal neuronal function and viability occurs before the onset of BRB abnormalities in diabetic patients and in experimental animals^[7-11]. Perhaps neuronal and BRB disorder is a more appropriate description for DR.

VEGF IN REGULATING BLOOD-RETINA BARRIER FUNCTION

VEGF-A or VEGF, a heparin-binding homodimeric glycoprotein^[12,13], belongs to a family of seven members, including VEGF-A to -F and placental growth factor and (PlGF). Each of them may also have several isoforms due to alternative splicing, which affects their solubility, and thus is responsible for their cellular localization. The most intensively studied and predominant isoform of VEGF-A in humans is VEGF-A₁₆₅. VEGFs exert their function through complicated receptor- and co-receptor-mediated signaling cascades, which involves VEGF receptor-1 (VEGFR1), VEGFR2, VEGFR3, neuropilin-1, neuropilin-2, vascular endothelial cadherin, and integrin^[14]. Much of the literature information regarding VEGFs in the eye is centered on the pathobiology of VEGF-A due to its high relevance to the pathogenesis of DR, retinopathy of prematurity (ROP), and age-related macular degeneration (AMD), leading causes of blindness.

VEGF is a potent mitogenic factor for endothelial proliferation and migration and tube formation during vessel development and is a major stimulator for embryogenesis, vasculogenesis, and angiogenesis^[13,15]. Disruption of a single VEGF allele is lethal in mice at embryonic day 11-12^[16,17]. VEGF is a major regulator of pathological neovascularization in proliferative DR^[18]. VEGF blockade has been shown to inhibit hypoxia-induced retinal angiogenesis^[19-21]. Due to its potent activity in inducing blood barrier hyperpermeability^[22-25], VEGF is regarded as a major contributor to the high level of blood-content leakage in DR^[26,27]. Overexpression of VEGF or its receptors, which causes disorganization of endothelial and RPE tight junctions^[25,28,29], is associated with diabetic macular edema^[30].

A major regulator for VEGF signaling is oxygen tension and VEGF expression is induced by hypoxia,

a pathological condition occurs after early stages of DR^[31]. While hypoxia-inducible factor-1 (HIF-1) is a critical regulator in this response^[32], its degradation is controlled by von-Hippel-Lindhal (VHL) suppressor protein^[33]. Therefore, HIF-1 (perhaps other HIFs) and VHL are key upstream regulators for VEGF-induced BRB breakdown in DR through VEGFR2^[13,15,34]. Although VEGF signaling may be a major pathogenic mechanism for DR, various growth factors, inflammatory cytokines, and prostaglandins may also affect the disease through VEGF signaling-dependent or independent mechanisms^[35-38]. As a result of intensive effort in VEGF pathobiology and pharmacology, anti-VEGF agents are utilized as a major strategy for the treatment of retinal neovascularization, BRB breakdown, and macula edema in DR.

CONDITIONAL VEGF DISRUPTION IN MÜLLER GLIA

VEGF is produced by several retinal cell-types. Müller glia and RPE cells are thought to produce high levels of VEGF^[39]. Indirect evidences obtained from *in vivo* localization and molecular biology approach with cell cultures suggest a significant role for VEGF in regulating BRB function in DR^[40,41]. However, the importance of MCD-VEGF in DR remained unclear for a long time after the initial discovery of VEGF as a potential pathogenic factor in DR. A major reason for this is that Müller glia only comprise a small portion of retinal cells and VEGF is expressed by multiple cell-types, which makes it difficult to detect Müller cell-specific VEGF expression by immunohistochemistry (IHC) or immunoblotting (IB). Additionally, more and more “new” VEGF functions have been identified since the discovery of its involvement in BRB function in the mid-1990s^[27,42]. While other retinal cells may not produce a high level of VEGF at a given time, it is almost impossible to pinpoint the local effect of VEGF produced by these cells, if VEGF action is blocked globally by genetic, immunological, biochemical, or pharmacological approaches. Therefore, cell-specific approach may be the “only” way to delineate the precise function of Müller cell-derived VEGF (MCD-VEGF). As Müller glia play such a critical role in general health and functions of the retina, a better understanding of their biology is paramount to the prevention and treatment of retinal diseases^[43]. For this purpose, several laboratories developed cell-specific genetic tools for Müller glia^[44], which is very helpful for dissecting the specific function of globally expressed proteins, such as VEGF, in Müller cells.

In a serendipity fashion while developing inducible Cre/*lox* system for the RPE using the promoter of human vitelliform macular dystrophy-2 (*Vmd2*) gene^[45,46], we identified one transgenic founder mouse that was capable of carrying out productive Cre-mediated excise recombination in Müller glia. This transgenic Cre-drive line provides an opportunity to generate conditional VEGF

Table 1 Alteration in protein expression/modification in diabetic or hypoxic Müller cell-specific KO mice

Model/time	Proteins/modification	Alteration
STZ-induced diabetes/6 mo	Albumin	Decrease (59%)
STZ-induced diabetes/2 mo	ICAM1	Decrease (62%)
STZ-induced diabetes/2 mo	Nitrotyrosine	Decrease (19%)
STZ-induced diabetes/6 mo	Occludin	Increase (60%)
STZ-induced diabetes/2 mo	pNF-κB p65	Decrease (48%)
STZ-induced diabetes/2 mo	TNFα	Decrease (53%)
STZ-induced diabetes/6 mo	VEGF	Decrease (51%)
STZ-induced diabetes/6 mo	ZO1	Increase (130%)
Oxygen-induced retinopathy	Albumin	Decrease (56%)
Oxygen-induced retinopathy	VEGF	Decrease (45%)
Oxygen-induced retinopathy	Occludin	Increase (35%)

STZ: Streptozotocin; VEGF: Vascular endothelial growth factor; ICAM1: Intercellular adhesion molecule-1; TNFα: Tumor necrosis factor-α; NF: Nuclear factor; ZO1: Zonula occludens-1.

knockout (CVKO) mice that disrupt VEGF expression mainly in Müller glia. The CVKO mice were generated by breeding this Müller glial Cre-drive line with a mouse line carrying *loxP*-flanked VEGF gene (*VEGF^{ff}*), called floxed VEGF mice^[45,47,48]. The degree of VEGF disruption in the Müller glia was assessed by IB with primary Müller cell cultures derived from the CVKO mice, which reduced VEGF production by 66%. This degree of VEGF knockout (KO) caused a near 50% reduction of total retinal VEGF in CVKO mice under normal conditions^[48,49]. To ascertain whether the production of MCD-VEGF was substantially decreased in CVKO mice in disease models^[49], we examined VEGF expression in diabetic and hypoxic models (see detail below). While diabetes and hypoxia doubled retinal VEGF in WT mice, the deletion of MCD-VEGF caused a near 50% decrease of VEGF overexpression in the retina under hypoxic or diabetic conditions (Table 1). These data were corroborated by IHC with CVKO mice^[48,49]. Considering the fact that Müller cells only comprise a small portion of retinal cells, our data undisputedly suggest that Müller glia are a major cellular origin of VEGF in mouse retinas.

MÜLLER CELL-DERIVED VEGF IN PROTEIN EXPRESSION/MODIFICATION AND RETINAL INFLAMMATION

To determine whether deletion of MCD-VEGF resulted in any significant changes in DR-associated gene expression, we performed IB analysis with retinal extracts from CVKO mice after diabetes was induced with streptozotocin (STZ). HIF1α is a major parameter for oxygen tension and the induction of HIF1α contributes to the increase in VEGF^[50]. To delineate the regulatory mechanisms between HIF1α and VEGF, we examined the expression level of HIF1α in hypoxic and diabetic CVKO mice. Although HIF1α was up-regulated significantly in hypoxic retinas at P14 (see detail below) and diabetic retinas (two month post-STZ injection) in WT controls, there was no apparent

Table 2 Pathological changes in diabetic or hypoxic Müller cell-specific KO mice

Model	Pathological changes	Alteration
STZ-induced diabetes	Leukocytosis	Decrease (75%)
STZ-induced diabetes	Vascular leakage	Decrease (60%)
STZ-induced diabetes	Acellular capillaries	Decrease (45%)
STZ-induced diabetes	Vascular leakage	Decrease (60.0%)
Oxygen-induced retinopathy	Pre-retinal neovascularization	Decrease (34%)
Oxygen-induced retinopathy	Neovascularization area	Decrease (40%)
Oxygen-induced retinopathy	Vaso-oblivation area	Decrease (30%)

STZ: Streptozotocin.

difference in the levels of retinal HIF1 α between the CVKO mice and WT controls under hypoxic and diabetic conditions^[48,49], suggesting that hypoxia/HIF1 α is an upstream regulator of VEGF produced by Müller glia.

Nuclear factor-kappa-B (NF- κ B) is a transcription factor and a major player in inducing early pathological changes, such as inflammation, in DR^[51,52]. To explore if MCD-VEGF regulated NF- κ B in diabetic retina, we examined the expression/phosphorylation of NF- κ B p65 subunit. While there was no detectable change in the total NF- κ B p65 level between the CVKO mice and WT controls, the loss of MCD-VEGF caused a dramatic decrease (48%) of phosphorylated (activated) NF- κ B p65 in the retina 2 mo after STZ-injection (Table 1). This result suggests that activated p65 is downstream of MCD-VEGF in DR^[49]. Nitric oxide (NO) is an important inflammatory mediator and its level is a representation of oxidative stress in the retina of diabetic patients and animals^[53-55]. Peroxynitrite is a highly reactive oxidant, which is formed by the rapid combination of NO with superoxide. Increased peroxynitrite formation may be directly linked to diabetes-induced VEGF overexpression and there is a possible loop effect of VEGF signaling and peroxynitrite formation^[56,57]. To determine the role of MCD-VEGF in protein nitration, we examined the level of nitrotyrosine, a biomarker of peroxynitrite, in retinal protein extracts from CVKO and control mice 2 mo after STZ-injection. The retinal extracts from diabetic CVKO mice demonstrated a 19% decrease of proteins with nitrotyrosine (Table 1), indicating that the disruption of MCD-VEGF reduced oxidative stress.

Inflammation is an early pathological response in DR. To identify the role of MCD-VEGF in retinal inflammation in DR, we examined the levels of pro-inflammatory markers in CVKO mice by IB analysis for intercellular adhesion molecule-1 (ICAM1) and tumor necrosis factor- α (TNF α), 2 mo after STZ-injection. Compared with controls, the CVKO mice showed 62.3% and 52.9% reduction of ICAM1 and TNF α (Table 1), respectively. These results suggest that the deletion of MCD-VEGF substantially inhibits inflammation in diabetic retinas^[49]. This notion is reinforced by the result from the leukostasis assay

showing a 75.0% reduction of adherent leukocytes, a cardinal feature of retinal inflammation in DR, in the retinal microvasculature of the CVKO mice 2 mo after STZ injection (Table 2). Collectively, these data point to a major role for MCD-VEGF in developing inflammation in DR.

MÜLLER CELL-DERIVED VEGF IN BRB FUNCTION AND INTEGRITY

As diabetic rodents usually do not develop retinal neovascularization^[58], the closest way to investigate this is to utilize oxygen induced retinopathy (OIR), a model mimicking an infant blinding disease, ROP. To examine the effect of MCD-VEGF on BRB function, the severity of retinal neovascularization was examined in CVKO mice subjected to OIR. The CVKO mice were placed in hyperoxia (75% oxygen) from postnatal day 7 (P7) to P12 and were then kept under normoxia for up to 5 d. In OIR model, the retina was relatively hypoxic at P14 (compared with that at P7-12), as judged by the abundance of HIF1 α . The level of retinal VEGF in hypoxic CVKO mice was decreased by 45% (Table 1). At P17, fluorescein angiography was performed and OIR-treated CVKO mice demonstrated 40% and 30% reductions in areas of retinal neovascularization and of vaso-oblivation, respectively (Table 2). Retinal sections from OIR-treated CVKO mice also showed a 34% reduction in the number of pre-retinal neovascular endothelia (Table 2). As a consequence of decreasing retinal neovascularization and unhealthy microvasculature, we observed a 56% reduction of OIR-induced vascular leakage in CVKO mice (Table 1), judged by IB for albumin. This observation was supported by IHC data^[48].

IB analysis with retinal and vitreous extracts prepared from PBS-perfused diabetic WT mice demonstrated a 1.5-fold increase in extravascular albumin 6 mo after STZ-injection. However, the disruption of MCD-VEGF caused a near 60% reduction of albumin leakage in age-matched diabetic mice (Table 1). Such a reduction can be visualized in the retinal flat-mounts of diabetic CVKO mice by intravenously injected fluorescein isothiocyanate-labeled albumin^[49].

To delineate the mechanistic insights of MCD-VEGF in diabetes-induced vascular leakage, we analyzed the levels of occludin and Zonula occludens-1 (ZO1), two major tight junction proteins, in the retina^[49]. While diabetes resulted in 39% and 58% decreases of occludin and ZO1, respectively, in WT animals, no alteration in occludin and ZO1 expression was observed in diabetic CVKO mice. As a result, the diabetic CVKO mice had 60% and 130% upregulation of occludin and ZO1 (Table 1), compared with that of diabetic WT controls. Our data indicated that the disruption of MCD-VEGF resulted in a significant reduction of diabetes-induced retinal vascular leakage by attenuating the depletion of occludin and ZO1. This result was supported by a 36% increase in the level

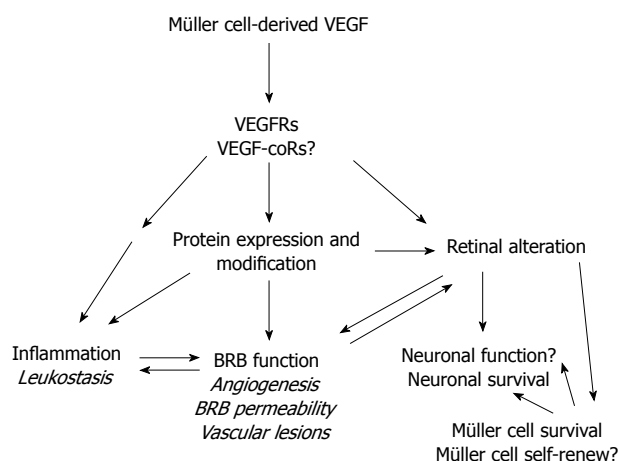


Figure 1 A simplified schematic diagram for the potential roles of Müller cell-derived-vascular endothelial growth factor in diabetic retinopathy. Alteration in pathological characteristics caused by MCD-VEGF is indicated in italic. Potential functions without direct proof by experimental and clinical data are indicated with a question marker. MCD-VEGF plays a significant role in causing retinal inflammation, neovascularization, vascular leakage, vascular lesion, and protein alteration and modification in the pathogenesis of DR. MCD-VEGF: Müller cell-derived-VEGF; DR: Diabetic retinopathy; VEGF: Vascular endothelial growth factor.

of occludin in the retina (Table 1) and by qualitative evidence from IHC in hypoxic CVKO mice generated with OIR^[48]. Collectively, our data suggest that MCD-VEGF was a major inducer of vascular leakage in OIR-treated hypoxic mice and in diabetic mice through VEGF signaling-induced decrease of tight junction integrity.

To assess retinal vascular lesions in diabetic CVKO mice, the number of acellular capillaries in trypsin digested retinal flat-mounts was examined 6 mo after diabetes was induced^[49]. The diabetic CVKO mice had 45% fewer acellular capillaries than that in controls (Table 2), suggesting that the loss of MCD-VEGF had a protective effect on retinal microvasculatures, which reduced vascular leakage through the endothelial barrier in DR.

MÜLLER CELL-DERIVED VEGF IN RETINAL DEVELOPMENT AND INTEGRITY

Disruption of MCD-VEGF in the CVKO mice did not affect the development of retinal and choroidal vasculatures and overall retina^[48], as determined by morphological analysis in retinal sections with light microscopy, functional test with electroretinography, and retinal and choroidal vascular density and morphological examination in retinal and RPE/choroidal flat-mounts with IHC and fluorescein angiography. Although negative results from conditional gene KO studies are not conclusive, our observation is somewhat expected as Müller glia are one of the last few cell-types to develop and KO of VEGF in neural retina during embryonic development results in abnormal retinal vessels^[59]. In our study, the loss of MCD-VEGF did not

affect retinal integrity in the aging CVKO mice under normal and diabetic mice^[49]. This result is seemingly contradictory to the observation that VEGF is a survival factor for retinal ganglion cells, photoreceptors, and Müller glia^[60,61]. The following may account for the “discrepancy”: the disruption of MCD-VEGF in the CVKO mice did not remove VEGF completely as several types of retinal cells produce permeable VEGF and a basal level of VEGF may be sufficient for physiological VEGF function, such as neuronal function and integrity in the retina. In addition, our studies did not blocked VEGF signaling in any retinal cells. However, the result that MCD-VEGF had no apparent role in retinal development and integrity provides an advantage to investigating the role of MCD-VEGF in CVKO mice. Since there was no distinguishable defect in the animals under normal conditions, any phenotypical difference between the CVKO and control mice can be attributed to the defects from deleting MCD-VEGF.

CONCLUSION

Work from others and our laboratories demonstrated a major role for MCD-VEGF in DR, as summarized in Figure 1. Our data clearly pinpoint that MCD-VEGF plays a major role in protein alteration/modification, inflammation, neovascularization, vascular leakage, and vascular lesion in DR. Our study also suggests that MCD-VEGF may be a downstream regulator of DR-related master regulator, such as HIF1 α /hypoxia, but upstream regulator for others, such as NF- κ B and peroxynitrite. We also need to keep in mind that DR is a multifactorial disease. Other growth factors and pro-inflammatory mediators may be involved in developing DR in a VEGF-independent manner. A better understanding of VEGF-dependent and -independent pathways is the key to new therapeutic strategies for intervening multiple drug targets simultaneously, since anti-VEGF strategy alone cannot prevent DR completely. The CVKO mice provide an excellent animal model in this new endeavor.

VEGF is a neural protectant and it has been shown to modulate neuronal function in the brain^[62]. Potentially, MCD-VEGF may also be involved in regulating neuronal integrity. With the development of tools in studying Müller glia and their relationship with neuronal survival in diabetes and hypoxia^[44,63,64], the role of MCD-VEGF in neuroprotection in the retina should be sorted out shortly. Current literature suggests that MCD-VEGF may have a critical role in maintaining Müller glia through autocrine in hypoxia and diabetes. However, it is not clear whether MCD-VEGF acts solely as trophic factor in the maintenance of Müller glia. Other mechanism, such as proliferation (Müller cell self-renew), may also be potentially important to the maintenance of retinal integrity. In principles, mammalian Müller cells are capable of dedifferentiation, proliferation, and differentiation into various retinal neurons under various conditions and

are considered as a major retinal stem cell^[65]. It will be fascinating if MCD-VEGF can act similarly as other growth factors in differentiating Müller cells to neurons for neuro-protection^[66].

Although there are many publications on VEGF or MCD-VEGF in DR, little is known about its actually signaling pathways. As discussed earlier, the presence of at least seven VEGF receptors and co-receptors accounts for the difficulties in revealing their mechanisms. Additionally, VEGF is a secreted protein and loss of VEGF produced by a single cell-type can be compensated by others. Delineating detailed mechanisms may greatly enhance our understanding to the pathogenesis, treatment, and neuronal function in DR, which is critical to the improvement and safety of current anti-VEGF strategy and to the design of new treatments for the disease.

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What neonatal complications should the pediatrician be aware of in case of maternal gestational diabetes?

Delphine Mitanchez, Catherine Yzydorczyk, Umberto Simeoni

Delphine Mitanchez, Division of Neonatology, Department of Perinatology, Armand Trousseau Hospital, 75012 Paris, France
 Catherine Yzydorczyk, Umberto Simeoni, Division of Pediatrics and DOHaD Laboratory, CHUV University Hospital and UNIL, 1011 Lausanne, Switzerland

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Correspondence to: Delphine Mitanchez, MD, PhD, Division of Neonatology, Department of Perinatology, Armand Trousseau Hospital, 26 Avenue du Docteur Arnold Netter, 75012 Paris, France. delphine.mitanchez@trs.aphp.fr
 Telephone: +33-1-44736191
 Fax: +33-1-44736892

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Abstract

In the epidemiologic context of maternal obesity and type 2 diabetes (T2D), the incidence of gestational diabetes has significantly increased in the last decades. Infants of diabetic mothers are prone to various

neonatal adverse outcomes, including metabolic and hematologic disorders, respiratory distress, cardiac disorders and neurologic impairment due to perinatal asphyxia and birth traumas, among others. Macrosomia is the most constant consequence of diabetes and its severity is mainly influenced by maternal blood glucose level. Neonatal hypoglycemia is the main metabolic disorder that should be prevented as soon as possible after birth. The severity of macrosomia and the maternal health condition have a strong impact on the frequency and the severity of adverse neonatal outcomes. Pregestational T2D and maternal obesity significantly increase the risk of perinatal death and birth defects. The high incidence of maternal hyperglycemia in developing countries, associated with the scarcity of maternal and neonatal care, seriously increase the burden of neonatal complications in these countries.

Key words: Birth defects; Hypoglycemia; Respiratory distress; Preterm; Perinatal mortality; Type 2 diabetes; Obesity

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Core tip: Increased mortality and morbidity are historically attributed to neonates of diabetic mothers. A discerning analysis of the literature shows that these adverse outcomes are uncommon among infants born from "pure" gestational diabetes mellitus (GDM) mothers, well managed during pregnancy. Macrosomia is the predominant adverse outcome and the main factor linked to neonatal complications. Poor maternal glycemic control, especially in the context of maternal type 2 diabetes and obesity increases the risk of all adverse neonatal outcomes, most strikingly the risk of perinatal mortality and birth defects. Developing strategies for screening and managing women with GDM must be encouraged notably in middle and low income countries and, also to limit the adverse effects on global health population in the future.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of any degree with onset or first recognition during pregnancy. In high income countries, but also in middle and low income countries, because of the spreading of industrialized lifestyle, the incidence of obesity and type 2 diabetes (T2D) has dramatically increased, and subsequently the incidence of GDM^[1].

In high-resource countries, progress has been made during the past fifty years regarding preconceptional care, screening and management of GDM. However, in low and middle-income countries, quality of antenatal care to detect and manage GDM, are often poorly available. As a consequence, the prenatal and neonatal burden of GDM may be paradoxically higher in these countries, although this point is not well documented^[2].

Much of the currently available knowledge on the consequences of maternal diabetes on the offspring has been provided by studies on type 1 diabetes (T1D), while the risks related to GDM, which is much more frequent, need to be clarified in order to improve and to adapt neonatal management^[3]. Moreover, extensive data suggest that the offspring of diabetic mothers is furthermore exposed to an increased risk of developing chronic, non-communicable diseases at adulthood^[4].

Neonatologists are facing first-line this new epidemiologic setting. This review addresses the currently available knowledge on short term consequences of GDM in neonates and focuses on situations with increased risks of neonatal adverse outcomes.

SHORT TERM OUTCOMES

Macrosomia

Macrosomia is the most constant complication in GDM. The concept of excessive fetal growth is expressed either by the word "macrosomia" or by the expression "large for gestational age" (LGA). Macrosomia is defined by a birth weight (BW) of 4000 or 4500 g and more, depending on the authors. However, in this definition, gestational age (GA) is not taken into account. The term LGA corresponds to a BW \geq 90th percentile or $> +2SD$ ($> 97^{\text{th}}$ percentile) for GA. This definition allows premature newborns with excessive fetal growth to be identified. Macrosomia in newborns of diabetic mothers is characterized by excess body fat, an increased muscle mass and organomegaly, without increase in brain size.

The Pedersen-Freinkel's hypothesis, expressed sixty years ago, suggested that fetal overgrowth is related to increased transplacental transfer of maternal glucose, which stimulates the release of insulin by fetal pancreatic beta cells^[5]. Insulin is a major factor of fetal growth and it up-regulates the Insulin-like Growth Factor (IGF) system, subsequently leading to fetal macrosomia. According to this hypothesis, different studies have characterized the link between maternal glycemia and neonatal macrosomia or fat mass^[6,7].

The HAPO study showed a continuous, positive association between maternal glycemia, fetal hyperinsulinism and BW^[8]. A linear and continuous relationship between body fat percentage in newborns, maternal glycaemia and fetal insulin levels has been found in this study^[9]. More recently, other mechanisms that may also contribute to fetal overgrowth were evoked, like maternal metabolic environment and placental modifications^[10]. In particular, maternal lipids availability and transport to the fetus may be enhanced in case of maternal diabetes^[11].

Hence, all types of maternal diabetes are risk factors for macrosomia. As discussed below, macrosomia is *per se* a cause increased neonatal adverse outcome and this point emphasizes the importance of recognizing the excess of growth, even in preterm infants. Treatment of GDM significantly reduces the rate of macrosomia^[12,13].

Preterm birth

A number of studies have reported an increased risk of preterm births in case of diabetes. However, data are not always available on the respective proportion of induced and spontaneous births, considering the increased maternal and fetal morbidity of diabetes during pregnancy. The benefits of early delivery to avoid fetal death or shoulder dystocia must be balanced against the morbidity linked to preterm birth, especially the respiratory morbidity.

The link between GDM and spontaneous preterm birth is still controversial. Hedderson *et al.*^[14] showed in a large cohort study that GDM was an independent risk factor for spontaneous preterm birth (RR = 1.42, 95%CI: 1.15-1.77). On the other hand, Yogeve *et al.*^[15] found that the rate of spontaneous preterm delivery was not increased in GDM compared to non-GDM patients. Nevertheless, both studies found a relationship between higher glucose values in the oral glucose tolerance test (OGTT) or higher mean blood glucose levels and preterm birth.

Metabolic disorders

Hypoglycemia: The link between macrosomia, increased cord C-peptide levels that reflects fetal insulin secretion, and neonatal hypoglycemia has long been known. The data collected by the HAPO study confirmed this relationship: neonatal hypoglycemia was strongly associated with elevated cord serum C-peptide levels^[16]. The infant of a diabetic mother is

at risk of transient hyperinsulinism, which prevents at birth the normal activation of metabolic pathways producing glucose and ketone bodies, and causes increased glucose consumption by tissues^[17].

The exact incidence of hypoglycemia in case of maternal diabetes is difficult to assess due to the various definitions used for neonatal hypoglycemia in the literature. The rate of intravenously treated hypoglycemia was reported between 5% to 7% in two large studies^[18,19]. Comparisons with the risk observed in healthy newborns are difficult also because monitoring of blood glucose at birth was different according to the mother was diabetic or not in most of the studies. At last, in many studies, blood glucose level in neonates is checked soon after birth, although the pathologic significance of low blood glucose levels immediately after birth, in the absence of specific symptoms, is still questioned. Indeed, an immediate fall in blood glucose concentration is observed after birth because of the interruption of placental supply, reaching a nadir between 1 and 2 h in healthy term infants^[20]. Normal levels at this period cannot be distinguished from abnormal ones in asymptomatic infants and the incidence of hypoglycemia is likely to be overestimated^[21]. From 3 h of age, blood glucose then rises spontaneously, even in the absence of any nutritional intake, due to the activation of metabolic regulatory pathways. Therefore, in the absence of abnormal clinical signs, the first blood glucose measurement is recommended after the second feed, which generally allows infants who cannot manage adequate early glucose homeostasis to be identified^[21].

There is currently no consensus on the indications for systematic glucose blood monitoring in asymptomatic infants born to diabetic mothers. It seems reasonable to consider that LGA or growth restricted infants (< 10th percentile) born to diabetic mother may benefit from blood glucose concentration check at 3 to 6 h intervals during the first day of life. On the other hand, normal-grown infants of mothers with diet-controlled GDM should not be monitored^[22].

For newborns with no clinical signs, therapeutic intervention can be considered starting at a threshold value of 0.36 g/L (2.0 mmol/L). Early and frequent breastfeeding remains the key in preventing hypoglycemia, whatever the infant's BW, as far as he/she is able to feed autonomously. Therefore, infants of diabetic mothers should be kept aside their mother, in the absence of significant complications requiring a transfer to a special care neonatal unit. Even in mildly or moderately symptomatic infants with low blood glucose levels, sustained breastfeeding, or eventually formula supplements should be tried first, provided a satisfactory clinical response is obtained^[22]. In case the infant is unable to feed, an IV glucose supplementation (3-6 mg/kg per hour) should be provided at constant rate of infusion, in order to avoid rebound hypoglycemia.

Hypocalcemia: Hypocalcemia can be defined by

plasma calcium concentration below 2 mmol/L or ionized calcium concentration below 1.1 mmol/L, regardless of GA or BW. Transient neonatal hypocalcemia has been mainly reported in neonates of pre-gestational insulin dependent- diabetic mothers and may be partly related to maternal hypomagnesemia and subsequent fetal hypomagnesemia. The severity of hypocalcemia also appeared to be related to the severity of maternal diabetes, as calcium concentration in the neonates was negatively related to maternal HbA1c levels^[23].

It seems that hypocalcemia is rarely of clinical significance, particularly in case of GDM, unless other complications are associated^[24].

The mechanism is still unclear but seems to involve an abnormal calcium phosphorus metabolism during pregnancy with a decrease in calcium and vitamin D concentrations especially during the third trimester. Some studies have reported an association between GDM and low maternal vitamin D status, particularly with poor blood glucose control. Conversely, there are growing evidences that women who develop GDM are more likely to be vitamin D deficient^[25]. Other factors like prematurity and perinatal asphyxia can contribute to low calcium levels^[26].

Therefore, there is no indication to screen healthy baby for hypocalcemia and hypomagnesemia. When treatment is indicated, it consists to give oral vitamin D supplements and calcium gluconate orally or intravenously (40-60 mg/kg per day) and magnesium treatment according to plasma level.

Hyperbilirubinemia: Hyperbilirubinemia is more frequently observed in infants born to diabetic mothers. It is not a serious complication if non-toxic levels are diagnosed and treated, which is usually the case. The risk of nuclear icterus, the severe form of hyperbilirubinemia, is not reported in cases of diabetes as being more frequent. In the HAPO study, hyperbilirubinemia was weakly associated with maternal blood glucose levels^[8]. Polycythemia could be one of the reasons, but additional mechanisms, such as preterm birth, poor liver conjugation are likely to be involved.

Hematologic disorders

It has been reported that infants of diabetic mothers may have polycythemia [hematocrit (Ht) higher than 65%]. Mechanisms evoked are reduced transplacental oxygen transport to the fetus and increased fetal oxygen consumption due to fetal hyperinsulinism. This may lead to fetal hypoxia and increased levels of fetal erythropoietin. However, no consistent correlation between plasma erythropoietin level and polycythemia has been reported in human. Increased insulin and IGFs levels can also increase red blood cells production. A strong positive correlation between maternal β -hydroxybutyrate levels and polycythemia was observed in a small observational study^[27].

Normovolemic polycythemia seen in infants from diabetic mother can lead to hyperviscosity. Early symptoms are unspecific, feeding problems, plethoric aspect, acro-cyanosis, lethargy, hypotonia, respiratory distress, jitteriness and irritability, seizure (due to multiple cerebral infarcts), necrotizing enterocolitis, hyperbilirubinaemia and hypoglycemia have all been found associated. Polycythemia may also favor deep vessels thrombosis. Hypoglycemia may be aggravated in infants from diabetic mothers in case of polycythemia, due to increased glucose consumption by the increased red cell mass. Partial exchange transfusion with saline solution should be performed in symptomatic infants according to the formula (volume exchanged in mL): $(Ht-55) \times \text{weight (kg)} \times 80/Ht$.

Respiratory disorders

The rate and the risk of respiratory distress syndrome (RDS) in cases of GDM cannot be accurately established, due to insufficient precise data^[28]. In a recent study from the French birth cohort in 2011, including 474 614 births, the risk for neonatal respiratory disorders was slightly but significantly increased in case of GDM [OR adjusted on mother's age and gestational age, 1.2 (1.1-1.3)] (personal data not yet published).

It is generally recognized that, besides RDS, infants born to diabetic mothers are exposed to increased risk of transient tachypnea of the newborn. This is more likely to happen after caesarian section due to delayed reduction of alveolar fluid at birth and when the infants have macrosomia. This was clearly showed in infants of T1D mothers^[29].

Diabetes, but also maternal body mass index (BMI), is associated with a higher risk of persistent pulmonary hypertension (PPH). However, other independent risk factors like macrosomia and caesarean deliveries might be in the causal pathway between diabetes, overweight and PPH^[30].

Cardiac disorders

Hypertrophic cardiomyopathy: Fetuses exposed to maternal hyperglycemia and hyperinsulinism, are prone to develop hypertrophic cardiomyopathy. It primarily affects the interventricular septum, but can extend to the myocardium in more severe cases^[31].

Myocardial hypertrophy has been reported in both pregestational diabetes and GDM with a wide range of frequencies (between 25% to 75% of infants born to diabetic mothers)^[32,33]. The incidence was lower in case of pure GDM comparing to pregestational diabetes^[34]. The most recent studies showed that good maternal glycemic control does not entirely prevent interventricular septum hypertrophy and minor fetal cardiac function impairment, regardless of the type of diabetes^[35,36]. Although myocardial hypertrophy is associated with an overall decrease in ventricular compliance and an increase in contractility of the left and right ventricles, it is most often asymptomatic. It can sometimes lead to severe morbidity and mortality,

according to the severity and the extension of cardiac hypertrophy. Major septal hypertrophy can lead to subaortic stenosis and secondary mitral insufficiency. It is usually considered that heart hypertrophy resolves anatomically within few months. However, the long term effect of diabetic cardiomyopathy on heart function remains to be elucidated.

Cardiac malformations: Some data supports that GDM carries a small but significantly increased of congenital defects (ORs between 1.1 and 1.3), but it is much lower than in women with pregestational diabetes^[37]. The malformations described are similar to those reported in pregestational diabetes, especially cardiovascular defects and anomalies involving the musculo-skeletal and central nervous systems^[38,39].

The most commonly reported cardiac malformations include transposition of the great arteries, double outlet right ventricle, truncus arteriosus, hypoplastic left heart syndrome and ventricular septal defects^[40].

Antenatal ultrasounds play an important role in monitoring fetal cardiac anatomy and function. Antenatal diagnosis of cardiac malformation is helpful to decide the place of birth when specific neonatal cardiologic care is needed. Babies of women with GDM should have an echocardiogram in the presence of clinical signs at birth associated with congenital heart malformations (cyanosis, murmur) or cardiomyopathy (heart failure).

Neurological impairments

Infants of diabetic mothers are prone to neurologic impairments, mainly due to perinatal asphyxia, birth traumas and metabolic disorders.

Perinatal asphyxia: Increased risk of perinatal asphyxia has been reported in diabetic pregnancies in a number of studies. The risks of perinatal asphyxia are increased in case of macrosomia, particularly when there is a shoulder dystocia^[28,41]. Impaired fetal environment characterized by fetal hypoxia has also been evoked as a contributing factor.

However, in cases of GDM, the incidence of perinatal asphyxia, defined by a 5-min Apgar score < 7, was very low (1%-2%) in a study including more than a thousand neonates of GDM mothers^[18]. In another study, umbilical arterial pH < 7.2 was about 15% in GDM group, comparable to the non-diabetic group^[42]. In both studies, the incidence was not influenced by the treatment of maternal diabetes.

Metabolic disorders: Glucose is the main energy substrate for the brain. In newborns, hypoglycemia can lead to the situation where brain energy metabolism cannot be sustained. The consequences of low blood glucose levels depend on the availability of other substrates, such as lactate and ketone bodies also used by the brain to provide energy. These alternative substrates are not routinely measured, and the normal

threshold values are unknown. Their availability also depends on the clinical and nutritional status of the infant.

For infants presenting with clinical signs compatible with hypoglycemia, like apnoea, hypotonia, jitteriness, apathy, hypothermia, tremors and seizures, treatment must ensure that blood glucose levels remain above 0.45 g/L (2.5 mmol/L). An IV bolus dose of glucose (150-200 mg/kg) should be administered urgently, followed by a constant rate infusion. It is necessary to check that thereafter blood glucose concentrations stabilize within normal ranges (20). In case of clinical signs, Cornblath *et al.*^[43] have suggested that the Whipple triad should be fulfilled: a low blood glucose concentration; signs consistent with hypoglycemia; and resolution of signs and symptoms after restoring blood glucose concentrations to normal values. Therefore, if symptoms persist despite adequate treatment, other causes should be investigated, since these symptoms are not specific.

Symptoms from hypocalcemia are similar to those observed in hypoglycemia, but usually present later, between 24-72 h of life^[31]. Then, blood calcium concentration should also be measured in the presence of symptoms suggestive of hypocalcemia.

Brachial plexus injuries: The spinal cord is vulnerable to birth trauma with symptoms related to palsies of the brachial plexus.

The most common type of brachial plexus injury, also called Erb's palsy, involved the cervical roots C5 to C7. The infant presents with internally rotated arm and flexed wrist. The second most common type called total plexus palsy involves cervical roots C5-C8 and sometimes thoracic root T1. The infant presents with a flaccid and insensitive arm and with clawed hand. Paralysis of the hemi-diaphragm is also observed when phrenic nerve is involved, leading to respiratory insufficiency and requirement of mechanical ventilation^[44].

Incidence of brachial plexus palsy in newborns of diabetic mothers is low, between 0.2% and 3%. As a consequence, the risk could not be accurately measured^[28].

Poor suckling: It has been shown in the early nineties that maternal GDM may impair neonatal behavior, leading to lethargy and hypotonia related to delayed neural maturation. More recently, poorer suckling patterns were found at day 3 only among infants of insulin-managed GDM mothers, but not in the diet-managed mothers^[45]. This study confirmed some degree of neurologic immaturity during the early neonatal period.

Digestive impairment

Apart from the difficulties to feed because of poor suckling pattern, neonates of diabetic mothers may also exhibit neonatal small left colon, a cause of

functional lower intestinal obstruction that can mimic Hirschsprung disease. The pathophysiology is unknown but it is significantly associated with maternal diabetes. The treatment is always conservative as long as intestinal perforation does not happen. Contrast enema is both diagnostic (abrupt transition zone at the splenic flexure) and curative, promoting the evacuation of meconium relieving the intestinal obstruction^[46].

FACTORS THAT INFLUENCE THE SEVERITY OF NEONATAL ADVERSE OUTCOME IN GDM

The adverse neonatal outcomes described above are not constant in all cases but they are significantly influenced by the quality of maternal care and by maternal health. Furthermore, most of these complications are more likely to happen in macrosomic infants.

Maternal conditions and neonatal outcomes

Impact of maternal blood glucose levels and pregestational T2D on neonatal outcomes:

Perinatal death, malformations and prematurity are mainly influenced by maternal glucose levels. As discussed above, there is also a linear relationship between glucose maternal level and the frequency of macrosomia^[8]. Furthermore, the analysis of the risk of fetal malformation and perinatal death in case of GDM shows that undiagnosed pre-pregnancy T2D has a substantial impact on these serious perinatal complications.

There is a relationship between the malformation rate and maternal fasting blood glucose level^[39,47]. This risk also increases with maternal BMI, and when GDM is diagnosed during early pregnancy^[48,49]. Most major malformations occur very early in gestation during the embryonic stage. In diabetic pregnancies, they are attributable to unstable periconceptional glycemia. Maternal hyperglycemia results in excess glucose metabolism in the developing embryo that may alter various molecular chain reactions: (1) altered cell lipid metabolism, notably the production of prostaglandin E2 involved in the patency of the ductus arteriosus in utero^[50]; (2) high glucose levels induce an excess production of reactive oxygen species which has been shown to cause oxidative stress and subsequently increase the risk for fetal malformations, notably neural tube defect^[51]; and also (3) high glucose levels induce the activation of many proteins involved in apoptotic cell death, including members of the caspase families^[52]. Although data on the molecular basis of diabetic embryopathy have improved during the last years, mechanisms are still incompletely understood^[53].

These clinical and physiopathologic data suggest that the increased risk of congenital defects in GDM reported in some studies is likely to be related to the inclusion of women with undiagnosed T2D in the GDM

groups^[54].

Unlike in pregestational diabetes, the increased rate of fetal deaths in the 2nd and 3rd trimesters of pregnancy is debatable in cases of GDM^[55,56]. In a large cohort study, the rate of mortality was 16.2/1000 in the GDM group vs 12.5/1000 in the general population. Six weeks after delivery, women diagnosed with GDM were re-classified by a post-partum glucose tolerance test. Women having diabetes on post-partum test were considered as "newly presenting T2D". When those women were excluded from the GDM group, perinatal mortality was 8.9/1000 in the "true" GDM group, which was similar to the general population. Mortality was the highest in the groups with T2D diagnosed before and after pregnancy (respectively, 39.1/1000 and 56.2/1000)^[57]. These data demonstrated that the increased risk of perinatal death reported in case of GDM in some studies, seems to be attributable to undiagnosed T2D.

Prematurity is one of the leading causes of neonatal death. As discussed above, higher maternal glucose levels were observed in case of prematurity in GDM pregnancies. Furthermore, one of the main causes of induced preterm delivery is maternal pre-eclampsia which is more commonly associated with T2D pregnancies^[58].

Impact of maternal obesity in the complications of GDM: Maternal obesity is associated with worse perinatal outcome even in glucose-tolerant women. Macrosomia is the main complication reported in overweight or obese women, independently of diabetes^[59-62]. It is well recognized that neonates born to obese women, even if normal glucose tolerant, have increased fat mass^[63]. As in GDM, increased adiposity at birth is related to maternal excess of glucose and lipids availability, and placental transfer to the fetus^[11].

The risk of fetal and infant deaths are two to three times greater for women with preconceptional obesity, after excluding pregnancies affected by congenital anomalies or pregestational diabetes^[64]. It was recently showed that even modest increases in maternal BMI were associated with increased risk of fetal death, stillbirth, and neonatal, perinatal, and infant death. The relative risk per 5-unit increase in maternal BMI ranged from 1.15 to 1.24^[65]. Maternal obesity is also associated with an increased risk of a range of structural anomalies, with the higher risk for neural tube defects^[66,67]. It is interesting to note that the risk of particular malformations such as omphalocele and diaphragmatic hernia is increased in obese pregnant women, but not in case of diabetes^[68,69].

There is a tight link between maternal obesity and diabetes in pregnancy. Indeed, the risk of GDM increases with maternal BMI^[70]. The overall population-attributable fraction of GDM related to overweight was estimated at 46.2%^[71].

The benefit effect of treatment of diabetes on neonatal outcomes is lower in obese women, even

if targeted levels of glycemic control are achieved. Furthermore, when GDM is untreated or poorly controlled, overweight and obese women have a higher risk of poor neonatal outcome, compared to normal weight GDM women^[42,72].

The combination of GDM and obesity shows a greater impact on pregnancy outcomes than either GDM or obesity alone. This cumulative risk was shown for macrosomia, newborn percent body fat and birth trauma^[73]. It was also reported for a composite neonatal outcome (BW > 4000 g, birth trauma, shoulder dystocia, hypoglycemia, or jaundice)^[74].

Effects of macrosomia on neonatal outcomes

It has long been reported that the delivery of macrosomic infants is associated with a higher risk for adverse neonatal morbidity such as birth injury, respiratory distress and hypoglycemia. Macrosomia (BW > 4500 g), regardless of the cause, is also in itself a risk factor for asphyxia and perinatal death^[75].

Macrosomia increases the risk of shoulder dystocia, regardless of the cause. In the study by Zhang *et al.*^[75], the risk of birth injury was the highest for infants with a birth weight 4500-4999 g and ≥ 5000 g, [ORs 2.4 (2.2-2.5) and 3.5 (3.0-4.2), respectively].

In the case of GDM, there is a particularly high risk of respiratory distress in newborns with a BW ≥ 4000 g, compared to those with a BW of less than 4000 g [OR = 3.1 (1.11-8.65)]^[76]. In the other study, the risk of respiratory complications increased with increasing BW ≥ 4000 g, irrespective of maternal diabetic status^[22]. Furthermore, it seems that clinically significant hypertrophic cardiomyopathy without concomitant fetal macrosomia is rarely observed^[40].

The analysis of the data collected by the HAPO study showed that neonatal hypoglycemia was strongly related to elevated cord C-peptide levels. High C-peptide levels are related to the importance of fetal hyperinsulinemia that favors fetal excess of growth. Therefore, infants with excessive size at birth are prone to develop hypoglycemia^[16]. It was shown that when BW was ≥ 4000 g the risk of hypoglycemia increased, but the risk was higher when BW ≥ 4000 g was associated with maternal GDM^[76]. In another study, the risk of hypoglycemia increased with increasing BW, irrespective of maternal diabetic status^[77].

SIZE OF THE BURDEN IN LOW INCOME COUNTRIES

The prevalence of risk factors for diabetes during pregnancy are increasing all around the world because of increasing incidence of T2D and obesity and the shift of age at onset of diabetes to younger age groups. T2D is an occult disease that can remain undiagnosed, especially in young women of reproductive age. A recent study reported an estimated global prevalence of hyperglycemia in pregnancy worldwide of 170/1000

live births in 2013. A majority of cases occurred in low and middle income countries (91.6%). The prevalence varies widely around the world. The South-East Asia region had the highest prevalence with 23% of live births, followed by the Middle East and North Africa region with 22%^[78].

A community-based prospective program in India, with universal screening for GDM, showed that the prevalence of GDM was 13.9%. The frequency varied widely across urban, semi-urban and rural areas, respectively 17.8%, 13.8% and 9.9%. The prevalence also varied according to maternal BMI. For BMI ≥ 25 mg/m², the incidence was up to 28.4%, 23.8% and 16.1% in urban, semi-urban and rural areas, respectively^[79].

A recent analysis of data from World Health Organization (WHO)'s Global Survey on maternal and perinatal outcomes in 23 developing countries described the prevalence of macrosomia, one of the main complications of maternal diabetes and obesity^[80]. There was a large variation in the prevalence of babies with BW ≥ 4 kg, ranging from 0.5% in India, to 15% in Algeria. Maternal diabetes and increased gestational BMI were significantly associated with macrosomia in all regions. For example, in Algeria, where 15% of the babies had a BW ≥ 4 kg, 25% of the mothers were obese (BMI ≥ 30 kg/m²). In Latin America countries, frequency of maternal obesity was more than 30% (Argentina, Mexico, and Paraguay).

It can then be estimated that the burden of neonatal complications is higher in developing countries than in high-income countries, because of the high incidence of maternal hyperglycemia and the absence of screening and treatment of maternal diabetes, and finally because of substandard neonatal care. This is probably even worst within the rural areas, because of limited financial and human resources.

CONCLUSION

It is indisputable that diabetes during pregnancy exposes the fetus and the neonates to increased adverse outcomes. These risks mainly depend on maternal health condition. Thus, awareness of maternal health prior and during pregnancy is essential to pediatricians to anticipate the severity of neonatal adverse outcome. Health systems in low income countries are often insufficiently structured to provide adequate screening and care to diabetic mothers. Such situations seriously increase the burden of adverse fetal and neonatal outcomes, probably still underestimated.

The current definition of GDM does not allow identifying pregestational diabetes from true GDM. The WHO recently proposed new criteria for the diagnosis and definition of hyperglycemia first detected in pregnancy which distinguishes the more serious diabetes in pregnancy from GDM^[81]. This is a considerable advance as we say that risks of serious complications for fetuses and the neonates are much

higher in true diabetes than in GDM. This will help to better understand the burden of hyperglycemia in pregnancy and its relationship with the growing prevalence of T2D. This will also probably allow in the future to determine precisely the risks linked to GDM compared to those linked to T2D. Such distinction will subsequently help better identifying risks in the neonatal period, but also later in life. Indeed, offspring of diabetic and obese women, or macrosomic infants, are more likely to be obese and to have diabetes and cardiovascular diseases in adulthood^[3,4]. These long-term consequences of diabetes in pregnancy are going to be the burden of further generations.

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Emerging links between type 2 diabetes and Alzheimer's disease

Gumpeny R Sridhar, Gumpeny Lakshmi, Gumpeny Nagamani

Gumpeny R Sridhar, Gumpeny Lakshmi, Gumpeny Nagamani, Endocrine and Diabetes Centre, Department of Obstetrics and Gynecology, Andhra Medical College, Visakhapatnam 530002, India

Author contributions: Sridhar GR, Lakshmi G and Nagamani G contributed equally to this paper.

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Correspondence to: Gumpeny R Sridhar, MD, DM, FACE, FRCP, Endocrine and Diabetes Centre, Department of Obstetrics and Gynecology, Andhra Medical College, 15-12-15 Krishnanagar, Visakhapatnam 530002, India. sridharvizag@gmail.com
 Telephone: +91-891-2566301
 Fax: +91-891-2509427

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Abstract

Type 2 diabetes mellitus and Alzheimer's disease are both associated with increasing age, and each increases the risk of development of the other. Epidemiological, clinical, biochemical and imaging studies have shown that elevated glucose levels and diabetes are associated

with cognitive dysfunction, the most prevalent cause of which is Alzheimer's disease. Cross sectional studies have clearly shown such an association, whereas longitudinal studies are equivocal, reflecting the many complex ways in which the two interact. Despite the dichotomy, common risk and etiological factors (obesity, dyslipidemia, insulin resistance, and sedentary habits) are recognized; correction of these by lifestyle changes and pharmacological agents can be expected to prevent or retard the progression of both diseases. Common pathogenic factors in both conditions span a broad sweep including chronic hyperglycemia *per se*, hyperinsulinemia, insulin resistance, acute hypoglycemic episodes, especially in the elderly, microvascular disease, fibrillar deposits (in brain in Alzheimer's disease and in pancreas in type 2 diabetes), altered insulin processing, inflammation, obesity, dyslipidemia, altered levels of insulin like growth factor and occurrence of variant forms of the protein butyrylcholinesterase. Of interest not only do lifestyle measures have a protective effect against the development of cognitive impairment due to Alzheimer's disease, but so do some of the pharmacological agents used in the treatment of diabetes such as insulin (especially when delivered intranasally), metformin, peroxisome proliferator-activated receptors γ agonists, glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Diabetes must be recognized as a risk for development of Alzheimer's disease; clinicians must ensure preventive care be given to control and postpone both conditions, and to identify cognitive impairment early to manage it appropriately.

Key words: Cognition; Insulin resistance; Insulin; Butyrylcholinesterase; Dementia

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Core tip: Type 2 diabetes mellitus is a risk factor for future development of Alzheimer's disease, the most prominent cause of cognitive failure in the elderly. Common pathogenic mechanisms underpin both

conditions. Therapeutic strategies in prevention (lifestyle changes) and pharmacological agents (biguanides, intranasal insulin, thiazolidinediones, glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors could also be useful against Alzheimer's disease.

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INTRODUCTION

The prevalence of diabetes mellitus is inexorably rising as evidenced by data from local, national and international studies^[1-3]. Improved care of diabetes as well as general increase of longevity are predictive of greater proportion of elderly in the general population. It is well known that the critical problems of the aged relate to impaired activities of daily living and of cognitive decline. It is also recognized that compared to persons without diabetes, those with diabetes have dementia two to three times more commonly^[4]. The economic and social burden on the health-care system as well as on care-providers would be enormous. It is essential if methods are available, to prevent or postpone the onset of both conditions; lifestyle changes appear to be effective in preventing both conditions^[5].

CAUSES OF DEMENTIA

Dementia may be broadly classed into Alzheimer's disease, vascular dementia, dementia with Lewy bodies and frontotemporal dementia^[4]. Of these, Alzheimer's disease is the most common type^[6].

Dementia in diabetes: Epidemiological studies

A number of epidemiological studies have shown an association of dementia with diabetes. In the Hisayama study (1995), the relative risk (RR) of Alzheimer's disease was 2.18 (95%CI: 0.97-4.9) and of vascular dementia 2.77 (95%CI: 2.59-2.97)^[7]. A later publication from the same study group (2011) showed an RR of 2.05 (95%CI: 1.18-3.57) for Alzheimer's disease and 1.82 for vascular dementia (95%CI: 0.89-3.71)^[8]. The Rochester Study (1997) reported differences in the risk of Alzheimer's disease based on gender: RR was 2.27 for men (95%CI: 1.55-3.31) and 1.37 for women (95%CI: 0.94-2.01)^[9]. The well cited Rotterdam Study (199) showed a relative risk of 1.9 for Alzheimer's disease (95%CI: 1.2-3.1)^[10] (Table 1).

Conflicting results: Cross sectional vs longitudinal studies

Although there is a consensus from cross-sectional

Table 1 Dementia and diabetes

Relative risk of AD	Relative risk of vascular dementia	Ref.
2.18	2.77	[7]
2.05	1.82	[8]
1.9	-	[10]

AD: Alzheimer's disease.

studies that hyperglycemia causes cognitive impairment, results from longitudinal studies are conflicting^[4]. Two recent longitudinal studies show up differences in the occurrence of Alzheimer's disease and glucose tolerance.

The Baltimore Longitudinal Study of aging prospectively assessed a cohort of community-dwelling individuals. An investigation was made to relate serial glucose intolerance, insulin resistance with brain β amyloid burden measured *in vivo* using carbon 11-labelled Pittsburgh Compound B. The latter is utilized to image β -amyloid ($A\beta$) *in vivo* with PET scan; ¹¹C-Pittsburgh compound-B (11C-PiB), a PET $A\beta$ ligand, is widely employed for early diagnosis of Alzheimer disease. It allows quantitative analysis of $A\beta$ burden. Derived as a carbon-11 labelled thioflavin-T amyloid dys, it binds to $A\beta$ plaques with high specificity and affinity. ¹¹C-Pittsburgh compound-B (11C-PiB), correlates with the rate of cerebral atrophy. Pathological process for Alzheimer's disease was established at autopsy^[11]. Analysis was carried out using grouped and continuous mixed-models analyses. The key result of the study was, there was *no* significant correlation of brain markers of Alzheimer with insulin resistance or glucose intolerance during the follow up period of 22.1 years (SD:8.0)^[11].

In contrast, a group from the University of Washington, which evaluated whether higher glucose levels increase the risk of dementia in those without diabetes, found that they did^[12]. Participants, without dementia were drawn from the Adult Changes in Thought study (839 men, 1228 women, mean baseline age 76 years). In all 35264 glucose levels and 10208 glycosylated hemoglobin levels were analyzed. They were followed up for a median of 6.8 years. 524 subjects developed dementia (74 of 243 with diabetes and 450 of 1228 without diabetes). Higher levels of average glucose levels were related to development of dementia in both groups, ie those with and without known diabetes. The authors conclude that "higher glucose levels may be a risk factor for dementia, even among persons without diabetes"^[12].

Can the conflicting results of these two rigorous, well-designed studies be resolved? The Baltimore study used both neuroimaging as well as autopsy to identify Alzheimer's pathological processes. The Adult Changes in Thought study performed a 6 year follow up in a large group of well-defined elderly. In the former, insulin resistance and glucose intolerance were not a risk factor for Alzheimer's changes; in the

latter, higher glucose levels even among those without diabetes may be a risk factor for dementia.

The apparent differences can be attributed to the variety of pathological changes in diabetes leading to dementia including Alzheimer's disease: chronic hyperglycemia, hypoglycemia (acute and recurrent), glycosylated of proteins, vascular disease, endothelial dysfunction, inflammation, altered blood brain barrier, dyslipidemia, insulin resistance, genetic predisposition, amyloid deposition and depression, among others^[13]. More sensitive methods of measuring brain volume as a surrogate of cognitive function may throw light^[4]. In addition, there is a flaw in using brain markers such as plaques in diagnosis of Alzheimer's disease: subjects may have amyloid plaques, yet display no symptoms of Alzheimer's disease throughout their life. This discrepancy between the presence of plaques and Alzheimers could play a role for a lack of finding a link between diabetes and Alzheimer's disease.

Hyperglycemia

Hyperglycemia is a recognized risk factor for cognitive impairment as shown by the ACCORD-MIND study and others^[14,15]. Biological reasons for such changes were ascribed to neural damage following advanced glycosylated end products and oxidative stress, osmotic stress damaging the blood brain barrier and resultant leak of toxic substances leading to further damage of nervous structures^[4]. In addition to chronic hyperglycemia as assessed by glycated hemoglobin, glycemic variability was also proposed to contribute to cognitive dysfunction. Measurements by continuous glucose monitoring revealed cognitive function was better correlated with diurnal variation in blood glucose^[16]. Post prandial glucose levels could also be a contributing factor, acting *via* oxidative stress^[4].

Hypoglycemia

Although severe hypoglycemia was shown to be associated with dementia in the elderly^[17], when hypoglycemia is avoided by careful treatment as in the DCCT/EDIC study, there was no association between hypoglycemia and cognitive dysfunction^[18]. In the elderly however, hypoglycemia, when coupled with atherosclerosis leads to organic brain damage which is often irreversible^[4].

Role of insulin in brain

Cognition may be affected not only by alterations in the level of glucose, but also *via* the action of insulin. Upon transport through the blood brain barrier, insulin binds to its receptors, and is involved in modulating cognitive function. A large number of insulin receptors occur in brain areas related to memory such as the hippocampus and cerebral cortex. In addition it also aids the release of β -amyloid peptide extracellularly, and increases the expression of the enzyme which degrades insulin, insulin degrading enzyme (IDE)^[4]. As the latter also degrades β -amyloid peptide, insulin deficiency results in accumulation of β -amyloid peptide.

Both hyperinsulinemia and hyperglycemia were shown to increase neuritic plaque formation^[19].

Information is being available about the origin of insulin in the brain and its role in cognition. Originally, brain was considered to be insulin insensitive because insulin did not influence the glucose uptake by the bulk brain. However insulin has been shown to be a neuroregulatory peptide playing a role in food intake and in monitoring the energy stores of the body^[20]. Interestingly a role for insulin in modulation of memory and cognition is also emerging. Its action has been observed in regions associated with reward recognition such as hippocampus, and in global cognition and memory. Rather than passing across the blood brain barrier from the periphery, insulin appears to be produced locally for action as a neurotransmitter, regulated by glucose levels. In addition a para-arteriolar pathway for transport at the level of microvasculature has been proposed^[20].

The role of insulin in the pathogenesis of Alzheimer disease has been described. Insulin can modulate A β peptide *in vitro*. The peptide is well known as a neuropathological hallmark of AD. Low levels of insulin in the brain can decrease the A β release into extracellular compartments. In addition hypoinsulinemia in the central nervous system can lower the levels of insulin-degrading enzyme, thereby impairing A β clearance. In all, chronic hyperinsulinemia in the peripheral circulation, along with decreased uptake of insulin into the brain can lead to dysregulation of A β and inflammation^[21,22].

Role of microvascular disease in cognitive decline

Studies have shown that retinopathy and nephropathy are associated with impairment of cognition^[22,23]. Small vessels in both organs arise from a similar embryonic antecedent and share similar structures; it is conceivable therefore that insults (*e.g.*, increased polyol pathway activity, myo-inositol dysmetabolism) result in similar adverse reactions in frontal lobe of the cerebral cortex leading to cognitive decline^[4]. However, evidence is not unequivocal. Retinopathy is related to microvascular changes in diabetes. Because the retina shares many features with the brain, both developmental, anatomical (*e.g.*, microvascular bed) and physiological (*e.g.*, blood-tissue barrier), changes in retina were suggested to presage brain pathological processes. Alzheimer's disease is known to involve the retina, such as the macula and the optic disc. It has been suggested that pathological changes in the retina such as macular deposits, reduced thickness of retinal nerve, cupping of optic disc and retinal microvascular changes may be related to cognitive dysfunction and Alzheimer disease^[24].

Insulin resistance and Alzheimer's disease

Downstream insulin signaling acts through a complex interplay involving phosphatidylinositol 3-kinase (P13K0 and mitogen activation protein kinase (MAPK).

The latter is associated with most metabolic effects of insulin. Unlike its peripheral effects, the action of insulin in the central nervous system is dependent on its crossing the blood brain barrier *via* direct transfer^[25] through an insulin receptor protein, which is selectively distributed. In addition there is also local synthesis of insulin in the CNS. Unlike peripheral insulin receptors, those in the CNS differ in terms of structure, function and size. They are highly populated in the olfactory bulb, hypothalamus, cortex, cerebellum, hippocampus and are expressed in both neurons and glia^[26].

The physiological effects of insulin in the brain are unlike those in the periphery: in an animal model it suppressed food intake and increased the level of glucose^[27], acting in a way as its own counterregulation^[25]. Compared to its weight, the brain depends on a larger amount of glucose for its metabolic needs compared to other tissues. Glucose reaches it *via* facilitated diffusion transported by glucose transport proteins. In the brain, insulin does not have a major effect on either the transport of glucose to the brain or its basal metabolism^[28,29]. While it is not a major regulator of glucose metabolism in the brain, insulin indirectly affects neurons by modulating neurotransmitter release, neural growth, tubulin activity, nerve survival and synaptic plasticity^[26]. In humans, insulin improves cognition independent of its effects on peripheral glucose^[30].

Whereas acute increases of insulin improve cognition, chronic hyperinsulinemia can adversely affect neuronal function *in vitro* by increasing susceptibility to toxin and stress-induced effects^[31]. Glycated proteins and inflammatory mediators could also have a pathogenic role^[25].

Protein aggregation, diabetes and Alzheimer's disease

Protein aggregation has been suggested to be an underlying pathogenic factor between type 2 diabetes and Alzheimer's disease^[32]. A number of hypotheses were proposed to explain why a biological protein can be transformed into a pathological entity with the ability to self-assemble: aging, high concentrations of the protein, mutation of amino acids or abnormal post-translational modification, modulated by environmental factors^[32].

Alzheimer's disease is associated with accumulation of neurofibrillary tangles and amyloid fibers leading to neuronal cell loss. Amyloid β peptides form from cleavage of amyloid β -protein precursor seen in plaques. It is organized as amyloid fibrils, which are linear aggregates^[32]. Diabetes is also characterized by localized and progressive amyloid deposition in the pancreatic β cell islets^[33]. Common features of amyloid deposited in both Alzheimer's disease and diabetes include: linear appearance, with a beta-sheet structure, which begin to form from spherical oligomers that can self-assemble^[34]. The islet amyloid peptide is secreted by β cells of the pancreas and

consists of 37 amino acids.

ApoE- ϵ 4

Expression of ApoE- ϵ 4, which is related to diabetes as well, increases the risk of early onset Alzheimer's disease. It has increased ability to deposit A β , which is neurotoxic, and also impair its clearance^[35]. ApoE- ϵ 4 is less protective against oxidative stress and leads to cholinergic dysfunction seen in Alzheimer's disease, besides modifying the cholesterol transporter protein ABCA1.

Other potential associations

In addition other associations are also being recognized as risk factors for both diabetes and Alzheimer's disease: weight gain, acting perhaps through defective leptin signaling and increased formation of advanced glycation end products, which could have a pathogenic role in the amyloid plaques deposited in the brain^[35]. Other emerging associations include disturbances in sleep and the circadian rhythm^[36,37], and iron overload in brain among persons with obesity^[38].

Insulin like growth factor

Animal studies have provided intriguing evidence that loss of insulin-like growth factors, along with insulin could lead to age-dependent brain atrophy with cognitive decline^[39]. Insulin and its growth factors maintain brain protein content; their replacement can prevent brain protein loss, cell degeneration and demyelination. Lack of insulin and growth factors, which are common to type 2 diabetes and to Alzheimer's disease could therefore play a role in their pathogenesis and provide therapeutic targets in their treatment^[39].

Butyrylcholinesterase

Butyrylcholinesterase, belonging to the esterase family of enzymes that also contain acetylcholinesterase^[40] has been evaluated in relation to insulin resistance, cardiovascular disease, obesity and dyslipidemia^[36]. Variant forms of the enzyme with little or no activity exist in isolated geographic populations^[41] with apparently no adverse health effects^[42]. Butyrylcholinesterase was studied in relation to both type 2 diabetes mellitus and to Alzheimer's disease^[43-45], with studies suggesting a possible protective effect against Alzheimer's disease and risk for fronto-temporal dementia^[46].

A number of hypothesis were put forward for the association of butyrylcholinesterase with type 2 diabetes mellitus and Alzheimer's disease^[33,36]. Plaques in the brains of individuals with dementia had higher levels of butyrylcholinesterase, which is also localized in neurofibrillary tangles. It was also shown to attenuate amyloid formation^[47].

Similarly in type 2 diabetes, butyrylcholinesterase may modify the expression of insulin resistance or by way of amyloid fibril deposition in β cells of the pancreas^[33]. It was shown to interact with amylin

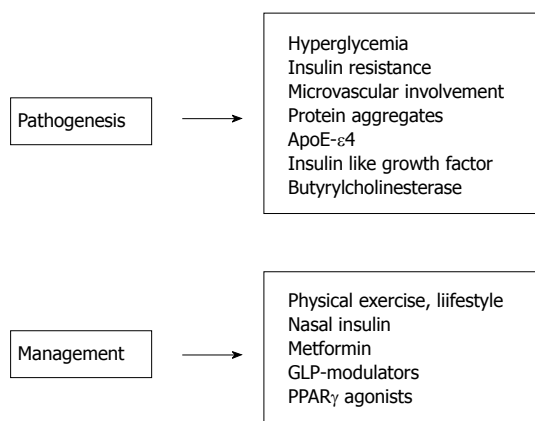


Figure 1 Links between Alzheimer's disease and type 2 diabetes mellitus.

and attenuate the formation of amylin fibril as well as its oligomer. When applied to cultured β cells, it was protective against amylin cytotoxicity^[47]. Butyrylcholinesterase was shown to participate in the progression of metabolic syndrome to type 2 diabetes mellitus. A majority of subjects with type 2 diabetes show extracellular deposits formed by islet amyloid polypeptide (IAP), adjacent to the β cells. Elevated levels of IAP are found in conditions of insulin resistance. Disturbed balance of sympathetic and parasympathetic nervous system could participate in metabolic syndrome. Lower vagal activity could in part be caused by increased hydrolysis of acetylcholine mediated by increased butyrylcholinesterase^[48]. Lowering of acetylcholinesterase results in reduced parasympathetic signals and increased ratio of sympathetic signals^[48]. In addition BChE was reported to attenuate the formation of A β amyloid fibrils^[47]. Essentially subjects with metabolic syndrome had elevated levels of BChE compared to those with type 2 diabetes and with controls. *In vitro* interaction of BChE was observed with amylin. It interacted with amylin, and attenuated the formation of both amylin fibril and oligomer formation, showing that it can protect cultured β cells from cytotoxicity due to amylin^[47]. Thus increased BChE seen in metabolic syndrome could protect pancreatic β cells by reducing toxic amylin oligomer formation^[47].

A bioinformatics study suggested the following sequences ($E < e^{-5}$) were associated with both type 2 diabetes and Alzheimer's disease: butyrylcholinesterase precursor K allele (NP_000046.1), acetylcholinesterase isoform E4-6 precursor (NP_000656.1) and apoptosis-related acetylcholinesterase (1B41|A). In an animal study, streptozotocin-induced diabetes was associated with elevated butyrylcholinesterase activity, lowered superoxide dismutase and impaired cognitive function assessed by Morris water maze method^[49]. Being low-grade inflammatory conditions, both type 2 diabetes mellitus and Alzheimer's may be target conditions for utilization of butyrylcholinesterase as a biomarker^[50], as well as a treatment target^[33].

The principal drugs currently available to manage

Alzheimer's disease act *via* modifying butyrylcholinesterase levels. Renewed interest in the possible role of "missing genes" in people who are apparently healthy may aid in uncovering new treatment modalities^[51]. Individuals with variant butyrylcholinesterase activity genes may be a potential group for such long-term follow up studies vis a vis their propensity or protection against diabetes and Alzheimer's disease^[40].

THERAPEUTIC IMPLICATIONS

The interest of linking type 2 diabetes mellitus and Alzheimer's disease lies not in science, but more in translational science: how understanding the common pathogenesis can help in prevention and treatment of both conditions. Other than the scope for future therapies, evidence is now available for the currently available drugs used in diabetes^[52] to have a modulatory effect on the cognitive decline due to Alzheimer's disease. Along with its action on AMPK, metformin has been recently shown to influence the incretin system by increasing the secretion of glucagon-like peptide-1 (GLP-1)^[53]. GLP-1 and GIP receptors are known to be expressed in the brain, and direct activation of these receptors may be a potential strategy to treat Alzheimer's disease^[54]. In addition, metformin also influences gut microbiome along with its other putative actions in the management of diabetes mellitus^[55]. The use of drugs used in diabetes mellitus such as metformin, GLP-1 mimetics (exenatide and liraglutide) and peroxisome proliferator-activated receptors γ agonists may all be of potential benefit in the prevention and management of Alzheimer disease^[56].

When a theoretical basis for insulin to affect brain responses was studied in clinical practice, a recent review of 8 published studies on effect of intranasal insulin on cognition, comprising 328 participants showed first of all, no significant adverse effects. Generally the authors concluded that "the limited clinical experience suggests potential beneficial cognitive effects of intranasal insulin"^[57]. In a single study, use of 20 IU intranasal insulin showed improved immediate recall in Apo ϵ 4(-) subjects but not in Apo ϵ 4(+) subjects.

Other than correcting hyperglycemia, some of the conventional antidiabetic agents were shown to affect cognition. Metformin protected brain against oxidative stress in rats, and by preventing apoptosis^[54]. However caution must be exercised because of metformin leading to increased biogenesis of Alzheimer's amyloid peptides^[58] and increased risk of cognitive impairment^[59]. Thiazolidinediones, which act at the nuclear receptors of insulin-sensitive tissues, affect transcription of genes affecting lipid and glucose metabolism. Early studies suggested that this group of drugs may favourably affect cognition, before the clinical use tapered due to their adverse drug effect profile.

Pharmacological agents modulating the incretin

system have now become mainstream in many markets world-wide. Pathological studies showed that sitagliptin lowered APP and A β deposition in the hippocampus of transgenic Alzheimer's disease mice^[53]. However whether it does so by lowering glucose levels or independently remains to be clarified. Interest arises because liraglutide, another drug in the same group had similar protective effects (Figure 1).

CONCLUSION

In conclusion, diabetes mellitus and Alzheimer's disease are both common and increasing in incidence in the aging population. Recent evidence has shown common pathogenic factors operating in both conditions. Thereby common preventive and therapeutic agents may be used in their prevention and treatment. Physicians caring for the elderly must be aware of the increased risk of the other when one condition is present. Common pathogenesis and therapeutic agents make it possible to manage both using similar lifestyle changes and pharmacological agents.

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Observational Study

Patient attitudes about financial incentives for diabetes self-management: A survey

Katherine S Blondon

Katherine S Blondon, Division of General Internal Medicine, University Hospitals of Geneva, 1205 Geneva, Switzerland

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Correspondence to: Katherine S Blondon, MD, PhD, Division of General Internal Medicine, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1205 Geneva, Switzerland. kblondon@uw.edu
 Telephone: +41-79-5534323
 Fax: +41-22-3729235

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Abstract

AIM: To study the acceptability of incentives for behavior

changes in individuals with diabetes, comparing financial incentives to self-rewards and non-financial incentives.

METHODS: A national online survey of United States adults with diabetes was conducted in March 2013 ($n = 153$). This survey was designed for this study, with iterative testing and modifications in a pilot population. We measured the demographics of individuals, their interest in incentives, as well as the perceived challenge of diabetes self-management tasks, and expectations of incentives to improve diabetes self-management (financial, non-financial and self-rewards). Using an ordered logistic regression model, we assessed the association between a 32-point score of the perceived challenge of the self-management tasks and the three types of rewards.

RESULTS: Ninety-six percent of individuals were interested in financial incentives, 60% in non-financial incentives and 72% in self-rewards. Patients were less likely to use financial incentives when they perceived the behavior to be more challenging (odds ratio of using financial incentives of 0.82 (95%CI: 0.72-0.93) for each point of the behavior score). While the effectiveness of incentives may vary according to the perceived level of challenge of each behavior, participants did not expect to need large amounts to motivate them to modify their behavior. The expected average amounts needed to motivate a 5 lb weight loss in our population and to maintain this weight change for a year was \$258 (interquartile range of \$10-100) and \$713 (interquartile range of \$25-250) for a 15 lb weight loss. The difference in mean amount estimates for 5 lb and 15 lb weight loss was significant ($P < 0.001$).

CONCLUSION: Individuals with diabetes are willing to consider financial incentives to improve diabetes self-management. Future studies are needed to explore incentive programs and their effectiveness for diabetes.

Key words: Patient incentives; Diabetes self-management; Motivation; Weight loss; Patient engagement

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Core tip: Patient incentives have shown potential in modifying behaviors such as smoking cessation or weight loss. This online survey for individuals with diabetes explores their attitude towards incentives (financial, non-financial and self-rewards) for diabetes self-management. Although nearly all participants showed positive expectations about financial incentives, they favored financial incentives for less challenging behaviors, and non-financial incentives for more challenging behaviors. This survey also enquired about expected amount of incentives, in particular for a 5 lb weight loss, maintained over a year.

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INTRODUCTION

Behavioral changes are key part of diabetes self-management^[1], but they are difficult to implement and maintain. Setting goals is a key step to behavior change. Findings from a group of Swedish investigators about patients' willingness to pay to improve their diabetes self-management emphasize the importance of certain goals for patients, such as weight loss, less frequent or severe hypoglycemic events and lower HbA1c levels^[2].

Financial incentives have shown potential in supporting patients to modify their behaviors^[3], such as for smoking cessation^[4] or weight loss^[5,6]. Acceptability and feasibility of incentives may vary with different health behaviors, as the complexity of the behaviors vary widely. The effect and type of incentives for one-time vaccinations^[7] may differ from incentives for repeated, constant efforts for weight loss^[8]. Financial incentives for health and wellness are being used by a rapidly growing number of employers, with nearly 90% companies using such measures in the 2013 survey from Fidelity Investments and the National Business Group on Health (57% increase since 2009)^[9]. While some authors have suggested using financial incentives for diabetes self-management behaviors, evidence for incentivized diabetes self-care is still scarce^[10]. There is to our knowledge only one study on patient incentives for diabetes self-management, comparing incentives to peer mentors or usual care^[11]. Although this study did not find a significant benefit, the sample was small and the reward design was solely based on the outcome (change in HbA1c value).

Incentives programs can reward different types of goals. Some programs provide outcome-based incentives, where rewards are given for achieved final results (e.g., HbA1c or BMI, body mass index). These outcome-based rewards can favor individuals who are healthier at baseline. Other programs reward the total amount of behavior change-related results, thus favoring individuals who are more obese, for example, who then have more weight to lose. Finally, some incentive programs reward the process, such as attendance at group sessions, or tracking and reporting results. These are more equitable, because it is more attainable for all participants. Furthermore, prior research suggests that process-based rewards may be more effective than outcome-based rewards, and that associating them could have an additional effect.

Incentives programs also differ in the rewards that are given. Some programs have financial incentives, others offer vouchers and discounts^[12], and yet others propose badges and stars without financial stakes. Self-rewards are another type of reward, which one gives oneself for reaching self-defined goals. Rewards are typically used to address present bias, the tendency to value small immediate rewards over large rewards in the distant future. Controversies about using financial incentives for long-term behavior change have been raised due to the "undermining effect"^[13,14]. Long-term behavior changes are driven by intrinsic motivation, or inherent motivation, rather than by extrinsic motivation, which are rewards that are external to the behavior^[15]. Prior studies on rewards have shown that the removal of extrinsic rewards can result in a decrease in intrinsic motivation, the so-called undermining effect^[13,14].

The goal of this study was to explore patients' expected responses to financial and non-financial incentives to improve their diabetes self-management. We hypothesized that expected responses to the type of incentive (financial, non-financial or self-reward) would differ with the perceived level of challenge of the incentivized behavior. We also wanted to explore the amount of money participants considered necessary to motivate behavior change. These results can help guide the creation and implementation of new patient-centered approaches for diabetes self-management.

MATERIALS AND METHODS

Setting

The survey was developed by the authors through an iterative process of editing and was tested on two groups of altogether 15 students and faculty at the University of Washington. The feedback led to modifications to simplify the survey, and resulted in a final set of 10 questions in addition to demographic information. Two individuals of the test groups had diabetes. The survey was available in English.

This online survey was launched to a panel of

Table 1 Overview of survey questions

Survey question themes
Three most difficult and three easiest diabetes self-management tasks:
Tracking health parameters
Choosing foods
Adapting medications or insulin
Affording healthcare
Adjusting meds and diet around unexpected events
Perceived challenges for living with diabetes (detailed in Table 2)
Perceived helpfulness of incentives for motivating healthier behaviors:
Cash rewards (weekly, monthly or yearly)
Tangible rewards, e.g., vouchers (weekly, monthly or yearly)
Decrease in future insurance premium
Non-financial rewards (badges, stars)
Social support (sharing with friends)
Self-reward (setting money aside for a self-set goal)
Potential sources of funding for the rewards (employer, insurance, self-paid)
Estimated amount needed to lose weight and keep it off for a year:
To lose 5 lb
To lose 15 lb

three hundred members of a commercial survey website in March 2013. This company has a large panel of international volunteer members who can be filtered according to personal information such as age or country of residence. Eligible members receive recruitment emails, and choose freely whether to respond or not. All participants gave their informed consent prior to their inclusion in the study. Participants who complete the proposed surveys receive website points, which can be exchanged for tangible gifts.

Recruitment

Our survey was administered in English, and was restricted to United States residents. It was open until 300 participants responded. We also filtered out individuals younger than 18 years old. The survey was open to individuals with type 1 or type 2 diabetes, but excluded those with gestational diabetes. We did not offer any supplementary compensation for completing the survey. (We had no involvement with the reward points that are part of the website). The study was approved by the Institutional Review Board of the University of Washington.

Measures

Participants completed the 10-question survey on diabetes self-management, goals and barriers (Table 1). We asked them to identify the three most difficult and the three easiest barriers among the following diabetes self-management tasks: tracking health, choosing foods, cooking appropriate meals, adapting medications or insulin, affording healthcare, adjusting meds and diet around unexpected events. We then asked them which diabetes-related behaviors were challenging: structuring a daily routine around diabetes management, impact on social relationships, thinking about diabetes all the time, social support from family, friends and workplace, changing foods, seeing

how others think of them, and understanding the relationships between glucose, diet and exercise.

We asked the participants to anticipate their responses to different rewards to help improve diabetes self-management: financial rewards, non-financial rewards and self-rewards. For the financial rewards, we asked participants to estimate the helpfulness of rewards, according to their frequency (weekly, monthly or annual) and type (cash, vouchers or reduced annual insurance premium) to improve their health behaviors. We used a 4-point scale with our predicted small sample to avoid having a neutral option. For the non-financial rewards, we enquired about the anticipated effect of receiving stars or badges, or sharing results with friends, in helping improve their behavior. Finally, we asked participants about the helpfulness of self-paid rewards (e.g., setting money aside for a self-set goal) in improving health behaviors.

We explored who the participants thought should pay for the rewards to improve their health behaviors (health insurance companies, employers or self-paid rewards). We adapted two questions from Long *et al*^[16]'s survey instrument, which were: "If you were overweight, how much money would you need to receive to persuade you to lose 5 pounds and keep it off for 1 year?" and likewise for a 15-pound weight loss. Finally, we explored their use of mobile technology (in general and for diabetes).

Statistical analysis

We conducted descriptive analyses describing means and distributions. We defined financial incentive as any cash incentive (weekly, monthly or yearly), any voucher or reduction in insurance premium. We considered recognition by badges or stars and sharing results with friends as a non-financial incentive. Helpfulness of incentives was a binary variable, defined as not helpful or helpful (somewhat helpful to very helpful). To explore associations between diabetes tasks and type of reward, we used a logistic regression model to compare expectations of self-management tasks perceived as difficult or easy. We proceeded similarly for the behaviors that are related to diabetes self-management, separating not helpful or somewhat challenging behaviors from those that are challenging. We then created a score ranging from 0 (easy) to 24 (hard) based on how difficult these behaviors were perceived to be (Table 2). Using a logistic regression model, we studied the association between the score and the three types of reward. In this model, we also analyzed the effect of age and of weight loss motivation, using the estimated amount needed to lose 5 lb and keep it off during a year. We used a cutoff of \$30000 to avoid bias from outliers in these analyses.

The percentage of complete cases was 76%. Missing covariate data were infrequent ($\leq 3\%$) other than for income (7%). Missing data were multiple-

Table 2 Challenges of diabetes self-management behaviors

Behavior	n (%)
Having to structure my daily routine around diabetes management	77 (50.3)
Coping with the impact of diabetes on my social relationships	64 (41.8)
Thinking about diabetes all the time	74 (48.4)
Having insufficient support from family and/or friends	52 (34.0)
Having insufficient support from my workplace	51 (33.3)
Seeing how other people think of me	88 (57.5)
Having to change what I eat	49 (32.0)
Understanding the relationships between glucose, diet and exercise	72 (47.1)
Overall mean	65.9 (43.1)

For the score, each behavior was rated 0 to 3 points (total of 0 to 24 points).

Table 3 Participant characteristics by type of diabetes

Diabetes	Type 1	Type 2 ¹	P-value
Value	17 (11.1)	136 (88.9)	
Female	6 (35.3)	71 (52.2)	0.20
Age (x)	39.6 ± 3.5	44.8 ± 1.3	0.18
United States. region			0.06
West	1 (5.9)	26 (19.1)	
Midwest	4 (23.5)	29 (21.3)	
Northeast	9 (52.9)	30 (22.1)	
South	3 (17.6)	51 (37.5)	
Hispanic	1 (7.6)	13 (9.3)	0.80
White race	14 (84.7)	120 (88.9)	0.14
Education (highest attained level)			0.98
High school	8 (47.1)	61 (44.9)	
College	5 (29.4)	40 (29.4)	
Graduate school	4 (23.5)	35 (25.7)	
Income > \$50000/yr	7 (42.9)	72 (52.9)	0.45
Current smoker	4 (21.8)	32 (23.5)	0.87
Smartphone user	14 (80.6)	53 (38.8)	0.005

¹Limited to white and black race. Data are expressed as mean ± SD or n (%).

imputed with 10 imputed datasets using imputation by chained-equations^[17]. The imputation model included the covariates used in all our analysis (with dependent variables), as well as the region of residence. Categorical variables were compared using χ^2 tests. *P* values from regression models were derived from Wald tests with robust standard errors. A *P*-value < 0.05 determined statistical significance. No interaction was tested. All analyses were conducted on Stata 11 (Stata Corporation, Texas).

RESULTS

Out of the 300 responders, 153 participants were eligible and consented to participate. Excluded participants differed from the inclusion group only by the higher proportion of female individuals (69.4% vs 50.4%). Age, region of residence, race, ethnicity, type of education and income were not statistically different among inclusion and exclusion groups.

The included participants had a mean age of 44.2

± 1.2 years, with 50.4% women. Graduate school education was achieved by 25.5%, and 34.1% had an annual income of > \$75,000/year. Nearly a quarter of the participants were smokers (23.0%) at the time of the survey. Smartphone ownership was 43.7%. We present the detailed participant characteristics by type of diabetes in Table 3. There were 11.1% individuals with type 1 diabetes and 88.9% individuals with type 2 diabetes. Although the mean age was not statistically different, participants with type 1 diabetes were significantly more likely to have a smartphone. Individuals with type 1 diabetes were located more in the northeast area of the United States. and less in the West and South areas.

Almost all participants (96.7%) had positive expectations from the use of incentives (financial or non-financial) to improve their diabetes self-management. Only six individuals were not interested in incentives. There was no significant difference in demographic characteristics (age, gender, race/ethnicity, education, income) among those interested in incentives or not. While all individuals with type 1 diabetes were interested in incentives, six individuals who were not interested in incentives had type 2 diabetes. Forty-five percent of participants with an interest in incentives were smartphone users, although there was no significant difference in smartphone use between those with or without interest in incentives.

Overall, the participants expected financial incentives to motivate themselves more than non-financial rewards (96.0% vs 60.0%), and 70.2% of individuals expected self-rewards to be helpful in improving diabetes self-management. The self-management tasks rated as the three easiest were: keeping track of health parameters, making food choices and cooking appropriate meals. The tasks considered most difficult were: affording diabetes costs, and adjusting diet and medications around unexpected events. Participants expected financial incentives to help improve food choices and healthcare costs the most, whereas self-rewards were expected to help improve adjustments to unexpected events the most. Non-financial incentives were expected to help improve adapting insulin doses the most.

We studied the participants' responses to the three types of incentives according to whether these factors were considered a challenge for diabetes self-management or not. We found that overall, 94% expected a positive outcome with financial incentives, compared with 60% with non-financial incentives, and 69% with self-rewards. Participants expected financial incentives to help improve the food habit changes the most, whereas non-financial incentives and self-rewards were expected to improve support from the workplace and impact on social relationships the most.

We assess the association of perceived level of challenge (0 is easy, 24 is challenging) and type of incentive (financial, non-financial and self-rewards) in Table 4. We found in the unadjusted analysis that for

Table 4 Effect of behavior score on the 3 types of rewards

Score	OR financial incentive (95%CI)	P-value	OR non-financial incentive (95%CI)	P-value	OR self-reward (95%CI)	P-value
Score	0.82 (0.72-0.93)	0.002	1.06 (1.01-1.10)	0.01	1.00 (0.96-1.04)	0.98
Score adjusted for age	0.82 (0.72-0.93)	0.002	1.06 (1.01-1.11)	0.01	1.00 (0.96-1.05)	0.94
Score adjusted for weight loss motivation ¹	0.83 (0.73-0.95)	0.005	1.07 (1.02-1.11)	0.006	1.01 (0.96-1.05)	0.75

¹Estimated amount needed to lose 5 lb and maintain it for a year.

an increase in the behavior score by one point, the odds ratio comparing expected response to financial incentives with no response to financial incentives would be 0.82 ($P = 0.002$). When comparing expected responses to non-financial incentives to no response to these incentives, we found an OR of 1.05 ($P = 0.01$). The level of perceived difficulty with these behaviors was not associated with expecting to respond to self-rewards in our dataset (OR = 1.00, $P = 0.98$). We obtained similar results when adjusting for age or for the weight loss motivation (assessed by the amount needed to lose 5 lb and keep it off for a year). This means that when these behaviors are perceived to be more difficult, the participant less expected to respond to financial incentives. Yet for non-financial incentives, when more behaviors are perceived to be difficult, participants expected to respond more to non-financial incentives.

We asked participants to provide an estimated amount needed to motivate losing 5 and maintaining it for a year, and the amount for a 15 lb weight loss. We excluded 2 outliers for each analysis, using a \$30000 cutoff. To motivate a 5 lb weight loss, participants gave estimates that ranged from \$0 (12 participants) to \$2.15 billion. On average, the estimated amount to motivate people to lose 5 lb was \$258, with a median of \$50 and interquartile range of \$10 to 100. When asked how much money participants needed to lose 15 lb and keep it off for a year, the range of responses was identical. They expected themselves to be motivated with an average of \$713 (median of \$100, IQR \$25-250). Only 8 participants responded with \$0 for this question. The difference between these two estimates was statistically significant ($P < 0.001$).

DISCUSSION

In this national web-based survey of individuals with diabetes, we explored perceptions of financial and non-financial incentives to improve diabetes self-management. We found that nearly all surveyed individuals were interested in incentives, with no difference in socioeconomic status, or demographic features (age, race, gender and SES). In fact, the only significant difference was in the type of diabetes, as all individuals with type 1 diabetes were interested in incentives.

Smartphones offer a unique opportunity to monitor behaviors^[18] and provide rewards. Smartphone use was

reported by nearly half of surveyed individuals interested in incentives and smartphone adoption is increasing rapidly^[19]. The easy availability of smartphones creates opportunities for low-effort tracking and immediate gratification through smartphone applications with reward systems. This short delay favors the efficacy of feedback for behavior changes^[20], and is a unique possibility offered by these devices. Many applications already exist for diabetes management^[21,22], including applications with non-financial reward systems^[23]. Applications with financial rewards are also available to motivate users to exercise, and recent developments like near field communication technologies facilitate money management on mobile devices. Using a smartphone app could also allow for better individualization of the reward program, by adapting to each user's stage of disease and self-management, and by identifying areas that are more challenging for each person.

Individuals overall were optimistic about the effectiveness of incentives, and expected financial incentives to be a stronger motivation than non-financial incentives for behavior change. Furthermore, when considering how difficult behavior changes were perceived to be, using a 32-point score, we found that participants expected to be significantly less likely to use financial incentives for more challenging behavior changes in the unadjusted analysis. In fact, participants were more likely to use non-financial incentives when facing the difficult behaviors. Interestingly, the perceived level of difficulty for behavior change was not associated with the use of self-rewards. These findings persisted after adjusting for age and weight loss motivation.

Our findings suggest that financial incentives could have a potential role to play in motivating select behavior changes for diabetes self-management. The different response by perceived level of challenge suggest that perhaps a combination of incentives is needed to improve the various self-management skills. In Polonsky *et al*^[24]'s recent study about perceived obstacles for glucose self-monitoring, avoidance behaviors (including forgetting, lack of time or reminders of diabetes) were predictors of a low frequency of glucose testing. Whether avoidance also predicts low success of other self-care behaviors is uncertain. Based on our findings, avoidance behaviors that are perceived to be less challenging could have a positive response to financial incentives, while more

challenging behaviors would require the use of non-financial incentives.

The amounts of financial incentives are important to consider. An interesting finding from our survey is the relatively low amounts of money that participants expected as incentive for weight loss (\$258 for the 5 lb weight loss and \$713 for the 15 lb weight loss), particularly if we consider that current out-of-pocket costs for diabetes are estimated at \$350-500/mo^[25,26]. This concurs with findings from another study which explored the use of financial incentives in diabetes, where participants suggested \$25 per month for tracking and reporting glucose results^[27]. Employers typically employ similar amounts for action-based incentives, or rewards for taking action (joining a weight loss program, for example) after going through a risk assessment^[28]. Prior research has found that very large amounts can lead to lower performances, because the individual feels pressure to perform well. Likewise, amounts that are too small lead to lower performances, even lower than those who do not have any incentive^[29]. These considerations suggest that our participants' intuitions about the reward amounts are in the right ballpark, although this needs to be evaluated empirically.

The strengths of our study include its focus on the use of incentives, both financial and non-financial, in a diabetic population. Although the relatively moderate number of participants may limit its generalizability, its web-based modality allowed us to recruit nationwide. This modality however also has its limitations, as the participants are self-selected, and received tokens in exchange for taking part in the survey (no compensation was given by the investigators, this was solely a feature of the survey company). Our population may therefore be disproportionately biased in their interest in incentives. A final limitation to our study lies in its design as a survey, as we assess the participants' expected response to incentives, which may differ from their actual response to incentives. Future studies are needed to confirm our findings, and to further explore the acceptability and feasibility of incentives for diabetes self-management in a larger population.

According to our nationwide survey, patients with diabetes are willing to consider using incentives, both financial and non-financial, to improve diabetes self-management. While the financial incentives may be more effective for behavior changes that are perceived as less challenging, non-financial incentives may be useful for the more challenging behaviors. Participants did not expect to need large amounts to motivate them to modify their behavior. Our findings suggest that the effectiveness of incentives could vary, and may depend on the perceived difficulty of the incentivized task. Future studies are needed to confirm these results in interventional studies on larger populations.

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COMMENTS

Background

Financial incentives have shown potential for modifying behaviors such as smoking cessation. Diabetes self-management requires many behavior changes, which may benefit from such incentives. Little is known about patient attitudes in response to incentives for diabetes self-management.

Research frontiers

Although incentives have been studied for certain behaviors, the current research hotspot is patient acceptance of incentives for diabetes self-management, in particular for the goals, type (financial, non-financial or self-reward) and amount of incentives.

Innovations and breakthroughs

Beyond patient attitudes and expectations about their response to incentives, this survey provides suggestions about goal-setting in reward programs, in relation to the perceived level of challenge of certain behaviors. Individualization of goals and rewards could be feasible with the exponential adoption of mobile devices, which could both track and reward behaviors ubiquitously.

Applications

The results of this study can help guide future interventional studies with incentives, both in goal-setting in terms of types of behaviors, types of rewards, and amounts for financial incentives.

Terminology

Financial incentives include all rewards relating to monetary rewards or equivalents such as vouchers (cash or discount for a future purchase, for example). Non-financial incentives are rewards such as badges or stars in social networks that provide recognition from others for an achieved feat. Self-rewards are rewards that a person gives themselves when they reach a predefined goal, often planned as small rewards that accumulate to reach a large, final reward.

Peer-review

In this well-written paper, the author investigates the patient willingness to change diabetes self-management behaviors in response to rewards. The reviewers found this approach to improve patient engagement interesting. The results can help guide the creation and implementation of new patient-centered approaches for diabetes self-management.

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Diabetic nephropathy in Africa: A systematic review

Jean Jacques N Noubiap, Jashira Naidoo, Andre P Kengne

Jean Jacques N Noubiap, Internal Medicine Unit, Edéa Regional Hospital, PO BOX 100 Edéa, Cameroon

Jashira Naidoo, Department of Medicine, Groote Schuur Hospital, University of Cape Town, 7925 Observatory, Cape Town, South Africa

Jashira Naidoo, Andre P Kengne, Non-Communicable Diseases Research Unit, South African Medical Research Council, 7505 Cape Town, South Africa

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Correspondence to: Andre P Kengne, Professor, Medical Research Council of South Africa, PO Box 19070 Tygerberg, 7505 Cape Town, South Africa. andre.kengne@mrc.ac.za
Telephone: +27-21-9380529
Fax: +27-21-9380460

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Abstract

AIM: To determine the prevalence and incidence of diabetic nephropathy in Africa.

METHODS: We performed a systematic narrative review of published literature following the MOOSE Guidelines for Meta-Analysis and Systematic Reviews

of Observational Studies. We searched PubMed-MEDLINE for all articles published in English and French languages between January 1994 and July 2014 using a predefined strategy based on the combination of relevant terms and the names of each of the 54 African countries and African sub-regions to capture the largest number of studies, and hand-searched the reference lists of retrieved articles. Included studies reported on the prevalence, incidence or determinants of chronic kidney disease (CKD) in people with diabetes within African countries.

RESULTS: Overall, we included 32 studies from 16 countries; two being population-based studies and the remaining being clinic-based surveys. Most of the studies (90.6%) were conducted in urban settings. Methods for assessing and classifying CKD varied widely. Measurement of urine protein was the most common method of assessing kidney damage (62.5% of studies). The overall prevalence of CKD varied from 11% to 83.7%. Incident event rates were 94.9% for proteinuria at 10 years of follow-up, 34.7% for end-stage renal disease at 5 years of follow-up and 18.4% for mortality from nephropathy at 20 years of follow-up. Duration of diabetes, blood pressure, advancing age, obesity and glucose control were the common determinants of kidney disease.

CONCLUSION: The burden of CKD is important among people with diabetes in Africa. High quality data from large population-based studies with validated measures of kidney function are still needed to better capture the magnitude and characteristics of diabetic nephropathy in Africa.

Key words: Diabetes; Diabetes nephropathy; Chronic kidney disease; Epidemiology; Prevalence; Incidence; Mortality; Africa; Systematic review

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Core tip: Chronic kidney disease is a serious health threat for people with diabetes in Africa, with prevalence

figures ranging from 11% to 83.7%. The incidence estimates suggest that 95% of people with diabetes may have proteinuria after 10 years from diabetes diagnosis; about 35% may develop end-stage renal disease after 5 years and 18% die from nephropathy after 20 years of disease duration. Hypertension, obesity, poor glycemic control and diabetes duration are the main risk factors of chronic kidney disease among diabetic patients in Africa. High quality data are needed to refine the epidemiology of diabetic nephropathy on the continent.

Noubiap JJN, Naidoo J, Kengne AP. Diabetic nephropathy in Africa: A systematic review. *World J Diabetes* 2015; 6(5): 759-773 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i5/759.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i5.759>

INTRODUCTION

Africa, like the rest of the world, is experiencing an increasing prevalence of diabetes alongside other non-communicable diseases, mainly as a result of urbanization, sedentary lifestyles, obesity and population growth and ageing^[1]. Estimates for 2013 by the International Diabetes Federation (IDF) indicate that the number of adults with diabetes in the world will expand by 55%, from 381.8 million in 2013 to 591.9 million in 2035^[2]. The largest increase of the population with diabetes will occur in sub-Saharan Africa, with a projected growth of 109.6%, from 19.8 million in 2013 to 41.5 million in 2035^[2].

Diabetes causes significant morbidity, disability and early mortality. Diabetes has been identified as a major contributor in several other important diseases, both non-communicable diseases such as cardiovascular disease and renal disease^[3,4], and communicable diseases such as invasive bacterial infections^[5,6]. Mortality attributable to diabetes in sub-Saharan Africa was estimated to account for 8.6% of the total death in 2013^[7]. Diabetic nephropathy (DN) is one of the most common complications of diabetes. The prevalence of DN is increasing steeply along with the diabetes epidemic^[8]. Approximately one third to half of patients with diabetes develops renal manifestations^[8-11]. DN is associated with increased premature mortality, end-stage renal disease and need to renal replacement therapy, cardiovascular diseases, and escalating health-care costs^[8].

DN has been suggested to be more frequent among patients with diabetes in Africa as compared to those in the developed world due to delayed diagnosis, limited screening and diagnostic resources, poor control of blood sugar and other risk factors, and inadequate treatment at an early stage^[7,12,13]. However, evidence to support the burden of kidney diseases in people with diabetes in Africa remains very patchy, and

we are not aware of any effort to synthesize existing data on the occurrence of kidney disease in African populations with diabetes. Accordingly, the aim of this review is to provide a comprehensive overview of the published evidence on the occurrence of nephropathy in African people with diabetes.

MATERIALS AND METHODS

Data sources and search strategy

A systematic narrative review of published literature was performed following the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies^[14]. We searched MEDLINE *via* PubMed for articles published in English and French on DN in Africa between January 1994 and July 2014, using a predefined strategy based on the combination of relevant terms and the names of each of the 54 African countries and African sub-regions to capture the largest number of studies. The data search was limited to human studies. The last search date was October 22, 2014. Search histories are provided in Table 1. Once duplicate references were removed the titles and abstracts of the references were screened. The references of included articles were scanned to identify additional articles of interest.

Study selection and data extraction

We included cross-sectional, case-control or cohort studies of subjects with diabetes mellitus resident in African countries reporting the prevalence or incidence or progression of DN. We excluded studies of populations of African origin residing outside Africa; case series (sample size less than 50 subjects), letters, comments and editorials; studies not published in English or French. Two investigators (JJNN, APK) independently identified articles and sequentially screened them for inclusion (Figure 1). Disagreements were solved by a third investigator (JN). Full text articles were reviewed by two investigators (JJNN and APK) who independently extracted data regarding study setting and design, study population characteristics and prevalence or incidence of DN.

RESULTS

We identified 730 articles, of which 73 were reviewed in full-text; 32 met the inclusion criteria (Figure 1)^[15-46].

Characteristics of included studies

Characteristics of the included studies are summarized in Table 2. The 32 studies were performed in 16 countries, with a geographical distribution covering all the African regions. However, more than half the studies [18 (56.3%)] were from South Africa (five), Nigeria (four), DR Congo (three) and Ethiopia (three).

Only two population-based studies were identified. In Democratic Republic of Congo, between March and April 2007, Makulo *et al*^[35] studied pathologic

Table 1 Search history PubMed

Search	Search terms	Hits
1	Diabetes[tw] OR Diabetes mellitus[tw] OR Type 1 diabetes[tw] OR Type 1 diabetes mellitus[tw] OR T1DM[tw] OR Type 2 diabetes[tw] OR Type 2 diabetes mellitus[tw] OR T2DM[tw] OR Hyperglycemia[tw] OR Glucose intolerance[tw]	445204
2	Renal insufficiency[tw] OR Renal failure[tw] OR Renal injury[tw] OR Renal disease[tw] OR Kidney insufficiency[tw] OR Kidney failure[tw] OR Kidney injury[tw] OR Kidney disease[tw] OR End-stage renal disease[tw] OR End-stage renal failure[tw] OR End-stage kidney disease[tw] OR End-stage kidney failure [tw] OR End stage renal disease[tw] OR End stage renal failure[tw] OR End stage kidney disease[tw] OR End stage kidney failure [tw] OR Microalbuminuria [tw] OR Micro-albuminuria OR Macroalbuminuria [tw] or Macro-albuminuria [tw]	154354
3	# 1 AND # 2	20388
4	Diabetic nephropathy [MeSH Terms]	19406
5	# 3 OR # 4	34221
6	(((((("Africa"[MeSH] OR Africa*[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR "Burkina Faso"[tw] OR Burundi[tw] OR Cameroon[tw] OR "Canary Islands"[tw] OR "Cape Verde"[tw] OR "Central African Republic"[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR "Democratic Republic of Congo"[tw] OR Djibouti[tw] OR Egypt[tw] OR "Equatorial Guinea"[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR "Guinea Bissau"[tw] OR "Ivory Coast"[tw] OR "Cote d'Ivoire"[tw] OR Jamahiriya[tw] OR Jamahiriya[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Libya[tw] OR Libia[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mayote[tw] OR Morocco[tw] OR Mozambique[tw] OR Mocambique[tw] OR Namibia[tw] OR Niger[tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR "Sao Tome"[tw] OR Senegal[tw] OR Seychelles[tw] OR "Sierra Leone"[tw] OR Somalia[tw] OR "South Africa"[tw] OR "St Helena"[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR "Western Sahara"[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw] OR "Central Africa"[tw] OR "Central African"[tw] OR "West Africa"[tw] OR "West African"[tw] OR "Western Africa"[tw] OR "Western African"[tw] OR "East Africa"[tw] OR "East African"[tw] OR "Eastern Africa"[tw] OR "Eastern African"[tw] OR "North Africa"[tw] OR "North African"[tw] OR "Northern Africa"[tw] OR "Northern African"[tw] OR "South African"[tw] OR "Southern Africa"[tw] OR "Southern African"[tw] OR "sub Saharan Africa"[tw] OR "sub Saharan African"[tw] OR "subSaharan Africa"[tw] OR "subSaharan African"[tw]) NOT ("guinea pig"[tw] OR "guinea pigs"[tw] OR "aspergillus niger"[tw])))	354928
7	# 5 AND # 6	1065
8	#4 Limits: 1994/01/01 to 2014/10/22 and studies done in Humans	918

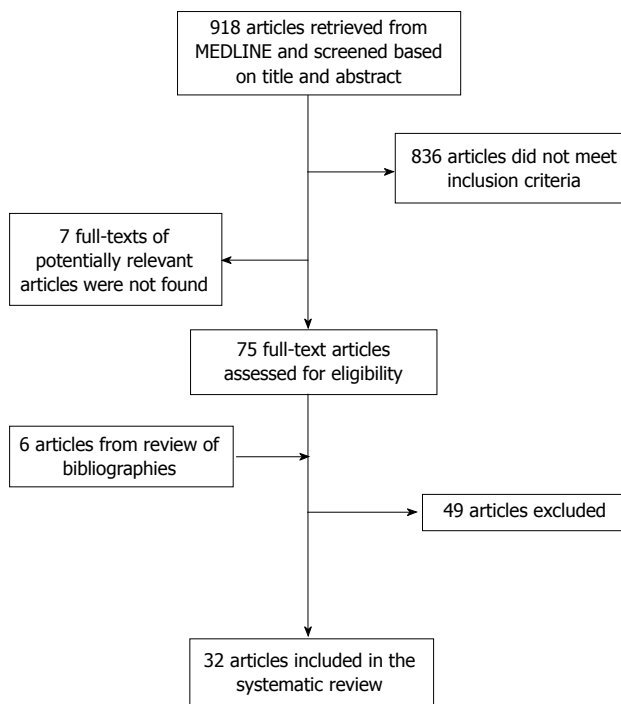


Figure 1 Flow diagram of study selection.

albuminuria among 81 diabetic patients identified through a population-based survey on the prevalence of diabetes involving 1898 participants^[35]. Pruijm *et al*^[39] in Seychelles in 2004, conducted a large-scale population-based estimate of the prevalence of microalbuminuria among 1218 adults. All other studies were clinic-based surveys conducted mostly in

diabetic clinics. There were three cohort studies (two prospective and one retrospective), one case-control study and the other 28 studies were cross-sectional with non-random sampling. Only three (9.4%) studies were conducted in rural settings.

Methods of assessment and classification of chronic kidney disease (CKD) varied widely. The studies assessed kidney function by urine protein [20 (62.5%) studies], urine albumin-to-creatinine ration (ACR) [9 (28.1%) studies], and estimation of glomerular filtration rate (GFR) by Cockcroft-Gault formula [3 (9.4%) studies] or by MDRD formula [4 (12.4%) studies]. Six studies (18.8%) measured kidney function by two methods, and renal biopsy was not performed in any study.

Prevalence of CKD

As depicted in Table 3, the overall prevalence of CKD varied from 11% in Tunisia to 83.7% in Tanzania^[20,29]. In studies where proteinuria was used to assess CKD, the prevalence varied from 5.3% in South Africa to 53.1% in Cameroon (study with a small sample size)^[32,44]. When considering the estimation of the GFR, the prevalence ranged from 4.6% in Tanzania to 43.1% in Nigeria (study with a small sample size)^[15,33].

Incidence of CKD

A study in South Africa investigated the long-term incidence of proteinuria among T2DM patients. After 12 years of follow-up or death, 94.9% (56/59) had a proteinuria with a mean duration from diabetes onset to proteinuria of 9.7 (5.9) years^[31]. In another study in South Africa, found that 18.4% of T1DM patients had

Table 2 General characteristics of studies of chronic kidney disease in people with diabetes in Africa

Ref.	Country	Period	Design	Setting	Sample size	Mean or median age (yr)	Male (%)	Type and duration of diabetes (yr)	Duration FUP	Method for CKD assessment		
										Proteinuria	MDRD	Cockcroft-Gault
Motala <i>et al</i> ^[37] , 2001	South Africa	Not precised	Retrospective cohort study	Clinic, urban	219	39.5 T1DM; 58.4 T2DM	19.6	16.10 T1DM; 18.6 T2DM	At least 10 yr	persistent proteinuria (Dipstick)		
Elbagir <i>et al</i> ^[26] , 1995	Sudan	Jan-July 1992	Cross-sectional, self-selected sampling	Clinic, urban	128	31.5 (15-75)	48.4	Insulin-treated; 9 (1-40)	NA	Proteinuria (Dipstick)		
Sobngwi <i>et al</i> ^[44] , 1999	Cameroon	Not precised	Cross-sectional, self-selected sampling	Clinic, urban	64	37.4 normotensive T1DM; 51.7 normotensive T2DM; 57.9 hypertensive T1DM	57.8	6.7 normotensive T1DM; 4.7 normotensive T2DM; 4.8 hypertensive T1DM	NA	Proteinuria (Dipstick)		
Katchunga <i>et al</i> ^[60] , 2010	DR congo	2005-2007	Cross-sectional, self-selected sampling	Clinic, urban	98	58 (10.4)	35.7	7.3 T2DM	NA		MDRD (corrected for Blacks)	
Choukem <i>et al</i> ^[22] , 2012	Cameroon	Jan 2008-Oct 2010	Cross-sectional, self-selected sampling	Clinic, urban	420	56.7	49	4 (1-9) T2DM	NA	Proteinuria (Dipstick)		
Keeton <i>et al</i> ^[61] , 2004	South Africa	Not precised	Prospective cohort, self-selected sampling	Clinic, urban	59	62	35.6	17.8 T2DM	12 yr			Urine ACR
Prujim <i>et al</i> ^[69] , 2008	Seychelles	2004	Cross-sectional; random sex and age-stratified sample	Population	1218 (whole sample, including diabetic patients)	Not precised	45.9	Newly diagnosed patients	NA			Urine ACR
Alebiosu ^[66] , 2003	Nigeria	Jan 2000	Cross-sectional, self-selected sampling	Clinic, urban	342	6.5 T1DM; 9.4 T2DM	53.8	26 T1DM; 53.4 T2DM	NA	Persistent proteinuria		
Bouaziz <i>et al</i> ^[20] , 2012	Tunisia	Jan 2008	Cross-sectional, self-selected sampling	Clinic, urban	73	59.3	23.3	T2DM 10.6	NA	Proteinuria		
Ajayi <i>et al</i> ^[13] , 2014	Nigeria	Not precised	Retrospective cross-sectional	Clinic, urban	65	Not available	Not available	T2DM	NA		MDRD	
Levitt <i>et al</i> ^[23] , 1997	South Africa	July-December 1992	Cross-sectional, stratified random sampling	Clinic, urban	243	56.4	38.3	8 T2DM and T1DM	NA	Persistent proteinuria		Urine ACR
Majaliwa <i>et al</i> ^[34] , 2007	Tanzania	June 2005-Feb 2006	Cross-sectional, self-selected sampling	Clinic, urban	99	12.6	42.4	4.76 T1DM	NA	Proteinuria		
Marshall <i>et al</i> ^[36] , 2013	Rwanda	June 2009-Nov 2010	Cross-sectional, self-selected sampling	Clinic, urban	286	18.6	46.5	3.4 T1DM	NA	Proteinuria		Urine ACR
Alebiosu <i>et al</i> ^[18] , 2003	Nigeria	Sept 1999-August 2002	Cross-sectional, self-selected sampling	Clinic, urban	465	Not precised	Not precised	T2DM	NA	Proteinuria		
Gill <i>et al</i> ^[28] , 2005	South Africa	From 1982 to 2002	Prospective cohort, self-selected sampling	Clinic, urban	88	22 at onset	52	T1DM	20 yr			
Djrolo <i>et al</i> ^[24] , 2001	Benin	Not indicated	Cross-sectional	Clinic, urban	152	53.3	65.8	T1DM and T2DM	NA	Proteinuria		

Rotchford <i>et al</i> ^[43] , 2002	South Africa	1999	Cross-sectional, self-selected sampling	Clinic, rural	253	56.5	26.9	42.2; T1DM and T2DM	NA	Urine ACR
Rissassi <i>et al</i> ^[42] , 2009	DR Congo	11 June 2008 to 30 July 2008	Cross-sectional, self-selected sampling	Clinic, urban	181	19.1	38.7	57.6 T1DM	NA	Urine ACR
Rahlenbeck <i>et al</i> ^[40] , 1997	Ethiopia	January - April 1995	Cross-sectional, self-selected sampling	Clinic, urban	170	31.4 T1DM; 56.7 T2DM	60	5.9 T1DM; 6.0 T2DM	NA	Proteinuria
Wanjohi <i>et al</i> ^[45] , 2002	Kenya	June 2000 - January 2001	Cross-sectional, self-selected sampling	Clinic, urban	100	53.7	37	10.3 T2DM	NA	Albuminuria
Nambuya <i>et al</i> ^[48] , 1996	Uganda	1 January 1993 - 10 August 1994	Cross-sectional, self-selected sampling	Clinic, urban/urban (origin of participants)	252	Not precised	46.4	45 (range 30-69) T2DM and T1DM	NA	Proteinuria
Rasmussen <i>et al</i> ^[49] , 2013	Zambia	February - April 2011	Cross-sectional, self-selected sampling	Clinic, rural	101	50 (range 50-68)	37.3	T2DM and T1DM	NA	Urine ACR
Bentata <i>et al</i> ^[19] , 2013	Morocco	From September 2006	Prospective cohort study	Clinic, urban	72	29.5	69.4	17 (11-20) T1DM	5 yr	Proteinuria MDRD
Gill <i>et al</i> ^[27] , 2008	Ethiopia	Not precised	Cross-sectional, self-selected sampling	Clinic, rural	105	41	70.5	7 T1DM and T2DM	NA	Urine ACR
Bouaid <i>et al</i> ^[21] , 2011	Tunisia	June 2006 - July 2008	Cross-sectional, self-selected sampling	Clinic, urban	689	60	39.3	11 T2DM	NA	Proteinuria
Janmohamed <i>et al</i> ^[29] , 2013	Tanzania	October 2011 - March 2012	Cross-sectional, self-selected sampling	Clinic, urban	369	54 (IQR 45-62)	46.6	6 (3-11) T1DM (6.2%) and T2DM (93.8%)	NA	Cockcroft-Gault
Danquah <i>et al</i> ^[23] , 2012	Ghana	August 2007 - June 2008	Cross-sectional, self-selected sampling	Clinic, urban	675	54.7	25	T2DM	NA	Proteinuria
Lutale <i>et al</i> ^[31] , 2007	Tanzania	July 2003 - March 2004	Cross-sectional, self-selected sampling	Clinic, urban	244	T1DM 21(range 4-44.8) T2DM 53 (range 23.5-85) 44.4	46.3	T1DM 3 (0-17) T2DM 4 (range 0-25) T1DM and T2DM; 53.4% less than 5 yr and 33.8% 5-9 yr	NA	Proteinuria
Worku <i>et al</i> ^[46] , 2010	Ethiopia	October 2008	Cross-sectional, self-selected sampling	Clinic, urban	305		62.9		NA	Proteinuria
Makulo <i>et al</i> ^[35] , 2010	DR Congo	30 March - 24 April 2007	Cross-sectional, self-selected sampling	Population-based, Urban	81	Not precised	Not precised		NA	MDRD Urine ACR
Eghan <i>et al</i> ^[25] , 2007	Ghana	January - July 2005	Cross-sectional, self-selected sampling	Clinic, urban	109	54.1	28	T1DM and T2DM 10.7	NA	Proteinuria
Alebiosu <i>et al</i> ^[17] , 2004	Nigeria	January 2000 - June 2001	Case (T2DM with persistent proteinuria-control (T2DM patients nephropathy)	Clinic, urban	162	53.4	50	T2DM 9.4 cases, 5.5 controls	NA	

ACR: Albumin-to-Creatinine Ratio; FUP: Follow-up; MDRD: Modification of diet renal disease; NA: Not applicable.

Table 3 Prevalence and incidence of chronic kidney disease in people with diabetes across studies in Africa

Ref.	Country	Sample size	Type of diabetes	Duration of follow-up	Diagnostic criteria for CKD	Prevalence	Incidence	Comments
Motala <i>et al</i> ^[37] , 2001	South Africa	219	T1DM and T2DM	16.10 (4.9) T1DM; 18.6 (5.7) T2DM; at least 10 yr	Persistent proteinuria (dipstick proteinuria on three or more consecutive occasions over 18 mo in the at absence of infection or cardiac failure)	Not applicable	24.6%	
Elbagir <i>et al</i> ^[26] , 1995	Sudan	128	Insulin-treated	Not applicable	Proteinuria (≥ 30 mg/dL)	22%	Not applicable	
Sobngwi <i>et al</i> ^[44] , 1999	Cameroon	64	T1DM and T2DM	Not applicable	Proteinuria	53.1%	Not applicable	
Katchunga <i>et al</i> ^[30] , 2010	DR Congo	98	T2DM	Not applicable	MDRD: CKD stage ≥ 2 according to the National Kidney foundation	18.1%	Not applicable	
Choukem <i>et al</i> ^[22] , 2012	Cameroon	420	T2DM	Not applicable	Proteinuria (30 mg/24 h)	31%	Not applicable	
Keeton <i>et al</i> ^[31] , 2004	South Africa	59	T2DM	12 yr	Urine Albumin-to-Creatinine Ratio (no detail)		After 12 yr of follow-up or death, 94.9% (56/59) had a proteinuria with a mean duration from diabetes onset to proteinuria of 9.7 (5.9) yr	83% (49/59) had an elevated SCr at the end of the study and in 66.1% (39/59) the SCr level had doubled during the study
Pruijm <i>et al</i> ^[39] , 2008	Seychelles	1218	All types	Not applicable	Microalbuminuria: Urine Albumin-to-Creatinine Ratio 3.4-33.9 mg albumin/mmol creatinine	36.1%	Not applicable	
Alebiosu ^[16] , 2003	Nigeria	342	T1DM and T2DM	Not applicable	Persistent proteinuria	28.4%	Not applicable	
Bouaziz <i>et al</i> ^[20] , 2012	Tunisia	73	T2DM	Not applicable	Microalbuminuria: < 2.8 g/mol for women and < 2.3 g/mol for men	11%	Not applicable	
Ajayi <i>et al</i> ^[15] , 2014	Nigeria	65	T2DM	Not applicable	MDRD: eGFR ≤ 60 mL/min per 1.73 m^2	43.1%	Not applicable	
Levitt <i>et al</i> ^[32] , 1997	South Africa	243	T2DM and T1DM	Not applicable	Urine Albumin-to-Creatinine Ratio > 3.4 mmol/mmol	36.7%	Not applicable	
					Persistent proteinuria (for at least 3 consecutive visits)	5.3%		
Majaliwa <i>et al</i> ^[34] , 2007	Tanzania	99	T1DM	Not applicable	Proteinuria (no detail)	29.3%	Not applicable	
Marshall <i>et al</i> ^[36] , 2013	Rwanda	286	T1DM	Not applicable	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g	Microalbuminuria: 21%; Macroalbuminuria: 5%	Not applicable	
					Macroalbuminuria or overt nephropathy: Urine Albumin-to-Creatinine Ratio ≥ 300 mg/g			
Alebiosu <i>et al</i> ^[18] , 2003	Nigeria	465	T2DM	Not applicable	Proteinuria and eGFR	41.1%	Not applicable	The method for the estimation of the GFR is not indicated
Gill <i>et al</i> ^[28] , 2005	South Africa	88	T1DM	20 yr	Persistent dipstick proteinuria		Death of renal cause after 20 yr = 18.4% (9/49)	Death due to chronic renal failure after 20 yr of follow-up was 9/49 (after exclusion of lost to follow)
Djrolo <i>et al</i> ^[24] , 2001	Benin	152	T1DM and T2DM	Not applicable	Proteinuria (no detail)	20%	Not applicable	
Rotchford <i>et al</i> ^[43] , 2002	South Africa	253	T1DM and T2DM	Not applicable	Microalbuminuria > 2.5 mg/mmol in men or 3.5 mg/mmol in women	46.4%	Not applicable	

Rissassi <i>et al</i> ^[42] , 2009	DR congo	181	T1DM	Not applicable	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g Macroalbuminuria: Urine Albumin-to-Creatinine Ratio \geq 300 mg/g	21.9% (microalbuminuria) and 7.3% (macroalbuminuria)	Not applicable	
Rahlenbeck <i>et al</i> ^[40] , 1997	Ethiopia	170	T1DM and T2DM	Not applicable	Microalbuminuria: > 30 mg/L Macroalbuminuria: > 300 mg/L	T1DM: 32% (microalbuminuria) and 15% (macroalbuminuria) T2DM: 37% (microalbuminuria) and 20% (macroalbuminuria)	Not applicable	
Wanjohi <i>et al</i> ^[45] , 2002	Kenya	100	T2DM	Not applicable	Proteinuria \geq 20 mg	26%	Not applicable	
Nambuya <i>et al</i> ^[38] , 1996	Uganda	252	T1DM and T2DM	Not applicable	Proteinuria (no detail)	17.1%	Not applicable	Newly diagnosed patients
Rasmussen <i>et al</i> ^[41] , 2013	Zambia	101	T1DM and T2DM	Not applicable	Microalbuminuria: ACR = 3.5-35.0 for women and 2.5-25.0 mg/mmol for men Macroalbuminuria were ACR > 35.0 for women and > 25.0 for men	Microalbuminuria: 23.8% Macroalbuminuria: 8.9%	Not applicable	There were 33 patients with diabetes alone, and 68 patients with diabetes and hypertension
Bentata <i>et al</i> ^[19] , 2013	Morocco	72	T1DM	5 yr	Microalbuminuria: albumin excretion rate 30-300 mg/24 h Macroalbuminuria: albumin excretion rate > 300 mg/24 h Nephrotic proteinuria: albumin excretion rate \geq 3000 mg/24 h Renal failure: eGFR < 60 mL/min (MDRD)	At the time of enrollement Microalbuminuria: 48.6% Macroalbuminuria: 36.1% Nephrotic proteinuria: 15.3%	The incidence of end stage renal disease after 5 yr: 34.7%	Urinary assays done on admission were repeated on three specimens at three-monthly intervals
Gill <i>et al</i> ^[27] , 2008	Ethiopia	105	T1DM and T2DM	Not applicable	Nephropathy: ACR > 25.0 mg/mmol and retinopathy present Microalbuminuria: ACR > 2.5 and < 25.0 mg/mmol in men and > 3.5 and < 25.0 mg/mmol in women	Nephropathy: 2% Microalbuminuria: 51%		Urinary ACR levels (to assess microalbuminuria and nephropathy) were done on 59 patients, as those with haematuria and/or urinary infection were excluded
Bouzzid <i>et al</i> ^[21] , 2011	Tunisia	689	T2DM	Not applicable	CKD: eGFR < 60 mL/min per 1.73 m ² (Cockcroft-Gault) Microalbuminuria: albumin excretion rate 30-300 mg/24 h Macroalbuminuria: albumin excretion rate > 300 mg/24 h	CKD: 19.8% Microalbuminuria: 13% Macroalbuminuria: 10.1%	Not applicable	Macroalbuminuria was significantly associated with CKD ($P < 0.00001$)
Janmohamed <i>et al</i> ^[29] , 2013	Tanzania	369	T1DM and T2DM	Not applicable	CKD: eGFR < 60 mL/min per 1.73 m ² (Cockcroft-Gault) or microalbuminuria (> 20 mg/L) or overt proteinuria	CKD: 83.7% eGFR < 60 mL/min per 1.73 m ² : 24.7% Microalbuminuria: 45.8% Overt proteinuria: 34.1%	Not applicable	
Danquah <i>et al</i> ^[23] , 2012	Ghana	671	T2DM	Not applicable	Proteinuria \geq 20 mg/L	43%	Not applicable	
Lutale <i>et al</i> ^[33] , 2007	Tanzania	244	T1DM and T2DM	Not applicable	Microalbuminuria: AER 20-200 μ g/min Macroalbuminuria: AER > 200 μ g/min Renal failure: eGFR < 60 mL/min per 1.73 m ²	Microalbuminuria: 12.1% (T1DM); 9.8% (T2DM) Macroalbuminuria: 1.1% (T1DM); 7.2% (T2DM) Renal failure: 4.6% (T1DM); 22% (T2DM)	Not applicable	

Worku <i>et al</i> ^[46] , 2010	Ethiopia	305	T1DM (38%) and T2DM (62%)	Not applicable	Proteinuria (no detail)	15.7%	Not applicable	
Makulo <i>et al</i> ^[35] , 2010	DR Congo	81	No precision	Not applicable	Microalbuminuria: ACR 30-299 mg/g Macroalbuminuria: ACR \geq 300 mg/g Renal failure: eGFR < 60 mL/min per 1.73 m ²	Microalbuminuria: 43.5% Macroalbuminuria: 12% Renal failure: 21.4%	Not applicable	
Eghan <i>et al</i> ^[25] , 2007	Ghana	109	T1DM and T2DM	Not applicable	Microalbuminuria: ACR 30-300 mg/g	43.1%	Not applicable	
Alebiosu <i>et al</i> ^[17] , 2004	Nigeria	162	T2DM	Not applicable	Not applicable	Not applicable	Not applicable	The study did not assess the prevalence or incidence of diabetic nephropathy, but its predictors

ACR: Albumin-to-Creatinine Ratio; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; CKD: Chronic kidney disease; eGFR: Epidermal growth factor receptor.

died from renal nephropathy after 20 years of follow-up^[28]. In a recent study in Morocco, the incidence of end-stage renal disease after 5 years was 34.7%^[19].

Risk factors of CKD

Twenty studies (62.5%) reported factors associated with CKD in diabetic patients (Table 4). However, in most studies the method to assess this association was imprecise. In cross-sectional studies, correlates of CKD included systolic and diastolic high blood pressure, long duration of diabetes, older age, dyslipidemia, obesity^[16-22,25,26,29-31,33,36,40,42-44,46]. In a study in Cameroon, T2DM patients with systolic hypertension and diastolic hypertension were respectively 1.45 (95%CI: 1.15-1.84; $P = 0.006$) and 1.33 (95%CI: 1.06-1.66; $P = 0.026$) times more likely to have nephropathy^[22]. Two studies in Rwanda and South Africa respectively showed that a one year increase in the duration of T1DM increased by 0.86 (95%CI: 0.77-0.96; $P = 0.008$) the odds of microalbuminuria^[36], and that T1DM and T2DM patients with a duration of diabetes greater than 10 years were 4.19 times (95%CI: 1.93-9.10; $P < 0.001$) more likely to have microalbuminuria^[43]. Poor glycemic control as measured by HbA1c was also a strong predictor of nephropathy. For instance, HbA1c level greater than 10% and 14% were respectively associated with a 2.6 fold (95%CI: 1.1-6.4) and a 4.69 (95%CI: 1.65-13.3; $P = 0.004$)^[42,43]. A 1 g/dL decrease in hemoglobin level has been found to be associated with end-stage renal disease (OR 3.18, 95%CI: 1.47-6.87; $P = 0.003$)^[19]. Studies in Nigeria showed that left ventricular hypertrophy, stroke, myocardial infarction and peripheral arterial disease were more frequent in T2DM patients with nephropathy, especially those with advanced stages^[17,18].

plication of diabetes and the leading cause of CKD in the developed world. The lack of renal registries means that there are no reliable statistics about the burden of CKD in people with diabetes in the majority of African countries. The current systematic review identified 32 relevant studies published over the last 20 years on kidney diseases in people with diabetes residing in Africa. Prevalence rates ranged from 11% to 83.7% for the overall CKD, 5.3% to 53.1% for CKD based on proteinuria, and 4.6% to 43.1% for CKD based on eGFR. Incident event rates were 94.9% for proteinuria at 10 years for follow-up, 34.7% for ERSD at 5 years of follow-up and 18.4% for mortality from nephropathy at 20 years of follow-up. Diagnosed duration of diabetes, blood pressure variables, advancing age, obesity and to some extent glucose control were the common determinants of kidney disease in people with diabetes. Studies were overwhelmingly hospital-based studies; half of them originated from four countries while variable definitions and methods for assessing nephropathy had been used across studies.

The most recent overview of CKD in populations within Africa was completed in 2012, and was restricted to sub-Saharan African Countries^[47]. This review identified 90 articles representing data from 21 countries, with over half of the studies originating from South Africa, Nigeria and Ethiopia alones. Across 21 studies deemed to be of medium to high quality by the investigators, the pooled prevalence of CKD was 13.9% (95%CI: 12.2-15.7), with substantial heterogeneity across studies. The prevalence in people with diabetes ranged from 4% to 24% based essentially on proteinuria defined CKD^[47]. In our review without applying quality criteria, we found much higher prevalence of CKD, regardless of the definition. In four studies published in 2013 for instance, the prevalence of microalbuminuria ranged between 21% and 45%. Although issues with the quality of the studies preclude direct comparisons, it is likely that nephropathy is

DISCUSSION

Diabetic nephropathy is a common and morbid com-

Table 4 Risk factors for chronic kidney disease in people with diabetes

Ref.	Country	Sample size	Type of diabetes	Diagnostic criteria for CKD	Risk factor	Measure of association		Factors adjusted for	Comments
						Effect size	P-value		
Motala <i>et al</i> ^[37] , 2001	South Africa	219	T1DM and T2DM	Persistent proteinuria	Not assessed				
Elbagir <i>et al</i> ^[44] , 1995	Sudan	128	Insulin-treated	Proteinuria	Age Duration of diabetes Systolic BP Diastolic BP Serum cholesterol Duration of diabetes Diastolic BP Hypertension		P = 0.006 P = 0.003 P = 0.0001 P = 0.001 P < 0.05 P = 0.04 P = 0.01 P = 0.04		
Sobngwi <i>et al</i> ^[44] , 1999	Cameroon	64	T1DM and T2DM	Proteinuria				Age, duration of diabetes, BMI	
Katchunga <i>et al</i> ^[30] , 2010	DR Congo	98	T2DM	MDRD (corrected for Blacks), CKD stage ≥ 1 according to the National Kidney foundation		aOR: 2.49 (0.98-6.34)			
Choukem <i>et al</i> ^[22] , 2012	Cameroon	420	T2DM	Proteinuria (30 mg/24 h)	Systolic BP Diastolic BP Pulse pressure Mean arterial pressure High entry serum creatinine BMI < 28 Severe retinopathy Mean glucose level of > 14 mmol/L	aOR: 1.45 (1.15-1.84) aOR: 1.33 (1.06-1.66) aOR: 1.35 (1.06-1.71) aOR: 1.42 (1.13-1.78)	P = 0.006 P = 0.026 P = 0.0007 P = 0.006 P < 0.006 P < 0.003 P < 0.002 P < 0.035	These are risk factors for death from chronic renal failure (compared with the patients who were still alive at follow-up)	
Keeton <i>et al</i> ^[31] , 2004	South Africa	59	T2DM	Urine Albumin-to-Creatinine Ratio (no detail)					By the end of study 47 of the 59 patients had died; the cause of death not established in 2 patients. Death was due to chronic renal failure in 17 cases Risk factors were investigated in the whole study population in both diabetics and non-diabetics
Prujijm <i>et al</i> ^[39] , 2008	Seychelles	1218	All types	Microalbuminuria: Urine Albumin-to-Creatinine Ratio 3.4-33.9 mg albumin/mmol creatinine Persistent proteinuria	Not assessed				
Alebiosu ^[46] , 2003	Nigeria	342	T1DM and T2DM	Persistent proteinuria	Not assessed				
Bouaziz <i>et al</i> ^[20] , 2012	Tunisia	73	T2DM	Microalbuminuria: < 2.8 g/mol for women and < 2.3 g/mol for men	Family history of nephropathy Smoking Insulin therapy Glitazones therapy Anti-hypertensives (not ACE inhibitor) Lipid-lowering agents Not assessed		P = 0.0289 P = 0.0056 P = 0.0310 P = 0.0115 P < 0.0001 P < 0.0001	Comparison of T2DM patients with nephropathy with those without nephropathy	
Ajayi <i>et al</i> ^[13] , 2014	Nigeria	65	T2DM	MDRD: eGFR ≤ 60 mL/min per 1.73 m ²					
Levitt <i>et al</i> ^[23] , 1997	South Africa	243	T2DM and T1DM	Urine Albumin-to-Creatinine Ratio > 3.4 mmol/mmol and Persistent proteinuria (for at least 3 consecutive visits)	Not assessed				

Majaliwa <i>et al</i> ^[34] , 2007	Tanzania	99	T1DM	Proteinuria (no detail)	Missing insulin doses		<i>P</i> = 0.045	Not available	
Marshall <i>et al</i> ^[36] , 2013	Rwanda	286	T1DM	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g	Age (increase)	aOR: 0.86, 95%CI: 0.77-0.96	<i>P</i> = 0.009	Each variable is adjusted for the others	These are risk factors of microalbuminuria. There was no factor associated to macroalbuminuria
					Duration of diabetes (one year increase)	aOR: 0.86, 95%CI: 0.77-0.96	<i>P</i> = 0.008		
					Diastolic BP (increase)	aOR: 0.86, 95%CI: 0.77-0.96	<i>P</i> = 0.004		
					HbA1c (increase)	aOR: 0.86, 95%CI: 0.77-0.96	<i>P</i> = 0.047		
Alebiosu <i>et al</i> ^[18] , 2003	Nigeria	465	T2DM	Proteinuria and eGFR (no detail)	Hypertension, left ventricular hypertrophy, stroke and myocardial infarction were more frequent in advanced stages of nephropathy	Not available	<i>P</i> < 0.05	Not available	Patients with advanced stages of nephropathy (IV and V) were compared with those with stages ≤ III
Gill <i>et al</i> ^[28] , 2005	South Africa	88	T1DM	Persistent dipstick proteinuria	Not assessed				
Djrolo <i>et al</i> ^[20] , 2001	Benin	152	T1DM and T2DM	Proteinuria (no detail)			Not available	Not available	Proteinuria was more frequent in insulin-treated patients compared those on oral antidiabetic treatment. The prevalence of proteinuria also increased with the duration of diabetes
Rotchford <i>et al</i> ^[43] , 2002	South Africa	253	T1DM and T2DM	Microalbuminuria > 2.5 mg/mmol in men or 3.5 mg/mmol in women	Duration of diabetes > 10 yr BMI > 33 HbA1c > 14% Hypertension	4.19 (1.93-9.10) 0.27 (0.08-0.48) 4.69 (1.65-13.3) 2.11 (1.07-4.17)	< 0.001 0.002 0.004 0.031	Model contains duration of diabetes, BMI, HbA1c, age and hypertension No precision	
Rissassi <i>et al</i> ^[42] , 2009	DR congo	181	T1DM	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g Macroalbuminuria: Urine Albumin-to-Creatinine Ratio ≥ 300 mg/g	Duration of diabetes > 5 yr Age > 18 yr HbA1c > 10%	4.1 (1.9-8.4) 2.9 (1.3-6.2) 2.6 (1.1-6.4)			
Rahlenbeck <i>et al</i> ^[40] , 1997	Ethiopia	170	T1DM and T2DM	albuminuria: > 30 mg/L	Duration of diabetes Systolic blood pressure	Beta = 0.061, SE = 0.018 for T1DM Beta = 0.027, SE = 0.005 for T1DM	< 0.001 < 0.001	Hypertensive patients excluded	
Wanjohi <i>et al</i> ^[45] , 2002	Kenya	100	T2DM	Proteinuria ≥ 20mg	None identified				
Nambuya <i>et al</i> ^[38] , 1996	Uganda	252	T1DM and T2DM	Proteinuria (no detail)	None assessed				
Rasmussen <i>et al</i> ^[41] , 2013	Zambia	101	T1DM and T2DM	Microalbuminuria: ACR = 3.5-35.0 for women and 2.5-25.0 mg/mmol for men Macroalbuminuria were ACR > 35.0 for women and > 25.0 for men	None assessed				
Bentata <i>et al</i> ^[19] , 2013	Maroc	72	T1DM	End-stage renal disease: eGFR < 15 mL/min	Hemoglobin blood (per 1 g/dL decrease) Diastolic blood pressure (per 1 mmHg increase)	3.18 (1.47-6.87) 1.15 (1.04-1.27)	0.003 0.006	No precision	These are independent risk factors for ESRD in type-1 diabetes patients with diabetic nephropathy

Gill <i>et al</i> ^[21] , 2008	Ethiopia	105	T1DM and T2DM	Nephropathy: ACR > 25.0 mg/ mmol and retinopathy present Microalbuminuria: ACR > 2.5 and < 25.0 mg/ mmol in men and > 3.5 and < 25.0 mg/ mmol in women Renal failure: creatinine clearance < 60 mL/ min (Cockcroft-Gault)	None assessed				
Bouziid <i>et al</i> ^[21] , 2011	Tunisia	689	T2DM		Older age Hypertension Long duration of diabetes Higher BMI Dyslipidemia Older age	Not provided	< 0.00001 < 0.00001 < 0.001 0.02 0.01 0.03		
Jannohamed <i>et al</i> ^[20] , 2013	Tanzania	369	T1DM and T2DM	CKD: eGFR < 60 mL/ min per 1.73 m ² (Cockcroft-Gault) or microalbuminuria (> 20 mg/ L) or overt proteinuria Proteinuria ≥ 20mg/ l		1.03 (1.00-1.05)		Adjustment made, but no precision	
Danquah <i>et al</i> ^[23] , 2012	Ghana	671	T2DM		Not assessed				
Lutale <i>et al</i> ^[33] , 2007	Tanzania	244	T1DM and T2DM	Abnormal proteinuria: AER > 20 µg/ min	Duration of diabetes Elevated systolic blood pressure	0.090 (0.049- 0.131) 0.012 (0.003-0.021)	< 0.0001 0.010	Predictors in the model: diabetes duration, Systolic BP, age, serum creatinine	Measure of association is β
Worku <i>et al</i> ^[46] , 2010	Ethiopia	305	T1DM and T2DM	Proteinuria (no detail)	Elevated serum creatinine Duration of diabetes T2DM on insulin	0.011 (0.002- 0.020) Not provided	0.016 0.001 0.018		
Makulo <i>et al</i> ^[35] , 2010	DR Congo	81	No precision	Microalbuminuria: ACR 30-299 mg/ g Macroalbuminuria: ACR ≥ 300 mg/ g Renal failure: eGFR < 60 mL/ min per 1.73 m ²	Not assessed				
Eghan <i>et al</i> ^[25] , 2007	Ghana	109	T1DM and T2DM	Microalbuminuria: ACR 30-300 mg/ g	Duration of diabetes Serum creatinine Blood urea nitrogen Urine potassium		0.04 0.05 0.01 0.0061	The associations were assessed by comparing patients with and without microalbuminuria	
Alebiosu <i>et al</i> ^[17] , 2004	Nigeria	162	T2DM	No precision	Duration of diabetes Serum total cholesterol Alcohol > 30 mg/ d Peripheral vascular disease Stroke		< 0.05 < 0.05 < 0.05 < 0.05 < 0.05	The study assessed the predictors of diabetic nephropathy comparing T2DM patients with and without nephropathy	

CKD: Chronic kidney disease; BMI: Body mass index; ACR: Albumin-to-Creatinine Ratio; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; eGFR: Epidermal growth factor receptor.

more frequent in population with diabetes within Africa than in developed countries. The review by Stanifer *et al.*^[47] also identified many challenges and limitations, which largely apply to the current study.

The most important aspect in assessing incidence and prevalence of diabetic nephropathy in Africa is currently different diagnostic criteria for CKD. There are no clear definitions on DN. The 2012 KDIGO CKD classification assesses diabetes related kidney changes according to urinary albumin-to-creatinine ratio based on early morning spot urine samples^[48]. Quantification of proteinuria in assessing CKD is controversial as no optimal test exists. The National Institute for Health and Clinical Excellence (NICE) guidance has recommended that an early morning urinary ACR should be preferred to other tests of proteinuria, because ACR offers greater sensitivity for the detecting lower, but clinically significant, levels of proteinuria^[49]. Almost all the studies included in our review utilized urine tests to diagnose CKD, but only nine studies used ACR. Inconsistencies in the way and manner of reaching a diagnosis of DN in Africans are explained at least in part by issues relating to availability and accessibility of screening or diagnostic tools. Swanepoel *et al.*^[50] have reviewed in detail some of the problems associated with nephrology in Africa and discussed the role of lack of amenities in diagnosing renal diseases. Another challenge to making the diagnosis of diabetic nephropathy in Africa is the degree to which other causes of chronic kidney disease have been excluded. A standard armamentarium of tests would include tests looking for HIV, hepatitis B and C, brief collagen screen, syphilis exclusion and other tests would have to be based on history and physical exam.

The classification of CKD is important in the definition of DN and has a few limitations that are universally acknowledged: eGFR underestimates kidney function and there is discordance in the estimates across different estimators^[51]; isolated microalbuminuria is a normal feature of aging, inflammation, vascular pathologies, smoking, diet and obesity which are all frequent in diabetes; decline in kidney function is an expected phenomenon with advanced age, just like diabetes risk increases with age. Further considerations to CKD classifications and DN definition limitations is that current guidelines take no notice of the single most important risk factor associated with CKD namely hypertension, which is present in over 50% of people with type 2 diabetes.

Risk factor association was not assessed in 12 of the 32 studies, however common risk factors included were hypertension, raised BMI, HbA1c and duration of diabetes. Despite advances in management over the last three decades, many people with diabetes still develop CKD. This may be partly explained by the poor achievement of blood pressure and blood glucose targets. Recently the JNC 8 guidelines have added to the controversy of various blood pressure targets needed for diabetic patients that would

assist in preventing progression to CKD. Optimal targets when reached, however have shown to aid in progression to progression. Another risk factor pertinent to the developing world is the socioeconomic status of individuals in the causative role of diabetic nephropathy. Weil *et al.*^[52], in 2010 reviewed factors associated with disadvantage that may increase the risk of diabetic kidney disease, and the barriers to care that hinder attempts to provide an adequate therapeutic response^[52].

Several mechanisms underlying the pathogenesis of diabetic nephropathy have been suggested and include glomerular hyperfiltration; hyperglycemia and the increased production of advanced glycation end products; hypoxia-inflammation and the activation of cytokines. Hyperfiltration commonly occur in early in the course of diabetes and involves glucose-dependent dilation of the afferent arteriolar dilation, and the enhanced filtration area secondary to the increase in the number of mesangial cells and capillary loops. Molecular level action involves vasoactive mediators like insulin-like growth factor 1, transforming growth factor beta, nitric oxide, prostaglandin, glucagon and vascular endothelial growth factor^[53]. Other hallmarks of diabetic nephropathy include nodular diabetic glomerulosclerosis and diffuse glomerulosclerosis, mediated at least in part by inflammatory processes and immune cells activity^[53]. Interstitial fibrosis and tubular atrophy are also seen early in DN, with the underlying pathogenetic mechanism being similar to those in progressive non diabetic renal disease^[54].

Diabetic nephropathy ultimately occurs only in susceptible individuals with diabetes; which susceptibility is determined by the combined effect of genetic predisposition and non-genetic factors. Genetic susceptibility to diabetic nephropathy is by nature polygenetic. Whole-genome scanning studies have identified several chromosomal regions linked with diabetic nephropathy; however, the pathophysiologic function of such genetic regions has yet to be fully elucidated. Genetic polymorphisms may explain the familial clustering of diabetic nephropathy^[55]. Some studies have suggested some detrimental effect of the double-deletion (DD) polymorphism of the angiotensin-converting enzyme (ACE) genotype on disease progression^[56]. Non-genetic determinants of diabetic nephropathy include among others socioeconomic factors, dietary factors, poor hyperglycemic control, hypertension, obesity and early life factors^[57,58]. Hypertension appears to be a strong correlate of disease progression in Black people^[59,60].

The current review has some limitations. Included studies were mostly based on small samples, with different study designs and most of the studies were cross sectional with only two being retrospective cohorts and one case-control. A large proportion were based in urban clinics with and most of the populations studied were that attending a general diabetic clinic and the results may not be generalizable

to primary care populations. Ideally chronic kidney disease should not be diagnosed on the basis of single measurements of serum creatinine and albuminuria, and standard baseline investigations are needed to exclude other causative kidney disease, although there is precedence for this in other studies in the West as well. Finally, detection of microalbuminuria was one the most frequent method to assess the presence of diabetic nephropathy. As microalbuminuria is more a quantitative estimate of endothelial/vascular dysfunction than of diabetic nephropathy, the incidence and prevalence rate of diabetic nephropathy have probably been overestimated when assessing kidney function by urine protein.

In conclusion, the current review gives a small glimpse of the larger numbers of CKD in diabetics in Africa compared to Western society. CKD is a substantial health burden among diabetic patients on the African continent, with prevalence varying from 11% to 83.7% depending on the method of assessment. Estimates suggest that 95% of diabetics may have proteinuria after a 10 years duration of diabetes, about 35% may have an end-stage renal disease after 5 years and 18% die from nephropathy after 20 years of disease duration. Risk factors of CKD include mainly hypertension, obesity, poor glycemic control and disease duration. Better surveillance of diabetes is a necessary first step toward its prevention and control, which is now recognized as an urgent priority. An electronic database in African regions would be ideal to assist in this entity although it is presumed that we are light years away from that. At a primary care level it is very plausible that with early detection, proper screening, and management, the impact of diabetic nephropathy may be better mitigated to lessen its impact on society and healthcare.

COMMENTS

Background

African countries are experiencing an epidemics of diabetes mellitus. Diabetic nephropathy is one the most frequent complications of diabetes mellitus. Several studies on the epidemiology of diabetic nephropathy have been conducted in Africa, but there is no previous published work which synthesizes evidences from this study to provide an overview of the disease on the continent.

Research frontiers

Epidemiological data on diabetic nephropathy in Africa are sparse. These data are important to quantify the magnitude of the disease and assist the formulation of strategies to reduce the impact of nephropathy on people with diabetes in Africa.

Innovations and breakthroughs

This review is the first to synthesize relevant data on diabetic nephropathy in Africa. The authors performed extensive electronic and manual bibliographic searches to determine the prevalence and incidence of diabetic nephropathy on the continent. Although the quality of data was not optimal, estimates suggest that the prevalence of diabetic nephropathy vary between 11%-83.7%. About one third of diabetic patients have end-stage renal disease after 5 years and about one fifth die from nephropathy after 20 years of disease duration. Hypertension, obesity, poor glycemic control and disease duration are the main risk factors of chronic kidney disease among diabetic patients in Africa.

Applications

This review shows that the burden of chronic kidney disease is important among people with diabetes in Africa. The findings will have implications for policy, practice and future research on diabetic nephropathy on the continent.

Terminology

Diabetic nephropathy is an alteration of the function of the kidneys due to diabetes mellitus. It is associated with substantial morbidity and mortality.

Peer-review

The authors of the present manuscript performed extensive electronic and manual bibliographic research to determine the prevalence and incidence of kidney disease in people with diabetes mellitus within countries in Africa. Overall the review is well written.

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Editorial Board Member of *World Journal of Diabetes*, Wayne H-H Sheu, MD, PhD, Professor, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung 40705, Taiwan

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

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Fax: +1-925-223-8243

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Incretin manipulation in diabetes management

Joseph M Pappachan, AV Raveendran, Rajagopalan Sriraman

Joseph M Pappachan, Department of Endocrinology and Diabetes, New Cross Hospital, the Royal Wolverhampton Hospital NHS Trust, WV10 0QP Wolverhampton, United Kingdom

AV Raveendran, Department of Medicine, Kottayam Medical College, Kerala 686008, India

Rajagopalan Sriraman, Department of Endocrinology, Lincoln County Hospital, LN2 5QY Lincoln, United Kingdom

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Correspondence to: Dr. Joseph M Pappachan, MD, MRCP (London), Department of Endocrinology and Diabetes, New Cross Hospital, the Royal Wolverhampton Hospital NHS Trust, Wolverhampton Road, WV10 0QP Wolverhampton, United Kingdom. drpappachan@yahoo.co.in
 Telephone: +44-1922-721172
 Fax: +44-1922-721172

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Abstract

Incretin-based therapies have revolutionized the medical management of type 2 diabetes mellitus (T2DM) in the 21st century. Glucagon-like peptide-1 (GLP-1) suppresses appetite and gastric motility, and has trophic effects on pancreas, cardio-protective and renal effects. GLP-1 analogues and dipeptidyl peptidase-4 inhibitors form the incretin-based therapies. Significant reduction of hemoglobin A1c when used as monotherapy and in combination regimens, favorable effects on body weight, and low risk of hypoglycemia are their unique therapeutic benefits. Their safety and tolerability are comparable to other anti-diabetic medications. Concern about elevated risk of pancreatitis has been discarded by two recent meta-analyses. This article discusses the therapeutic manipulation of incretin system for the management of T2DM.

Key words: Incretin hormones; Incretin-based therapies; Glucagon-like peptide-1 analogues; Dipeptidyl peptidase-4 inhibitors; Pancreatitis

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Core tip: Development of multiple pharmaceutical agents by the manipulation of incretin hormone system provided the global scientific fraternity several drugs for the management of type 2 diabetes mellitus (T2DM) in recent years. These agents, the glucagon-like peptide-1 analogues and dipeptidyl peptidase-4 inhibitors, form the incretin-based therapies that benefited T2DM patients with significant reduction of hemoglobin A1c, low risk of hypoglycemia, favorable effects on management of overweight and obesity, and enhanced efficacy in combination regimens for glycemic management with other anti-diabetics. Two recent meta-analyses discarded the concern about elevated pancreatitis risk. The article discusses the incretin-based therapies for the management of T2DM.

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INTRODUCTION

Incretins are gut hormones secreted in response to meals that modify the biological mechanisms of glucose homeostasis in the body mainly through their effects on the pancreatic endocrine function^[1,2]. Glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two major incretin hormones identified to have major effects on carbohydrate metabolism. Although the concept of incretin effect on glucose homeostasis was introduced as early as 1930s^[3], the biological effects of incretins were well-established only in the past 3-4 decades^[2]. Approximately 70% of β -cell insulin secretion is controlled by GIP and GLP-1^[2]. Native GLP-1 has a very short biological half-life (1-2 min only) being rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) to an inactive molecule that terminates its incretin effect. Research on the biological manipulation of incretin system in animal models in the past few decades showed promising results with development of multiple pharmaceutical agents quite useful in the management of obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome towards the turn of 20th century. This paper discusses an overview of incretin manipulation for the management T2DM.

BIOLOGICAL EFFECTS OF GLP-1

GLP-1 is a peptide hormone secreted from the entero-endocrine L cells located within the gastrointestinal mucosa (mainly the ileum) that act as nutrient sensors, which release GLP-1 in response to luminal nutrients such as sugars, amino acids, and fatty acids^[4]. The secreted GLP-1 binds to the GLP-1 receptors (GLP-1R) distributed widely in various body tissues such as the pancreatic islets, brain, heart, kidney, and the gastrointestinal tract. The binding of GLP-1 to islet cell GLP-1R results in amplification of insulin secretion by the pancreas. This property of augmented insulin secretion in response to gut hormone release related to meal intake is termed as "the incretin effect"^[4]. However, the effects of GLP-1R activation in most other tissues still remain elusive.

GLP-1 also has trophic effects on the pancreatic β -cells^[4,5]. It has been found to stimulate beta-cell proliferation, enhance the differentiation of progenitor cells in the pancreatic duct epithelium into new β -cells, and inhibit apoptosis of the β -cells^[4]. Fasting and meal-related hyper-secretion of glucagon was demonstrated in patients with T2DM, and GLP-1 was found to be

a strong inhibitor of glucagon secretion^[4]. The exact mechanism of this effect is unknown. Local increase in insulin levels around the α -cells in response to GLP-1R stimulation and the GLP-1-stimulated somatostatin secretion are thought to be responsible for the inhibition of glucagon secretion^[4].

GLP-1 possesses the property of inhibition of gastric motility, gastrin-induced acid secretion in the stomach, and the pancreatic secretion^[4,6]. The gastric inhibitory effects of GLP-1 are thought to be mediated through the vagus nerve. GLP-1 also possesses central effects in the brainstem and hypothalamus through which it modulates the appetite, satiety and eating behavior in animals and human beings^[4]. GLP-1 also has cardio-protective and renal effects. The physiological aspects of incretin bio-effects are depicted diagrammatically in the Figure 1.

PHARMACOLOGICAL MANIPULATION OF INCRETIN SYSTEM

Attempts for the pharmacological manipulation of GLP-1 and DPP-4 molecules were areas of immense research interest among the scientific fraternity over the past few 3 to 4 decades that resulted in development of multiple medications, which revolutionized the modern management of T2DM. Through the bio-modulation of GLP-1 molecules to counteract the ultra-short half-life of native GLP-1, a class of drugs termed GLP-1 analogues was invented (also termed as incretin mimetics or incretin analogues). Development of inhibitors of the DPP-4 enzyme resulted in production of multiple drugs that prolong the effects of endogenously synthesized incretin molecules termed as incretin enhancers. These two classes of drugs form the incretin-based therapies which are commonly used in the management of T2DM.

With a significant effect on reduction of hemoglobin A1c (HbA1c), favorable effects on body weight especially in obese T2 diabetic, and a relatively low risk of hypoglycemia^[7], these drugs were well accepted by diabetologists and internists in the past few years^[1]. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recently recommended incretin-based therapies as important second line agents for management of T2DM^[7,8]. Newer molecules with different therapeutic and pharmacodynamic profiles are being added to this class of drugs.

GLP-1 ANALOGUES

Native GLP-1 is 30 amino acid polypeptide hormone that is rapidly degraded by the DPP-4 enzyme. To counteract the ultra-short half-life, alterations in the molecular structure of GLP-1 were attempted resulting in successful invention of a few GLP-1 analogues in

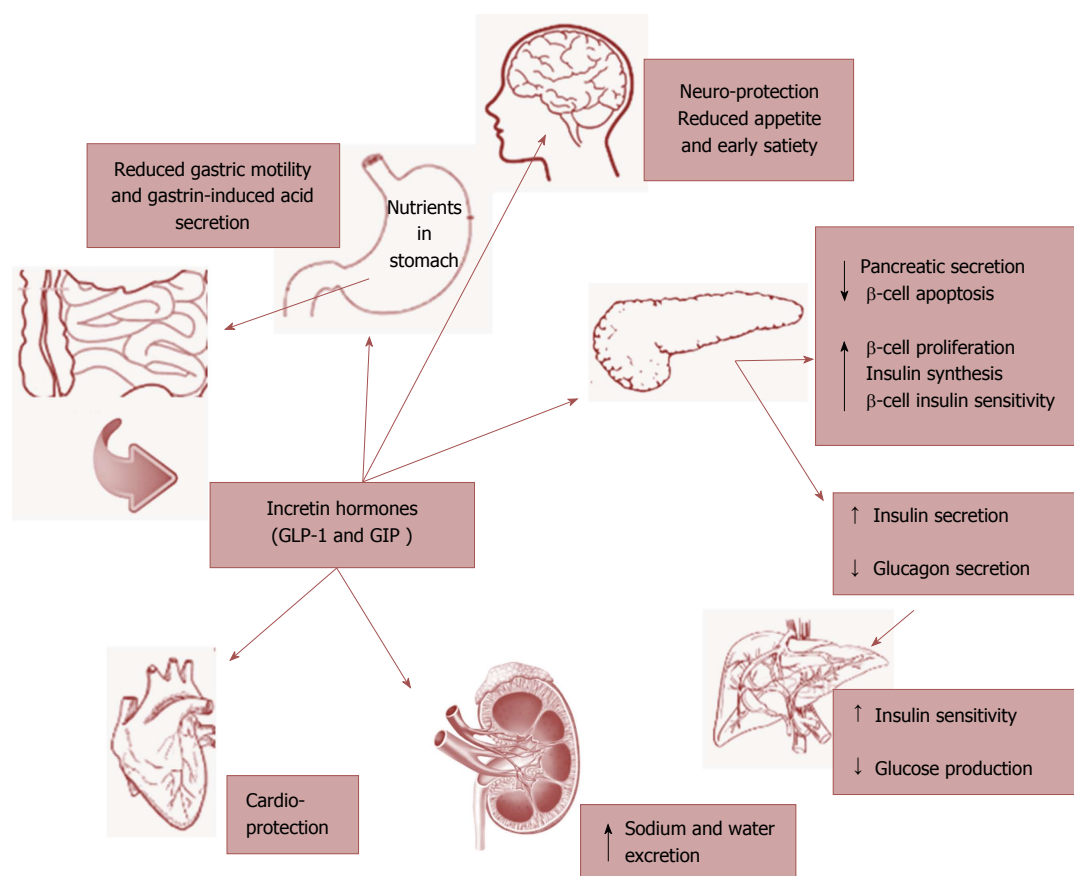


Figure 1 Physiological aspects of incretin hormones in the body. GLP-1: Glucagon-like peptide-1; GIP: Glucose-dependent insulinotropic polypeptide.

recent years.

Exenatide

Exenatide is the first among these molecules that gained approval from the United States Food and Drug Administration (FDA) in 2005^[9]. The drug, isolated from saliva of the reptile Gila monster (*Heloderma suspectum*), has 39 aminoacids with a 53% structural homology to natural GLP-1^[9,10]. It has a plasma half-life of 3-4 h, and is excreted by glomerular filtration with subsequent degradation^[10].

Exenatide improved glycemic control in patients with T2DM, not responsive to lifestyle modification, and medications such as sulfonylureas, metformin and thiazolidinediones, either alone or in combinations^[9,11]. Exenatide treatment showed improvements in both the fasting and post-prandial hyperglycemia in T2DM patients^[12]. In a recent meta-analysis examining the efficacy of the drug compared to placebo from nine clinical trials, the weighted mean difference of mean variation of hemoglobin A1c (Δ HbA1c) for all included data for exenatide 5 μ g twice daily or its equivalent long acting dosage form was -0.68% [95%CI: -0.89 to -0.48 ($P < 0.0001$)] and for exenatide 10 μ g twice daily or its equivalent long-acting dosage form was -0.99% [95%CI: -1.18 to -0.8 ($P < 0.0001$)]^[13]. The weighted mean difference of mean variation of BW (Δ BW) for

5 μ g twice daily or its equivalent long acting dosage form in eight of the trials was -0.56 kg [95%CI: -0.07 to -0.06 ($P = 0.0002$)] and for exenatide 10 μ g twice daily or its equivalent long-acting dosage form in twelve trials was -1.24 kg [95%CI: -1.69 to -0.78 ($P < 0.0001$)]. Other observed benefits were reduction of systolic and diastolic blood pressures, and total cholesterol and low density lipoprotein (LDL)^[13].

Being structurally different from native GLP-1, development of antibodies to exenatide on long-term treatment is common. Low-titre anti-exenatide antibodies were observed in 32% of cases on twice daily regimen and in 45% cases on once weekly regimen^[14]. However, a significant effect on therapeutic efficacy was not evident in most cases. Higher antibody titres were less common (5% and 12% respectively), and increasing titres were associated with a reduction in average efficacy that was statistically significant for exenatide once weekly preparation^[14]. Apart from injection-site reactions, there were no observed safety issues with anti-exenatide antibodies.

Liraglutide

The drug is manufactured using recombinant DNA technology, and is with a 97% structural homology to human GLP-1^[15]. Therefore, the molecule can be used effectively in patients with reduced response

to exenatide therapy after prolonged use because of antibody production. The estimated mean differences in HbA1c reduction with liraglutide 1.2 mg and 1.8 mg daily compared to placebo were -1.01% (95%CI: -1.18 to -0.85) and -1.18% (95%CI: -1.32 to -1.04) respectively in a recent meta-analysis^[16]. The Liraglutide Effect and Action in Diabetes (LEAD) program trial showed that mono-therapy with 1.2 mg and 1.8 mg of the drug was associated with a mean 2.1 kg and 2.5 kg weight reduction respectively, compared with a mean 1.1 kg weight gain among patients on glimepiride ($P < 0.001$) treatment^[17].

The other observed benefits were: a mean reduction in systolic blood pressure of 2.59 mmHg ($P = 0.0008$) and 2.49 mmHg ($P = 0.003$) from baseline for liraglutide 1.2 mg and 1.8 mg respectively at 26 wk of treatment^[15], improvement of β -cell function^[18], reduction of total cholesterol and LDL, improvement of non-alcoholic fatty liver disease (NAFLD)^[19], and improvement of cardiovascular risk markers^[15].

Lixisenatide

Plasma half-life of lixisenatide is 3 h similar to that of exenatide^[20], although it can be used as a single daily subcutaneous injection. Treatment with the drug resulted in a HbA1c reduction of 0.9%, body weight reduction of -3.62 kg (95%CI: -5.86 to -1.36) without significant risk of hypoglycemia compared to insulin^[21]. Lixisenatide was also shown to improve NAFLD (number needed to treat: 14 patients, $P = 0.042$)^[22]. Although slightly less effective than exenatide in terms of lowering HbA1c levels and weight reduction, lixisenatide use can be more convenient in comparison to exenatide as it has less hypoglycemia risk and gastrointestinal side effects, and the ease of once-daily administration^[23].

Albiglutide

Albiglutide is a DPP4-resistant human GLP-1 manufactured by fusion of the molecule with recombinant human albumin^[24]. With a plasma half-life of approximately 5 d, the drug has the advantage of being administered once weekly. The drug recently received FDA approval in the United States for management of T2DM. Albiglutide can be used as a monotherapy or as an add-on therapy to metformin, sulfonylureas, insulin glargine and thiazolidinediones. Superior clinical efficacy compared to sitagliptin, and glimepiride, and non-inferiority to insulins (glargine and lispro) with HbA1c reduction of 0.55% to 0.9% and weight reduction up to 1.21 kg were reported with the use in T2DM cases^[25]. Although gastrointestinal side effects were less common, efficacy in reducing HbA1c and body weight were less pronounced compared to liraglutide.

Dulaglutide

This new long acting GLP-1 analogue received recent

FDA approval for use in T2DM. The plasma half-life of dulaglutide is approximately 4 d, with a once weekly dosing advantage^[26]. Efficacy of once weekly regimen was reported to be superior to: metformin monotherapy, sitagliptin as add-on to metformin, and exenatide as add-on to metformin and pioglitazone, with a safety profile similar to other GLP-1 analogues^[27]. The plasma half-lives, dosage range and common side effects of GLP-1 analogues are shown in Table 1.

DPP-4 INHIBITORS

DPP-4 inhibitors increase the endogenously secreted GLP-1 and GIP concentrations by inhibiting the bio-degradation of these hormones by the DPP-4 enzyme, and thereby enhancing the incretin effect. In patients with T2DM these drugs are effective both as monotherapy and as add-on therapy to sulphonylureas, metformin, thiazolidinediones and insulin. In general, DPP-4 inhibitors are weight neutral, making them favorable options in the management of overweight and obese T2DM patients.

Sitagliptin

Sitagliptin is first among the DPP-4 inhibitors that received FDA approval in 2006. The drug has good oral bio-availability, a half-life of 10-12 h (with once daily dosing advantage), and is eliminated mainly through the kidneys necessitating dose reduction in renal impairment^[28]. Sitagliptin improves both fasting and postprandial hyperglycemia in T2DM patients. HbA1c reduction of up to 0.94% has been reported when sitagliptin is used as a monotherapy and better reduction in combination regimens. A recent meta-analysis concluded that sitagliptin had comparable efficacy to metformin in reduction of HbA1c and body weight, and improvement of β -cell function, although inferior to metformin in improvement of insulin sensitivity^[29].

Vildagliptin

When used as a monotherapy, this molecule showed glycemic control comparable to sulfonylureas and thiazolidinediones, with the advantages of fewer hypoglycemic episodes and lesser body weight gain^[30]. Additional favorable effects on pancreatic α - and β -cell function compared to sulphonylureas were noted with the drug. The plasma half-life of vildagliptin is 1.5-4.5 h and the elimination is mainly through hepatic hydrolysis^[28]. HbA1c reduction of 0.5%-1% has been reported with the drug use in T2DM. Use in combination with metformin, further improves glycemic control when metformin monotherapy is insufficient, with good tolerability and safety^[30]. Combination regimens with other oral anti-diabetic medications and insulins are also effective and well tolerated.

Table 1 Plasma half-lives, dosage range, average hemoglobin A1c and body weight reduction, and common side effects of glucagon-like peptide-1 analogues

Drug	Plasma $\frac{1}{2}$ -life	Dosage	HbA1c reduction	Weight reduction	Adverse effects	Other special features
Exenatide	3-4 h	5-10 mcg twice daily s.c, 60 min prior to meal	0.68%-0.99%	0.56-1.24 kg	Nausea, diarrhoea, headache, pancreatitis, injection site nodule/reaction, formation of anti-exenatide antibody	Not recommended if Creatinine clearance is < 30 mL/min
Exenatide ER	2 wk	2 mg s.c once weekly	0.99%	1.24 kg	Nausea, diarrhoea, vomiting, pancreatitis, injection site nodule/reaction	Injection at any time independent of meals
Liraglutide	13 h	0.6-1.8 mg s.c once daily	1.01%- 1.18%	2.1-2.5 kg	Nausea, diarrhoea, headache, pancreatitis, injection site reaction, formation of anti-liraglutide antibody and naso-pharyngitis	Store in refrigerator (36-46 ° F) Injection at any time independent of meals
Lixisenatide	3 h	20 mcg, once daily s.c	0.90%	3.62 kg	Nausea, diarrhoea, vomiting, pancreatitis	
Albiglutide	5 d	30-50 mg s.c once weekly	0.55%- 0.9%	1.21 kg	Upper respiratory infection, diarrhoea, injection site reaction, hypersensitivity, pancreatitis	Administer on the same day of the week
Dulaglutide	4 d	0.75-1.5 mg s.c once weekly	0.99%- 1.3%	-	Nausea, diarrhoea, vomiting, increased amylase and lipase levels, abdominal pain, injection site reaction, hyper-sensitivity and pancreatitis	

HbA1c: Hemoglobin A1c.

Saxagliptin

The drug received FDA approval in 2009 for use in patients with T2DM. When used as monotherapy at a maximum dose of 5 mg, saxagliptin caused a mean HbA1c reduction of 0.8% with significant improvement of fasting hyperglycemia, and with other categories of oral anti-diabetics, an additional mean HbA1c reduction by 0.6%-0.7%^[31]. The plasma half-life is 2.5 h and elimination is mainly by hepatic and renal clearance^[28].

Linagliptin

Linagliptin is primarily excreted *via* bile and therefore safe to be used in T2DM patients with renal impairment. With a reasonable safety profile, low hypoglycemia risk, HbA1c reduction ranging from 0.6% to 0.8% and weight neutrality, the drug became popular in the recent years^[32]. Additional benefits such as improvement of wound healing, reduction of hepatic steatosis, decrease in the infarct size following myocardial infarction and ischemic stroke, improvement of vascular function, and reduction of albuminuria are claimed with linagliptin use in pre-clinical studies that needs further research in large randomized controlled trials. Linagliptin has relatively low oral bio-availability compared to other DPP-4 molecules (15%-50%) and the plasma half-life of the drug is 12 h^[32].

Alogliptin

This new DPP-4 molecule has a plasma half-life of about 21 h, and can be administered once daily^[33]. Elimination is mainly through kidneys that necessitates dose reduction in advanced renal disease. Alogliptin is safe and well tolerated. A mean HbA1c reduction of 0.6% is reported with monotherapy^[28,33], and additional reduction in combination regimens with other anti-

diabetics^[33].

Teneligliptin

Teneligliptin is one of the latest additions to the class of DPP-4 inhibitors. A recent study revealed that the drug administration was associated with significant elevations of postprandial active GLP-1 and GIP levels, lowering of postprandial hyperglycemia, 24-h mean blood glucose levels, and mean amplitude of glycemic excursions without hypoglycemia^[34]. A significant elevation in early-phase insulin release and a reduction in postprandial glucagon surge were also observed. Even short-term teneligliptin treatment was found to be beneficial in patients with T2DM. HbA1c reduction of about 1%, improvement of β -cell function, insulin sensitivity, and adverse lipid parameters are the benefits claimed in a clinical trial^[35].

Anagliptin

The drug is still being evaluated in phase III clinical trials and is expected to be available for clinical use soon. Mean HbA1c reduction of $-0.85\% \pm 0.70\%$, reduction in the fasting proinsulin/ insulin ratio, and improvement of insulin secretion were observed when used as an add-on therapy to metformin (all effects comparable with sitagliptin) in a recent multi-center clinical trial^[36]. Safety profile and efficacy were also comparable with sitagliptin.

The dosage ranges and common side effects of DPP-4 inhibitors are shown in Table 2.

SAFETY ISSUES/CONCERNS ABOUT INCRETIN-BASED THERAPIES

A lot of discussions on the safety of incretin-based

Table 2 Plasma half-lives, dosage ranges, average hemoglobin A1c reduction and common side effects of dipeptidyl peptidase-4 inhibitors

Drug	Plasma half-life	Dose	HbA1c reduction	Adverse effects	Other remarks
Sitagliptin	12.4 h	100 mg PO daily	0.94%	Nasopharyngitis, diarrhea, headache, constipation, oedema, hypersensitivity, pancreatitis, elevation of hepatic enzymes	Use with caution in renal, hepatic or cardiac failure
Vildagliptin	90 mts - by terminal elimination	50-100 mg/ daily PO	0.5%-1%	Headache, nasopharyngitis, cough, constipation, dizziness, and increased sweating	Dose reduction with CYP450 3A4/5 inhibitors
Saxagliptin	2.5 h	2.5-5 mg/PO daily	0.8%	Urinary and upper respiratory infections, headache, edema, purpuric rash, hypersensitivity, pancreatitis and angio-edema	
Linagliptin	12 h	5 mg PO daily	0.6%-0.8%	Nasopharyngitis, dyslipidemia, pancreatitis	Monitor LFT and stop if elevated
Teneligliptin	24.2 h	20-40 mg PO daily	0.78%	Constipation, QT interval prolongation, hypoglycaemia and elevation of alanine aminotransferase and γ -glutamyltransferases	
Alogliptin	21 h	25 mg PO daily	0.6%	Hypoglycemia, nasopharyngitis, headache and pancreatitis	
Anagliptin	4.37 h - by terminal elimination	100 mg PO daily	0.85%	Not available	

HbA1c: Hemoglobin A1c.

therapies occurred recently following multiple case reports and the data from the United States FDA adverse events reporting system about the risk for pancreatic damage^[1]. Two recent meta-analyses showed reassuring results without significant risk of pancreatitis favoring incretin-based therapies^[37,38]. However, the potential long-term effects of chronic GLP-1R stimulation and its effects on pancreatic enzyme synthesis and the probability of evoking inflammatory response in the pancreas are not clear at the moment.

The other important concern is about the potential to induce neoplasia by these drugs. Significant β -cell hyperplasia, co-expression of insulin and glucagon from β -cells, hyperplasia of α -cells, increased proliferation markers, and excess prevalence of pre-neoplastic lesions were found in pancreas specimens of organ donors previously treated with incretin-based medication for T2DM^[39]. Concerns about elevated risk of pancreatic and thyroid cancer in animal models and human beings^[40] need further clarification by long-term studies and drug safety monitoring.

CONCLUSION

Incretin-based therapies are promising tools for the management of T2DM, especially in overweight and obese individuals. Favorable effects on body weight, significant reduction of HbA1c levels and the relatively low risk of hypoglycemia make them attractive therapeutic options in the day to day management of T2DM patients. Newer GLP-1 analogues and DPP-4 inhibitors are being added to this class of medications recently. Concern about elevated risk of pancreatitis is not obvious at the moment, based on results from two large meta-analyses. However, long-term effects of

these medications on pancreatitis risk and cancer risk still need vigilant monitoring.

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Gestational diabetes mellitus: An update on the current international diagnostic criteria

Mukesh M Agarwal

Mukesh M Agarwal, Department of Pathology, College of Medicine, UAE University, Al Ain, United Arab Emirates

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Correspondence to: Mukesh M Agarwal, MD, FCAP, Department of Pathology, College of Medicine, UAE University, P.O. Box 17666, Al Ain, United Arab Emirates. magarwal7@gmail.com
 Telephone: +971-3-7672000
 Fax: +971-3-7671966

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Abstract

The approach to screening and diagnosis of gestational diabetes mellitus (GDM) around the world is disorderly. The protocols for diagnosis vary not only in-between countries, but also within countries. Furthermore, in any country, this disparity occurs in-between its hospitals and often exists within a single hospital. There are many reasons for these differences. There is the lack of

an international consensus among preeminent health organizations (*e.g.*, American College of Gynecologists and World Health Organization). Often there is a disagreement between the country's national diabetes organization, its local health society and its regional obstetric organization with each one recommending a different option for approaching GDM. Sometimes the causes for following an alternate approach are very obvious, *e.g.*, a resource strapped hospital is unable to follow the ivory-tower demanding recommendation of its obstetric organization. But more often than not, the rationale for following or not following a guideline, or following different guideline within the same geographic area is without any perceivable explanation. This review is an attempt to understand the problems afflicting the screening and diagnosis of GDM globally. It traces the major temporal changes in the diagnostic criteria of (1) some respected health organizations; and (2) a few selected countries. With an understanding of the reasons for this disparity, a way forward can be found to reach the ultimate goal: a single global guideline for GDM followed worldwide.

Key words: Gestational diabetes; Criteria; Screening; Diagnosis; Global

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Core tip: Globally, the screening and diagnosis of gestational diabetes mellitus (GDM) is idiosyncratic. This disarray is independent of whether a country is affluent (*e.g.*, Denmark) or relatively poor (*e.g.*, Bangladesh). The reason is that not just the international but also the national medical and obstetric organizations in a country advise a multitude of approaches to GDM. This confuses the primary providers of obstetric care, who need one clear, evidence-based, global recommendation. Despite all the differences, in the near future, the light at the end of the tunnel for providing such a universal global GDM guideline is bright.

Agarwal MM. Gestational diabetes mellitus: An update on the current international diagnostic criteria. *World J Diabetes* 2015; 6(6): 782-791 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i6/782.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i6.782>

INTRODUCTION

Since it is one the commonest metabolic problems of pregnancy, an accurate diagnosis of gestational diabetes mellitus (GDM), *i.e.*, high plasma glucose first identified during pregnancy, is critical to the care of pregnant women. Five decades ago, GDM was used to detect pregnant women who were at a higher risk of developing type 2 diabetes mellitus (DM2) after childbirth^[1]. Currently, GDM is used to predict morbidity in index pregnancy; many trials have confirmed that it is related to multiple maternal and fetal complications like preeclampsia, caesarean sections and birth injuries^[2]. Thus, missing GDM has grim implications, personal for individual women and epidemiological for the entire population. Women with diabetes mellitus who become pregnant have more harmful complications (due to the severe hyperglycemia since early pregnancy) compared to pregnant women developing mild hyperglycemia in late pregnancy. The former have diabetes in pregnancy while the latter are diagnosed with GDM. Thus, GDM implies a milder form of hyperglycemia seen generally in late pregnancy, which usually, but not always, reverts to normal after delivery.

The screening of GDM is done by assessing the clinical risk factors or by the 50-g glucose challenge test (GCT). The diagnosis of GDM is made by the 75-g or 100-g oral glucose tolerance test (OGTT). A screen followed by the diagnostic OGTT (in screen positive patients) is called the two-step approach, while OGTT directly without screen is called the one-step approach. The two-step and the one-step screening methods are also known as the selective and universal screening methods, respectively. The various preeminent health organizations recommend different glucose cut-offs for the OGTT; as a result, there many international diagnostic criteria are available for diagnosis^[3]. More often than not, the gynecologic, medical and health associations within any one country support distinctly diverse schemes for GDM causing major differences in the approach to GDM. Thus, the scourge of gestational diabetes mellitus (GDM) is the diversity of processes accessible for its screening and diagnosis. The variation in the diagnostic thresholds advocated by these venerable organizations, when applied to the same OGTT, results in major discrepancies in prevalence and the women classified with GDM^[3]. Misclassifying women with GDM will result in excessive treatment of many women without GDM and no treatment of many

women with GDM again iterating the need for correct classification.

This review traces the progress in the major international diagnostic criteria worldwide. It looks at the changes in practices of GDM screening and diagnosis in selected countries of the world to show that most countries face similar problems caused by the multitude of criteria available. An understanding of the reasons for the disparity is critical to formulate plans for the ideal goal: a single global approach to GDM.

MAJOR GDM DIAGNOSTIC CRITERIA: DEVELOPMENT

World Health Organization criteria

The World Health Organization (WHO) provides guidelines for numerous communicable and non-communicable diseases. GDM is no exception and due to the worldwide reach and authority of the WHO, the WHO criteria for GDM^[4] are popular globally. In 1965, the WHO Expert Committee on Diabetes Mellitus published the first guideline on diabetes mellitus. They defined gestational diabetes as "hyperglycemia of diabetic levels occurring during pregnancy". After these initial attempts to define GDM, new follow-up WHO guidelines were published in 1980, 1985, 1999 and 2013.

In 1980, the WHO recommended the OGTT for diagnosis of DM2 in non-pregnant adults using 2 values: fasting plasma glucose and the 2-h plasma glucose levels after 75-g of oral glucose. For convenience, common thresholds were applied to both pregnant women and non-pregnant adults; thus, the diagnosis of GDM was applied if a woman was pregnant instead of DM2 for the non-pregnant. In 1985, the glucose values were made more precise by rounding to the nearest tenth of a millimole (rather than the nearest millimole). In 1997, for the diagnosis of diabetes, the ADA lowered the fasting plasma glucose (FPG) cut-off to 7.0 mmol/L (from 7.8 mmol/L). In 1999, the WHO followed suit applying the same FPG criteria as recommended by the ADA to the OGTT. The WHO has always applied the same criteria to the pregnant and non-pregnant women even though common thresholds for pregnant and non-pregnant have been shown to be erroneous^[5]. However, due to the ease of use, simplicity and global clout, the WHO criteria for have remained popular in most countries of the world.

The current global diabetes epidemic has resulted in many younger women in the child bearing age to get DM2. Due to the more severe fetal and maternal complications resulting from such diabetes mellitus antedating pregnancy, in 2013, the WHO^[6] has divided hyperglycemia in pregnancy as follows: (1) Diabetes in pregnancy: Pre-gestational diabetes (PGD) or

pregnancy occurring in a women with known diabetes, and Overt diabetes - diabetes first detected during pregnancy; and (2) Gestational diabetes mellitus.

Essentially, this latest WHO 2013 guideline has endorsed the International Association of Diabetes and Pregnancy Study Groups (IADPSG 2010) criteria (see below).

International Association of Diabetes and Pregnancy Study Groups criteria

In 1998, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) were created to find a common consensus between many national and international groups addressing diabetes in pregnancy. Delegates from over 40 countries met to review the results of the elaborate Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study^[7].

In 2010, IADPSG recommended universal screening of all pregnant women with the 75-g oral glucose tolerance test (OGTT)^[8]. They used 1.75 odds of having complications seen in the HAPO study and proposed new thresholds for the 75-g OGTT. Thus, the IADPSG criteria had the possibility to be accepted all by the preeminent medical, endocrine and health organizations worldwide. However, as of 2014, as will be pointed out, despite the IADPSG guideline being agreed to by many global health groups, one worldwide guideline remains elusive.

American Diabetes Association criteria

Due to its geographical location and authority, the American Diabetes Association (ADA) criteria are widely used in United States, Canada and Mexico. In 1964, O'Sullivan and Mahan^[9] recommended using the 4 sample, 3-h 100-g OGTT for diagnosis of GDM; the glucose thresholds were established from a cohort involving 752 women. With time, as glucose measuring techniques evolved, Carpenter and Coustan (C and C)^[10] modified O'Sullivan's recommended glucose thresholds by adjusting for (1) the non-glucose reducing elements in blood; and (2) converting whole blood glucose values to the higher plasma glucose values. So the C and C thresholds were modified to be aligned to the newer glucose enzymatic methods for quantifying plasma glucose. The ADA incorporated the C and C thresholds for the 100-g, 3-h OGTT in their recommendations in 2000. Thus, a two-step approach was popular in North America, *i.e.*, 50-g GCT screen followed by 100-g OGTT if the screen GCT was positive. In 2003, the ADA also accepted the one-step approach of using the 75-g OGTT for the screening and diagnosis of GDM, especially in high-risk populations, since it was deemed more cost-effective. For the thresholds, the C and C cut-offs were used leaving the 3-h glucose value of the 100-g OGTT, which is not collected in the 2-h, 75-g OGTT. In 2011, the ADA accepted the recommendations of the

International Association of Diabetes and Pregnancy Study Groups (IADPSG)^[8], *i.e.*, using the 75-g OGTT on all women as a one-step screening and diagnostic method eliminating the need for the 50-g GCT. In 2013, after the American College of Obstetricians and Gynecologists (ACOG)^[11] refused to accept the IADPSG criteria at the conference organized by the National Institute of Health^[12]; in 2014, the ADA relented reversing its earlier stance and accepted both the one-step and two-step as a methods to screen and diagnose GDM agreeing with the ACOG (see below) and IADPSG recommendations.

ACOG criteria

The ACOG has always endorsed the two-step approach to GDM. In 1986, ACOG recommended the 50-g 1-h screening test for "women at risk," which in 2001 was changed to "all" women but excluding women at very low risk. In 2011, though the ADA approved the IADPSG; the ACOG had concerns that GDM prevalence would rise from 5%-7% to 18% - a three-fold increase. The ACOG had doubts that the increase in prevalence would have clinically significant improvements in maternal and neonatal outcomes in the "additional" women identified and treated with GDM. So, in its August 2013 bulletin, it has retained the two-step procedure using the thresholds (for the 100-g OGTT) of the National Diabetes Data Group (NDDG)^[13] or C and C criteria for the 100-g OGTT^[10].

Canadian criteria

In Canada, the Canadian Diabetes Association (CDA)^[14] and the Society of the Obstetricians and Gynecologists of Canada (SOGC) publish recommendations for GDM. Like the ADA and the ACOG in United States, their approaches have been dissimilar though they have shared many common ideas. CDA has been regularly using the latest research to update their recommendations - their latest guidelines were released in 2013^[15]. The SOGC recommendations of 2002 have not been modified; therefore, the SOGC has lagged behind in providing recommendations for GDM after 2002. It advocated either no screening as an option or using screening with a 50-g GCT with women having positive screens to undergo an OGTT (100-g or 75-g). These guidelines are completely out of date and need an update using research from the recent trials.

The CDA has consistently advocated screening all (*i.e.*, universal screening) women as any form of risk-factor screening, though cheaper, would always miss some patients with GDM. The 75-g OGTT CDA thresholds have been much higher than the C and C criteria originally approved by the ADA; thus, the strict CDA criteria for the 75-g OGTT always identified less women with GDM when compared to other criteria^[3]. The latest guideline, CDA 2013, recommends scr-

Table 1 Comparison of screening and diagnostic criteria of gestational diabetes^[58]

Area	Advising body	Year	Advise for screening	Method of screening (positive cut-off \geq)	Glucose load, g	Glucose thresholds (mmol/L)				Number of OGTT values for diagnosis \geq
						Fasting	1-h	2-h	3-h	
North America	NDDG	1979	None	50-g GCT (7.8)	100	5.8	10.5	9.2	8.0	2
	ADA	2003	All but for those at low risk	50-g GCT (7.8)	100	5.3	10.0	8.6	7.8	2
					75	5.3	10.0	8.6	-	2
	C and C	1982	None	-	100	5.3	10.0	8.6	7.8	2
	IADPSG	2010	All	75-g OGTT	75	5.1	10.0	8.5	-	2
	CDA	2003	All	50-g GCT (7.8)	75	5.3	10.6	8.9	-	2
	CDA	2013	All	50-g GCT (7.8)	75	5.3	10.6	9.0	-	1
South America	SOGC	2002	All except low risk	50-g GCT (7.8)	100	5.3	10.0	8.6	7.8	2
					75	5.3	10.0	8.6	-	2
	BSD	2007	All	FPG (4.7)	75	-	7.0	-	7.8	1
Europe	BSD	2014	All	FPG (4.7)	75	5.1	10.0	8.5	-	1
	NICE	2015	Clinical risk	75-g OGTT	75	5.6	-	7.8	-	1
	EASD	1991	NS	NS	75	5.5 or 6.0	-	-	9.0	1
Asia	JDS	2013	All	50-g GCT (7.8)	75	5.1	10.0	8.5	-	2
	DIPSI	2009	-	-	75	-	-	7.8	-	1
Australasia	ADIPS	2014	All, unless resources limited	75-g OGTT	75	5.1	10.0	8.5	-	1
	NZSSD	1998	All	50-g GCT (7.8) 75-g (8.0)	75	5.5	-	9.0	-	1
Global criteria	WHO	2013	All	75-g OGTT	75	5.1	10.0	8.5	-	1

ADA: American Diabetes Organization; ADIPS: Australian Diabetes in Pregnancy Society; BSD: Brazilian Society of Diabetes; CDA: Canadian Diabetes Association; C and C: Carpenter and Coustan; EASD: European Association for the Study of Diabetes; DIPSI: Diabetes in Pregnancy Study group in India; IDF: International Diabetes Federation; FPG: Fasting plasma glucose; JDS: Japan Diabetes Society; NDDG: National Diabetes Data Group; NZSSD: New Zealand Society for the Study of Diabetes; NICE: National Institute for Health and Care Excellence; NS: Not specified; RPG: Random plasma glucose; SOGC: Society of Obstetricians and Gynecologists of Canada; WHO: World Health Organization.

creening high-risk women with the 50-g GCT in early pregnancy. All women should undergo the GCT between 24-28 wk, and if between 7.8-11.0 mmol/L, they should undergo the 75-g OGTT using thresholds recommended by them (Table 1).

The cut-offs of CDA 2013 are similar to the IADPSG 2010 thresholds since they use the odds ratio of 2.0 and 1.75, respectively, based on adverse outcomes of the HAPO data. In their latest guideline^[15] the CDA claims that their 2003 and 2013 thresholds are very similar. However, their resulting prevalence will be very different. The reason for the disparity is that, though the thresholds are similar, the number of thresholds needed for diagnosis are different (one vs two) - a fact not so obvious in their guideline.

Thus, it can be seen the CDA has kept with the evolution of GDM research, suggesting higher thresholds for diagnosis. Though it has reluctantly agreed with the IADPSG as an alternative some of its members have been very skeptical of the IADPSG guidelines^[16]. CDA also differs in that it recommends a 50-g GCT screen on all women followed by the 75-g OGTT.

European Association for the Study of Diabetes criteria

In 1991, the European Association for the Study of Diabetes (EASD) published diagnostic criteria for GDM^[17]. It accepted the 1996 the Pregnancy and Neonatal Care Group^[18] glucose thresholds of the 75-g OGTT (either FPG \geq 6.0 mmol/L or 2-h plasma

venous glucose \geq 9.0 mmol/L) for GDM diagnosis. Nevertheless, the EASD has not recommended any changes in their diagnostic criteria for GDM despite new epidemiological data and numerous randomized trials; thus, the EASD recommendations have not been modified or changed since the last 20 years. Unfortunately, they still are used in some countries of Europe^[19].

Australasian Diabetes in Pregnancy Society criteria

In 1991, the Australasian Diabetes in Pregnancy Society (ADIPS) endorsed its first directives for GDM^[20]. They modified the popular WHO GDM for 75-g OGTT based on opinion of the experts. Subsequently, their recommendations were modified in 1998^[21]. The ADIPS accepted both selective (if the resources were limited) or universal screening (if the resources were adequate) with a 50-g GCT or 75-g OGTT (Table 1). In 2013, the ADIPS issued new guidelines after considering the available evidence like HAPO study and other clinical trials. In fact, they accepted the WHO 2013 (same as IADPSG 2010) with a few caveats. They recommend not using the term "Overt diabetes" as suggested by the IADPSG for marked hyperglycemia first discovered in pregnancy. At booking, they have a list of risk factors for diabetes and recommend that all women with these risk factors undergo a 75-g OGTT and clinical judgment should be used for further work-up^[22].

New Zealand Society for the Study of Diabetes criteria

Until recently, the Australasian (ADIPS) 1998 guidelines were common for both Australia and New Zealand. The excessive number of women diagnosed with GDM would strain the limited resources of New Zealand. So, to diagnose less women with GDM, the New Zealand Society for the Study of Diabetes (NZSSD) raised the 2-h cutoffs for the 75-g OGTT from 8.0 mmol/L to 9.0 mmol/L. This change shows how many changes in the criteria were made on an “ad-hoc” basis.

In 2014, The New Zealand Ministry of Health published a clinical practice guideline: Screening, Diagnosis and Management of Gestational Diabetes in New Zealand^[23]. Twenty international and national guidelines and position statements were identified and critically appraised. Their recommendation: a HbA1c should be ordered at booking and at 24-28 wk, depending on the result of the HbA1c, a 50-g GCT or an OGTT may be done (cut-offs $F \geq 5.5$ mmol/L or 2-h ≥ 9.0 mmol/L) (Table 1). Thus, they have not accepted the WHO 2013/IADPSG 2010 criteria for GDM like the ADIPS, which has accepted them.

Japan Diabetes Society criteria

In Japan, the Japan Diabetes Society (JDS) has kept up with the new research in diabetes and GDM. It published new guidelines three times between 1970-1995 critically evaluating guidelines of major organizations like ADA and WHO. Originally, JDS adhered to local Japanese criteria (derived from healthy pregnancies) suggested by the Committee for Nutrition and Metabolism of the Japan Society of Obstetrics and Gynecology (JSOG)^[24] and the JDS continued endorsing this approach to GDM established in the early 1980s^[24]. In 2013, the JDS released Evidence-based Practice Guideline for the Treatment for Diabetes in Japan 2013^[25]. Essentially, it accepted the IADPSG criteria for the diagnosis of GDM. Hence it can be appreciated that Japan has kept up with the latest developments on GDM something not done by many modern European countries (like Sweden).

Brazilian Society of Diabetes criteria

The Brazilian Society of Diabetes (BSD) accepted the fasting plasma glucose (FPG) as a screening test for GDM at booking and at 24-28 wk gestation^[26]. These recommendations were based on a landmark study published in a preeminent diabetes journal^[27]. As per these recommendations, a FPG ≥ 4.7 mmol/L and < 5.0 mmol/L needed a diagnostic OGTT using the C and C criteria for diagnosis. In 2010, a Brazilian Consensus guideline endorsed these guidelines^[28]. The use of FPG in Brazil has been recently been authenticated by a recent Brazilian study^[29]. The IADPSG has been accepted as the diagnostic method in Brazil^[30].

International Diabetes Federation guidelines

In 2009, the International Diabetes Federation (IDF)^[31]

acknowledged that many strategies were available. It logically stated that any GDM definition must take into account 3 risk factors: perinatal morbidity and mortality in index pregnancy, the mother developing type 2 diabetes, and intra-uterine epigenetic programming of the developing fetus. At that time, it accepted both the two-step or one-step methods of the ADA and WHO, respectively. However, the IDF had a preference for the 75-g OGTT because it used less glucose and was of shorter duration. Currently, they have accepted the current WHO 2013/IADPSG criteria^[30].

GDM APPROACH: CONTINENTS AND SELECTED COUNTRIES

The differences in algorithms for GDM by major international bodies translate into variation in the practices within individual countries. Similar trends are found in most countries for the screening and diagnosis of GDM. The practices in some specific selected countries, segregated by continents, are discussed below.

Europe

Buckley *et al*^[19] reviewed the screening practices all over Europe. They looked at 185 sources of information from 23 European countries. The screening methods varied from risk-factor screening (Norway); 50-g universal GCT (Finland, Poland, Austria); random plasma glucose (United Kingdom, Plymouth). Most countries used WHO 1999 criteria or the Carpenter and Coustan criteria for diagnosis as applied to the 75-g or 100-g OGTT. Some countries (like Hungary) used the universal one-step, 75-g OGTT (WHO 1999) for diagnosis while others (like Italy) used universal one-step, 100-g OGTT (C and C) for diagnosis. The authors conclude that global agreement on screening and diagnostic methods would lead to better detection and treatment; only more well-designed research would inform us about the best practice methods in screening and diagnosis of GDM.

United Kingdom

The national guidelines for GDM in United Kingdom were established only after 2008. The clinician had to decide if screening was needed or not^[32]. The National Institute for Health and Clinical Excellence (NICE) guidelines were originally issued in March 2008. These have been replaced by recommendations in February 2015^[33] and essentially, the NICE does not accept the IADPSG criteria. They continue to recommend using clinical risk factors for screening. GDM is diagnosed if on a FPG ≥ 5.6 mmol/L or a 2-h glucose after a 75-g OGTT is ≥ 7.8 mmol/L (Table 1). The OGTT should be done in the first or second trimester depending on the clinical need. These guidelines have been motivated by

the latest research and cost of treatment of GDM^[33].

The Scottish Intercollegiate Guidelines network (SIGN) in their 2010 recommendations advise (at booking) screening with clinical risk factors, with HbA1c or fasting glucose; at 24-28 wk, while all high-risk women should undergo a 75-g OGTT with the IADPSG criteria used for diagnosis; All low risk women at 24-28 wk, should undergo the fasting plasma glucose^[34].

In January 2011, the Royal College of Obstetricians and Gynaecologists discussed the overall strategies for GDM including NICE and IADPSG. However, they do not provide any recommendations on how to screen and diagnose GDM^[35].

This inconsistency of multiple approaches to GDM is reflected in practices at ground level. In an older United Kingdom survey by mail^[36], the screening practices were very varied: fasting and random plasma glucose and glycosuria were all used showing the heterogeneity in screening for GDM. Various cutoffs were used for diagnosis using the 75-g OGTT - a fact iterated in the latest 2015 NICE guidelines^[33]. Thus, it can be seen that in a country like United Kingdom, which has well-developed health system, there is no consistency in the approach to GDM. However, with time there should be more uniformity in the approach to GDM once there is more international agreement.

Italy

Many Italian organizations like Italian Society of Diabetology (SID) and the Italian Association of Diabetologists (AMD) set standards for diabetes in Italy in 2007^[37]. They agreed to use the C and C criteria for diagnosis, which were also endorsed by the ADA. Currently, the Italian Institute of health recommends using risk-factors for screening of GDM. Thus, even recently, whether universal or risk factors screening should be done has been debated in Italy^[38]. However, currently, the IADPSG guidelines have been accepted in Italy^[31].

Sweden

The screening and diagnostic criteria for GDM in Sweden have been evolving over time. In 1985, repeated random blood glucose measurements were popular for GDM diagnosis. Since 1991, the hand-held Hemocue spectrophotometers have been popular in Sweden. These use capillary whole blood for measuring glucose; even the OGTT samples, instead of plasma venous glucose, tend to utilize capillary whole blood for convenience. Currently, there is no consensus in Sweden and over 4 methods are used to screen and diagnose GDM as shown by a recent study^[39]; the authors recommend that IADPSG should be adapted. Thus in Sweden, even in 2015, there is a huge variation in the approach to GDM; furthermore, popular use of Hemocue complicates the diagnosis of

GDM. Thus, Sweden is no different when compared to other European countries in having no consistency for the diagnosis of GDM.

Belgium

A survey (May 2012-January 2013) of 45 obstetrical centers from Belgium showed that 56% used screening before 24 wk based on clinical methods^[40]. At 24 wk, the commonest strategy (56%) was the two-step method (GCT + 100-g OGTT) with C and C (52%) or NDDG (4%) criteria for diagnosis. The remaining used IADPSG (33%) or WHO 1999 (2%) or C and C (9%). Belgium, like other European countries, also has no uniformity in the approach to GDM.

Germany

Like most other countries, the screening and diagnosis of GDM in Germany has been inconsistent. The German Society of Obstetrics and Gynecology and the German Diabetes Association (DDG) made some attempts at formulation guidelines for GDM in 2001. However, obstetricians either do not screen, or often carry on risk factor screening. Currently, the IADPSG has been approved formally in Germany by the German Diabetes Association^[41]. This should result in consistency in screening and diagnosis of GDM in Germany over time.

Asia

Tutino *et al*^[42] recently reviewed the situation of diabetes and pregnancy in Asia. Since the prevalence in Asia varies extensively due to lack of uniform diagnostic criteria, the authors stress the importance of a unified approach to GDM. Hyperglycemia in pregnancy has its highest prevalence in South-Est Asia, where one-fourth pregnancies are affected vs one-seventh, globally. Asians develop GDM at a lower BMI and type 2 DM occurs at a much younger age. With urbanization, GDM prevalence is becoming an epidemic. The IADPSG has been adapted by some Asian countries, although it remains a challenge to implement in low-resource settings. So, local modifications have been suggested.

China

The Ministry of health in China published its guidelines for testing and diagnosis of gestational diabetes in 2011^[43]. It recommends the fasting plasma glucose or 2-h venous glucose post 75 g OGTT at the first prenatal visit to rule out diabetes antedating pregnancy using standard diagnostic criteria for diagnosis of diabetes in the non-pregnant.

The diagnosis of GDM is made by a single step 75-g 2 h. OGTT done between 24 and 28 wk of gestation. The cut points for diagnosis of GDM are those of the IADPSG.

To reduce the number of OGTTs, it has been suggested that the FPG test may be done first - a

concept originally described by us, which was adapted by the Chinese Ministry of Health. If the FPG value is less than 4.4 mmol/L no further testing is needed. For values above 5.1 mmol/L a diagnosis of GDM is made without an OGTT. Pregnant women with fasting glucose values between 4.4 and 5.1 mmol/L must undergo a 75-g OGTT to further rule in or rule out GDM. This concept has been tested in China^[44]. Using this algorithm, only half of pregnant women would be required to undergo the formal OGTT.

India

Asian Indians are considered to be at the highest risk for gestational diabetes. In India there is a (1) high burden and a rising prevalence of diabetes; (2) constraint of resources; and (3) high rate of deliveries (27 million/year). Considering these factors and using local studies, the Diabetes in Pregnancy Study group in India (DIPSI) has developed practical usable recommendations for diagnosis of GDM in the community^[45]. This guideline has been recognized by the Ministry of Health, Government of India, the Federation of Obstetrics and Gynecological Societies of India (FOGSI) and the Association of Physicians of India (API).

Testing for GDM is recommended twice during antenatal care. The first testing should be done during first antenatal contact as early as possible in pregnancy. The second testing should be ideally done during 24-28 wk of pregnancy if the first test is negative. If women present beyond 28 wk of pregnancy, only one test is to be done at the first point of contact.

A single step is recommended by measuring plasma glucose 2 h after ingestion of 75-g glucose irrespective of the last meal (fasting or non-fasting). In the absence of available laboratory facilities a standardized glucometer may be used to evaluate plasma glucose. A glucose level of ≥ 7.8 mmol/L is the cut off for diagnosis of GDM. This test is called the DIPSI Test.

The older WHO 1999 criteria are very popular in many Asian countries^[46,47]. The latest guidelines of Sri Lanka recommend either the DIPSI or the IADPSG guidelines^[48]. However, other countries like Thailand use mostly use the two-step approach (the diagnostic criteria of the NDDG or C and C) or WHO 1999 criteria (75-g OGTT)^[49]. As can be appreciated in Asia, like the rest of the world, the approach between and within countries is not uniform.

Australia

As detailed earlier, the latest guidelines in Australia have been modified in November 2014. Essentially, with a few caveats, the ADIPS has accepted the guidelines of IADPSG.

Africa

The data from Africa about GDM is limited. Like Asia,

due to its global reach and acceptance, the WHO 1999 criteria are widely used in many African countries^[50-53]. Recently, Macaulay *et al*^[54] reviewed GDM in Africa. They found 14 useful papers from 60 studies. Six African countries, representing 11% of African continent, were Ethiopia, Morocco, Mozambique, Nigeria, South Africa and Tanzania. Major variation in methods was present between countries. Morocco used 100-g OGTT with C and C criteria. Mozambique used their own diagnostic criteria. Much heterogeneity between countries was present within countries. Six studies from Nigeria used 75 or 100-g OGTT for diagnosis with varying criteria. One center used 50-g GCT for diagnosis of GDM. Similar heterogeneity was found in 4 studies from South Africa. The authors conclude that there is a paucity of information about GDM from Africa and stress the importance of more research. This is crucial given the public health burden of obesity and diabetes. Only then, effective public health measures can be planned.

Nigeria

In 2011 (modified 2013), a national guideline on diabetes was published in Nigeria^[55]. The recommendations include (1) risk assessment at booking; (2) A one-step (75-g OGTT) or two-step method (50-g GCT with 100-g OGTT) using C and C criteria for diagnosis. Despite the availability of a guideline on GDM, practice varies across obstetric units in Nigeria. There are many gaps in the guideline as it is not appropriate for use in all circumstances; there is no recommendation on the screening/diagnostic approach for women outside tertiary care facilities as GCT/OGTT are not available in primary health care settings.

Thus, Nigeria reflects some of the problems seen with GDM screening in Africa and stresses the importance of addressing the specific needs of the sub-Saharan Africa region. The critical gaps were potentially due to only endocrinologists in the guideline development team. It also emphasizes that international guidelines cannot just be applied to poor countries in Africa.

South America

Like Africa, there is little published data from South America, which consists of 14 independent countries. However, much data comes from Brazil and Chile, and Argentina^[27,56,57]. In fact, Brazil is the leader in diabetes and gestational diabetes research. A literature search from individual countries from South America (Venezuela, Columbia, Peru, Uruguay, Ecuador, Bolivia, Paraguay) yields almost no results. Thus, it is difficult to get information on the approach to screening and diagnosis to GDM in South America.

WAY FORWARD

As can be appreciated, the availability of multiple criteria for the screening and diagnosis of GDM have resulted in an almost ad-hoc approach to GDM. Most (but not all) organizations have been updating their

criteria over time; however, the hospitals following recommendations of a preeminent association often lag behind in updating their approach to follow the latest guideline. This adds to the already disorderly situation. Major international bodies are aware of this problem. Thus, some of these organizations are working to convince all the major international professional organizations to come to a consensus. A major effort has been undertaken by the International Federation of Gynecology and Obstetrics (FIGO), which has members from 125 gynecology and obstetric organizations worldwide, to achieve consensus on GDM. Ideally, the answer may lie in a well-funded trial like the HAPO. Till that happens, the approach will have to be by consensus. As of 2015, worldwide, the most accepted criterion is of the IADPSG 2010^[30] and as it becomes more approved - we may achieve the much desired consensus for one guideline.

CONCLUSION

As can be appreciated from this review, the screening and diagnostic criteria for GDM throughout the world are summarized by one word: chaotic. Many international and regional guidelines have lagged behind the current research. The need for a single global guideline has been repeatedly stressed^[57] and this consistency is essential to avoid confusing primary care-givers of GDM. The primary care-givers of pregnant women look to the authorities and expert committees for guidance. Unfortunately, the experts continue to provide no clarity. With over five decades of research and hindsight, we must develop a single useful guideline for GDM: it is high-time.

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Diagnostic and prognostic utility of non-invasive imaging in diabetes management

Cristina Barsanti, Francesca Lenzerini, Claudia Kusmic

Cristina Barsanti, Francesca Lenzerini, Claudia Kusmic, Institute of Clinical Physiology, Italian National Research Council, 56124 Pisa, Italy

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Correspondence to: Claudia Kusmic, PhD, Institute of Clinical Physiology, Italian National Research Council, Via G. Moruzzi 1, 56124 Pisa, Italy. kusmic@ifc.cnr.it
 Telephone: +39-50-3153306

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Abstract

Medical imaging technologies are acquiring an increasing relevance to assist clinicians in diagnosis and to guide management and therapeutic treatment of patients, thanks to their non-invasive and high resolution pro-

perties. Computed tomography, magnetic resonance imaging, and ultrasonography are the most used imaging modalities to provide detailed morphological reconstructions of tissues and organs. In addition, the use of contrast dyes or radionuclide-labeled tracers permits to get functional and quantitative information about tissue physiology and metabolism in normal and disease state. In recent years, the development of multimodal and hybrid imaging techniques is coming to be the new frontier of medical imaging for the possibility to overcome limitations of single modalities and to obtain physiological and pathophysiological measurements within an accurate anatomical framework. Moreover, the employment of molecular probes, such as ligands or antibodies, allows a selective *in vivo* targeting of biomolecules involved in specific cellular processes, so expanding the potentialities of imaging techniques for clinical and research applications. This review is aimed to give a survey of characteristics of main diagnostic non-invasive imaging techniques. Current clinical appliances and future perspectives of imaging in the diagnostic and prognostic assessment of diabetic complications affecting different organ systems will be particularly addressed.

Key words: Medical non-invasive imaging; Diabetes; Diabetic complications; Molecular imaging; Multimodal imaging; Hybrid scanners

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Core tip: Non-invasive imaging techniques are increasingly employed in every medical field, both for diagnostic purposes and for monitoring of pathological progression and/or efficacy of treatments. Several imaging modalities are currently available to provide structural and functional information about tissue and organ physiology, and thanks to technical improvements and development of hybrid devices, multimodal imaging combining advantages of different techniques offers now new potentialities for research and clinics. Aim of

this review is to overview the principal features of most used diagnostic imaging modalities and to explore main current and forthcoming applications for the study and management of diabetes and its complications.

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INTRODUCTION

While X-ray diagnostic imaging has been in use for more than 100 years, it is in the last 40-45 years that imaging has made a great impact on healthcare due to the development of several modalities. Medical imaging technologies may be roughly divided into structural and functional imaging categories. The former entails the assessment of anatomical and morphological features of tissues and organs. Computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) scans are the prototypal and the most used non-invasive technologies for this imaging class. However, structural imaging alone may not provide the clinician or researcher with all the necessary information to fully characterize the pathophysiology of diseases. As such, functional imaging has come into existence and is comprised of a multitude of non-invasive, quantitative imaging techniques that are currently in use to study tissue and organ physiology, to probe molecular processes, and to study pathophysiological molecules and metabolites. The parallel development of specific contrast agents has significantly helped to improve the signal to noise ratio of the acquired images, and to gather both structural and functional information during the same scan sequence or within the same modality. Functional imaging is mainly achieved through the use of CT, MRI, and US, as well as through positron emission tomography (PET), single-photon emission computed tomography (SPECT), and optical imaging. Complementary information from structural and functional imaging can assist to determine the nature, location and extent of disease in patients, to guide interventions and to monitor the effects of treatment. Just for an example, MRI can be used to quantitatively determine the three-dimensional structure of an organ or tumour mass and by using the contrast agent, *e.g.*, gadolinium, it is also possible to monitor the blood flow which is an indicator of its functional state.

An accurate visual representation of the anatomy and sometimes of the functional state of the patient has been a goal of clinicians for several decades in many medical fields, although this aspect is often still neglected in diabetic patients. Nevertheless, the rapid rise in the prevalence of diabetes to 382 million

individuals worldwide during the last 20 years and the expected rise to 592 million by 2030^[1] has global implications and requires paradigm-shifting approaches to diagnosis, treatment monitoring, and prevention. Over the long term, hyperglycaemic conditions can lead to serious diseases affecting the cardiovascular system, eyes, kidneys, nerves, and teeth^[2-6]. In addition, people with diabetes also have a higher risk of developing infections, cognitive impairment and dementia^[7,8], and lower limb amputations^[9].

The present review aims to overview the current principal diagnostic appliances of imaging in the field of diabetes and its complications. In addition, mention will be also deserved to molecular and multimodal imaging, the two more recent approaches to non-invasive imaging tests that, by progressing in parallel with advancements in molecular pathology and with refinement of techniques, represent the new frontier of medical imaging and management of patients with diabetes.

DIAGNOSTIC IMAGING APPROACHES

A short analysis and comparison of the most employed techniques in diagnostic imaging can be of help in the evaluation of the approaches deserving advanced research with a view to both present application and future clinical translation. Table 1 sums up the main characteristics of the principal imaging modalities.

SPECT and PET

In nuclear medicine images of various body parts are produced by using small amount of radioactive tracers, administered intravenously or orally. Then, external detectors capture and form images from the radiation emitted by the radiopharmaceuticals.

There are two main nuclear imaging modalities: SPECT and PET, characterized by a very high sensitivity range (femto- to picomolar concentration range) but a limited spatial resolution (Table 1). Typical SPECT radionuclides are γ photon emitters (Table 2) and they are usually employed to label tracers of blood flow such as N-isopropyl-¹²³I-iodoamphetamine (¹²³I-IMP) and ^{99m}Tc-hexamethyl-propylene amine oxime (^{99m}Tc-HMPAO). Different SPECT radioisotopes can have one or more energy emission lines, therefore several processes can theoretically be imaged simultaneously by setting SPECT scanners at different energy windows. Among the limits of SPECT imaging there are the low temporal resolution, the limited number of available radiopharmaceuticals, and the difficulty to achieve absolute quantitative information due to lack of attenuation and scatter corrections necessary at the time of image reconstruction^[10].

PET differs from SPECT in that it relies on nuclides that are neutron-deficient, positron (β^+) emitters, with shorter half-lives (Table 2). It offers several advantages over SPECT. First of all, the large number of available

Table 1 Relevant features of the most common imaging modalities

Imaging modality	Anatomy	Metabolism/function	Spatial resolution	Weakness
SPECT	Poor	Yes	0.3-3 mm	Radiation
PET	Poor	Yes	1-4 mm	Radiation
CT	Yes	Yes	0.5-1 mm	Radiation
MRI	Yes	Yes	50-500 μ m	Expensive
Ultrasound	Yes	Yes	Approximately 200 μ m	Poor depth penetration
Optical	Poor	Yes	0.1-10 mm	Poor depth penetration

SPECT: Single-photon emission computed tomography; PET: Positron emission tomography; CT: Computed tomography; MRI: Magnetic resonance imaging.

radiolabeled compounds allows to image a large variety of functional cellular processes such as glucose and amino acid metabolism, neurotransmission, receptor affinity, gene expression, cell and molecular targeting. Moreover, the possibility of corrections at the time of image reconstruction allows quantitative measurements^[11]. However, one of the main disadvantages of PET is that all radionuclides decay at the same energy (photon energy of 511 KeV)^[11]. Therefore, it is not possible to simultaneously discriminate between different radiotracers at different energy windows. Furthermore, the short half-life of radioisotopes restrains the clinical use of PET mainly at those clinical centers which are equipped with a cyclotron. For this reason, radiopharmaceuticals with longer half-lives, such as ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) and ¹⁸F-fluoro-6-thia-heptadecanoic acid (¹⁸F-FTHA), have been implemented to assess glucose and fatty acid metabolism, respectively^[12].

X-ray CT

A CT scan consists of an X-ray beam (generated by an external source) passing through the body where a portion of the X-rays are either absorbed or scattered by the internal structures and organs, while the remaining X-ray pattern is transmitted to a rotating detector along multiple linear paths to create cross-sectional pictures of the body^[13]. CT scan involves a higher radiation dose than the conventional radiography. However, the radiation dose for a particular study depends on multiple factors: volume scanned, number and type of scan sequences, the desired resolution and image quality.

On the basis of the high spatial resolution (Table 1), CT scans can provide detailed information to diagnose, plan treatment for, and evaluate many conditions in adults and children. Additionally, the detailed images provided by CT scans may eliminate the need for exploratory surgery. CT scans are very good at imaging bone, soft tissue and blood vessel, even if the use of dyes with high atomic numbers is sometimes useful to

Table 2 Main nuclides used in nuclear medicine to label radiopharmaceuticals

SPECT			PET		
Nuclide	Half-life	γ (KeV%)	Nuclide	Half-life	β^+ (KeV%)
^{99m} Tc	6.02 h	89	¹⁸ F	109.8 min	96.9
¹¹¹ In	2.83 d	90.2	¹¹ C	20.4 min	99.7
¹²³ I	13.2 h	83	¹³ N	9.98 min	99.8
¹²⁵ I	60.14 d	6.5	¹⁵ O	2.03 min	99.9
			¹²⁴ I	4.18 d	25.0
			⁶⁴ Cu	12.7 h	17.9
			⁶⁸ Ga	68 min	90.0
			⁸² Rb	1.2 min	99.9

Radiation types: γ : Isomeric transition (gamma) decay; β^+ : Positron decay; KeV%: Percentage of energy per decay. SPECT: Single-photon emission computed tomography; PET: Positron emission tomography.

improve soft tissue contrast. Iodine-based compounds (classified into non-ionic and ionic) are mainly used as water soluble CT contrast agent to be injected intravascularly or into any sinus or body cavity, and can also give an indication of the renal function (*e.g.*, kidney filtration). Concerns about CT scans include the risks from exposure to ionizing radiation and possible allergic or toxic reactions to the intravenous contrast agents. The overall adverse reactions occur in 1% to 3% of people with non-ionic contrast agents and in 4% to 12% with ionic contrast agents^[14]. Skin rashes may appear within a week to 3% of people^[15].

Two of the most prevalent clinical and diagnostic applications, especially on the cardiovascular field, are the CT angiogram (CTA) and artery calcium scoring. The former can be used to view arteries and veins and requires contrast dye injected into the bloodstream. CTA images can be 3D reconstructed to overview all the organ vasculature and can be rotated and viewed from all angles.

As the artery calcium score test is concerned, it does not use X-ray contrast, and pictures are taken to look for the presence of calcium depots in the blood vessels, mainly in the coronary arteries. Calcium deposits are a very specific sign of coronary artery disease, as they are associated with cholesterol and scar tissue buildup in the arteries. While the amount of calcium in the arteries increases with age, patients who have significantly elevated amounts of calcium depots are at increased risk to heart attacks or other cardiovascular complications^[16].

Magnetic resonance

MRI uses strong magnetic fields and pulses of radio waves to produce cross-sectional images of organs and internal structures in the body at high spatial resolution (Table 1). Because the signal detected varies depending on the water content and local magnetic properties of a particular area of the body, MRI provides an excellent soft tissue contrast. Unfortunately, its sensitivity is low (micro- to millimolar

concentration range) and at present there are a limited number of ligands. The physical basis of magnetic MRI is the quantum interaction between a nuclear spin of certain atoms (^1H , ^{13}C , ^{19}F , ^{23}Na , ^{31}P and others) and an external magnetic field. MRI scanner detects the radio frequency signal which is emitted only by excited atoms in the body, when they are perturbed by an applied pulse in the range of the radio waves. Image contrast depends on three parameters: the proton density, the longitudinal relaxation time which corresponds to the energy transfer between excited spins and tissue (T1, spin-lattice relaxation time), and the transverse relaxation time which is related to the decay of magnetization by interaction between nuclei (T2, spin-spin relaxation time)^[17]. Variable image contrast can be achieved by using different pulse sequences and by changing the imaging parameters. Signal intensities on T1, T2, and proton density-weighted images relate to specific tissue characteristics. Moreover, it is possible to employ contrast agents that magnetically modify the proton spin environment to provide a positive enhancement (T1-targeted), mostly achieved by gadolinium chelates, or negative enhancement (T2- and T2*-targeted probes), by using paramagnetic ultra-small particles of iron oxide (USPIOs)^[18].

In 1990, Ogawa *et al.*^[19] discovered that deoxy-hemoglobin acts as a natural contrast agent to study brain activity on the basis of changes in blood flow, thus providing a functional value to MRI. Functional MRI based on the blood-oxygen-level-dependent (BOLD) contrast is applied both in the research field and, to a lesser extent, in the clinical arena. In this latter case, it is used to anatomically map the brain and detect the effects of tumors, stroke, head and brain injury, or diseases such as Alzheimer's^[20-22].

Although MRI does not use ionizing radiation and no harmful side-effects are known to be associated with temporary exposure to the strong magnetic field, there are important safety concerns to consider before performing or undergoing an MRI scan. The magnet, indeed, may cause pacemakers (and any other implanted medical devices that contain metal) to malfunction or heat up during the exam.

While MRI provides information on the spatial location and local chemical environment of protons, proton magnetic resonance spectroscopy (^1H -MRS) is a non-invasive technique providing biochemical information about tissues. ^1H -MRS is based on the principle that the resonance frequency of protons is also dependent on their chemical environment (e.g., protons have a slightly different resonance frequency in lipids than in water). Therefore, protons can be visualized at a specific chemical shift (peak position along the X-axis) depending on their chemical environment. The panel of metabolites that can be recognized by MRS include some amino acids, neurotransmitters, choline, lactate, lipids, creatinine, and myo-inositol. MRS is currently used to investigate brain and metabolic disease^[23-25].

Ultrasound imaging

Diagnostic ultrasound, or US, is an imaging method that uses high-frequency sound waves (1 to 12 MHz) and their echoes to produce relatively precise images of structures within the body (Table 1). The transducer probe is the main part of an ultrasound machine. It generates and receives sound waves using a principle called the piezoelectric effect. The sound waves travel into the body and hit a boundary between tissues (e.g., between fluid and soft tissues or between soft tissues and bone). Some of the sound waves get reflected back to the probe, while some others travel on further until they reach another boundary and get reflected. The machine calculates the distance from the probe to the tissue or organ (boundaries) by using the speed of sound in tissue and the time of each echo's return, and then displays a 2D-image based on the echoes' intensity. Transducer probes come in many shapes, sizes and frequency of emitted sound waves. This latter parameter determines how deep the sound waves penetrate into the body, and so affects the resolution of the image.

Contrast enhanced ultrasound extends ultrasound techniques to the exploitation of gas-filled microspheres [microbubbles (MB)] as an ultrasound contrast medium. MB are commercially available for clinical use in cardiovascular imaging, being confined by their size to the intravascular space. Their proven clinical tolerability, along with the advantages of real-time imaging, high spatial resolution, and the relatively low cost of equipment renders molecular targeting of MB an attractive option for future development from its current preclinical stage to the actual clinical application^[26-29]. A variant of US is based upon the Doppler effect (Doppler US). When the object reflecting the ultrasound waves is moving, it changes the frequency of the echoes as a function of its velocity. Doppler US measures the change in frequency of the echoes to calculate how fast the object is moving, and it is mostly used to measure the rate of blood flow.

Optical imaging

Optical imaging is based on the detection of molecular emission in the electromagnetic spectrum (visible and near-infrared) by a high sensitive and high resolution charge-coupled device digital camera. It extends over a wide range on the imaging resolution scale (Table 1) and is often complementary to other imaging modalities.

Optical imaging offers a number of important advantages over the existing radiological imaging techniques. It uses non-ionizing radiation, which significantly reduces patient radiation exposure, provide high sensitivity detection (pico- to nanomolar concentrations) and allows for repeated studies over time. Moreover, optical imaging has the potential to differentiate among soft tissues, and between native tissues and tissue labeled with contrast media (either endogenous or exogenous compounds), using their

different photon absorption or scattering profiles at different wavelengths. Optical imaging encompasses a host of light-based imaging modalities, including diffuse optical tomography (DOT), optical coherence tomography (OCT), and hyper-spectral imaging, that holds great potential for improving disease prevention, diagnosis, and treatment in healthcare facilities.

DOT modality utilizes red and near-infrared light (λ 650-900 nm) to probe the optical properties of tissues. By measuring the spatio-temporal variations of transmitted and back-scattered light intensities, it is possible to image regional variations in the chemical concentration of specific molecules to detect cellular physiological changes (e.g., neuronal activation). The limited spatial resolution (approximately 1 cm) of DOT is balanced by a high temporal resolution (approximately 10 ms), a potential large optical penetration depth (up to several cm) which depends on the characteristics of the light source and by the light-transmittance of the tissue, a high intrinsic contrast associated with hemoglobin (contrast factor of 10-100 in most soft tissues), and the capability of spectral discrimination of multiple chromophores. DOT has been used in multiple thick tissue imaging appliances, including brain functional imaging, breast cancer imaging, and tissue oxygenation analysis. Methods to improve DOT imaging performance by combining multi-modality information such as from X-ray, CT and MRI are also being explored^[30-33].

OCT detects light that has been back-scattered from structures at a particular depth by exploiting constructive and destructive interference between the returning light and a reference beam. It is a technique for obtaining sub-surface images (up to 2 mm), now in use in a variety of applications, including art conservation, artery disease, and diagnosis of diabetic retinopathy^[34].

HIS imaging, or imaging spectroscopy, represents a hybrid modality for optical imaging which combines the power of conventional digital imaging and spectroscopy. Indeed, it provides a three-dimensional matrix, or image cube, merging information coming from every pixel of the entire 2D-image with the optical spectrum over a large number of wavelengths (typically tens to hundreds)^[35]. The high spatial and spectral resolution offered by HIS allows to detect and quantify tissue environment in the early stages of disease progression. In the medical and clinical scenarios, HIS has exhibited a great potential in the early diagnosis of several forms of cancer, peripheral artery disease, burn wounds, diabetic foot, and ischemic tissue pathology^[36-39].

Hybrid techniques for multimodal imaging

To overcome single modality limitations (e.g., the strong variation in sensitivity, spatial and/or temporal resolution, and quantitative analysis capabilities), multimodal imaging which combines techniques

with complementary strength has grown up fast in clinical practice. Most of the examinations, however, are performed on separate machines, with some drawbacks that can impact on the diagnostic accuracy: an inaccurate anatomic matching due to patient repositioning, side-by-side or co-registration of images, time-consuming and expensive processes. In the last decade, the development of combined PET-CT integrated systems has revolutionized the concept of hybrid imaging^[40]. On the success of PET-CT hybrid scanners, more recently also SPECT-CT hybrid devices have been introduced^[41]. However, there are also some negative aspects in using CT as complementary anatomical imaging modality, such as the additional radiation to the patient and the poor soft tissue contrast in the absence of contrast agents. These shortcomings do not apply to MRI and, hence, the idea to combine PET and MRI in a unique device. Hybrid PET-MRI scanners are currently available mainly for preclinical studies on small animals, and in a proof-of-principle phase for clinical applications. Hybrid bimodal PET-MRI imaging is attracting great interest because, unlike PET-CT that requires sequential acquisition of PET and CT images, it makes available the simultaneous acquisition of PET and MRI images, and potentially performs dynamic imaging to obtain valuable functional information within an accurate anatomical framework^[42-44].

Targeted molecular imaging

The significant advancement provided by molecular targeting of imaging probes with respect to the traditional diagnostic techniques of morphological, functional or metabolic imaging runs parallel to the advancements in the hybrid imaging systems^[45,46]. Molecular imaging approach allows the selective *in vivo* targeting of biomolecules that are specifically expressed in cellular processes contributing to the development of a variety of disease states. It requires the availability of appropriate molecular probes, composed by a label system that can be visualized by imaging devices and a ligand that recognizes and binds the molecular target (e.g., antibody, peptide, small synthetic or natural molecules). The application of targeted molecular imaging has already proven valuable in clinical oncological practice for early detection and diagnosis as well as in prognosis^[47]. Despite its great capability, however, molecular imaging approach in other medical fields is still mainly confined to the laboratory settings and almost exclusively used in preclinical studies. Many areas of research are very active in this field, especially studies centered on the detection of pre-disease states or molecular states that occur before typical disease symptoms are overt^[48,49]. Other important areas of interest are the imaging of gene expression and the development of novel biomarkers^[50,51]. Nevertheless, at present there are some barriers to a widespread clinical translation

Table 3 Synopsis of imaging modalities and their applications in diabetes

	SPECT/PET	CT	MR	US	Optical	Application
Pancreas (β -cell function)	$[^{18}\text{F}]$ -tracer		MRI		Luminescence	Mainly preclinical ^[61,62]
Pancreas (transplant/ inflammation)	$[^{18}\text{F}]$ -tracer		MRI	High-frequency		Preclinical and clinical ^[63-68]
Kidney			MRI/BOLD-MRI	B-mode/Doppler		Clinical ^[70-78]
Brain	$[^{123}\text{I}]$ - and $[^{99}\text{Tc}]$ -tracer		MRI/MRS	Doppler		Clinical ^[22,82,83]
Vessels/ atherosclerosis	$[^{18}\text{F}]$ -tracer	Angio-CT	MRI	B-mode		Clinical ^[85-95,100-106]
Ulcerations	$[^{99}\text{Tc}]$ -tracer	Hybrid SPECT/CT	MRI	Doppler	HIS	Clinical ^[113,114,116,117]
Heart	$[^{18}\text{F}]$ -, $[^{123}\text{I}]$ - $[^{11}\text{C}]$ -tracer	Hybrid PET/CT	MRI/MRS	Doppler		Clinical ^[95,120-126,130,131,134,135]
Visceral fat		CT/dual energy CT	MRI/MRS	B-mode		Clinical ^[142,145,146]

SPECT: Single-photon emission computed tomography; PET: Positron emission tomography; CT: Computed tomography; MRI: Magnetic resonance imaging; US: Ultrasound; MRS: Magnetic resonance spectroscopy; BOLD: Blood-oxygen-level-dependent; HIS: Hyperspectral imaging.

of molecular imaging: the paucity of approved molecular imaging agents; the difficulty of combining the suitable characteristics of the probe (feasibility of synthesis, pharmacokinetics, high binding efficacy and specificity) with the lack of toxicity in patients; the reduced interest for industrial investment considering that, by its very nature, molecular imaging (as well as personalized medicine) decreases the size of the possible patient set from a commercial point of view^[52,53].

MEDICAL AND CLINICAL APPLICATIONS OF IMAGING IN DIABETES

A general view of the different non-invasive imaging approaches and their applications on the medical and clinical settings is offered in this section. Some advantages and drawbacks of alternative or combined approaches are also described. Table 3 sums up the most employed imaging modalities in the diagnosis and monitoring of diabetic complications and end-organ damage.

Pancreatic islet and beta cells imaging

The loss of functional β -cells is decisive in the development of both type 1 (T1D) and type 2 (T2D) diabetes. T1D is characterized by an autoimmune reaction against pancreatic β -cells, while T2D leads to β -cell dysfunction due to insulin resistance^[54,55].

The possibility to non invasively imaging the severity and the extent of a critical mass of β -cell destruction could significantly aid in the diagnosis and treatment of diabetes. However, imaging of β -cells is a major challenge due to the small size of pancreatic islets, the low density distribution of islets throughout the pancreas, and the scarce inherent contrast from the surrounding tissues.

A variety of currently available imaging techniques, including MRI^[56,57], bioluminescence imaging^[58,59], and nuclear imaging (PET and SPECT) have been tested for the study of β -cell diseases^[60]. The majority of the

cited studies was carried out on animal models, and even though the translational potential of some of the methods is hampered by the depth of the pancreas in the human body, in many cases the possibility of a clinical transfer embodies a real opportunity.

Since zinc plays a critical role in the biosynthesis and secretion of insulin, Lubag *et al.*^[61] demonstrated the feasibility of utilizing zinc-responsive T1-contrast MRI for monitoring islet β -cells function in animal models. Currently, there are two approaches that have been developed for monitoring β -cell function using MRI: manganese-enhanced and a zinc-responsive contrast agent^[62].

Moreover, MRI proved to be useful at diagnosing and monitoring immune cell infiltration of the pancreas^[63-66] by using superparamagnetic iron oxide nanoparticles as T2-weighted negative contrast agent.

Very recent preclinical studies proposed the use of PET imaging to evaluate the loss of pancreatic islet cells in a rodent model of T1D, using $[^{18}\text{F}]$ -fallypride, a dopamine D2/D3 receptor radiotracer^[67].

Finally, PET, MRI and US have been used in several trials to investigate the efficacy of different imaging modalities for visualizing transplanted islets^[68]. Since these methodologies have different advantages and disadvantages, their use in combination is recommended for accurate assessment of the condition of transplanted islets^[69].

Diabetic nephropathy and kidney imaging

In diabetic patients, renal functional deterioration is the result of heterogeneous renal structural changes and represents 35%-40% of new cases of renal insufficiency requiring dialysis. Renal damage occurs in multiple stages and diagnostic tests that help to identify early stages of kidney alterations will provide significant benefits to get the disease under control. In the early stages of diabetic nephropathy, kidney size may be enlarged from hyperfiltration^[70-72]. With progression of kidney disease in diabetes, the kidneys diminish in size due to glomerulo-sclerosis^[70]. US

imaging is typically performed to assess kidney size^[73]. Moreover, a renal ultrasound examination can reveal hyperechogenicity that is suggestive of chronic kidney disease, and can assist in ruling out any obstruction. In addition, Doppler US can support in both the evaluation of renal parenchymal perfusion and the computation of renal resistance parameters to assess endothelial dysfunction and microvascular impairment in the kidneys of diabetic patients^[74].

Also quantitative diffusion-weighted MRI and BOLD-MRI can play a role in the evaluation of renal disease^[75-77] and may facilitate the development of more effective follow-up and treatment modalities^[78].

Brain imaging in diabetes

The technique of choice to image the brain is MRI that spans from coarse anatomical studies of atrophic areas to the more detailed investigations of both functional and structural alterations in both gray and white matter of specific cerebral areas. It has been suggested that the risk of decreases in cognitive ability, usually associated with aging, is increased in type 2 diabetic patients. Some recent and comprehensive reviews focused on the relationship between diabetes and brain abnormalities^[22,79]. A consistent number of studies were aimed to measure volumetric differences in diabetic population by MRI. During aging, a global brain atrophy with an average decline in the brain volume of 0.1%-0.5% per year is physiological^[80,81]. However, brain volume in T2D patients is reduced of 0.5%-2.0% relative to controls^[22], correspondent to an extra 2-5 years of normal aging. Brain atrophy can be generalized or focal, with the medial temporal lobe mainly involved^[22], and can be related to either the white or the gray matter, or both. Ryan *et al.*^[79] reported that insulin resistance is a predictor of gray matter atrophy and cognitive impairment. Moreover, the analyses of T2-weighted MR brain images of diabetic patients revealed micro- and macrovascular alterations induced by the chronic inflammation associated with hyperglycemia, which could play a role in the hyperintense lesions of the white matter observed^[82]. Several MRI studies indicated a significant correlation between T2D and brain infarct, mostly lacunar necrosis^[22], and such parameters as insulin resistance, nephropathy, diabetes duration and high systolic blood pressure are suggested as main determinants of the increased occurrence of brain infarcts in the diabetic population^[22].

At present, there are few studies on brain metabolism of diabetic patients by MR spectroscopy^[23-24,83]. They were mainly addressed to determine the alterations of the resonance peaks of brain metabolites and neurotransmitters under different conditions of cognitive impairment^[22].

Brain microvascular function can be investigated by imaging of cerebral blood flow and cerebrovascular reactivity. The former is assessed by SPECT (using

¹²³I- or ⁹⁹Tc- labeled tracers), MRI (phase-contrast or arterial spin-labeling modalities) and transcranial Doppler US. Cerebrovascular reactivity, assessed by MRI or transcranial Doppler US, is the measure of the microvascular reserve defined as the increase of blood flow under maximal cerebral vasodilation induced by acetazolamide or CO₂. At present, however, studies on cerebral blood flow and cerebrovascular reactivity in patients with T2DM show conflicting results^[22].

Imaging of vasculature and vascular changes

Non-invasive techniques provide information on macrovascular anatomy, as well as on functional parameters concerning vessel flows, tissue perfusion, microcirculation, all of which are affected by complications concurring in the high morbidity and mortality on diabetic patients.

Ultrasonography is mainly used to assess the atherosclerotic burden in non coronary arteries. Doppler US has been successfully employed for an early and accurate characterization of the vasculopathy of lower limb arteries (a strong risk factor in the development of diabetic foot ulcers)^[84], thus favoring the prevention or delay of foot complications, especially amputation. Moreover, the measurement of the carotid intima-media thickness (IMT) by US has been demonstrated a useful marker of the progression of atherosclerosis throughout the body, and an excellent predictor of cardiovascular events even in diabetic population^[85,86]. Furthermore, carotid IMT can be used to evaluate the efficacy of new treatments^[87-90].

At present, techniques based on CT technology, such as coronary artery calcium scoring and coronary multi-slice CT angiography, are considered the most robust imaging techniques for non-invasive visualization of coronary atherosclerosis, assessment of plaque composition and level of calcification^[91-93]. Also MRI is emerging as an important modality to assess atherosclerotic plaque burden and morphology in non coronary arteries^[94,95].

Nevertheless, because altered vessel morphology may be ambiguous, the ability to non invasively evaluate molecular and cellular pathological processes becomes crucial in terms of early detection and preventive treatment. The use of functional and molecular imaging approaches will provide valuable diagnostic tools. Recently, by using MRI in experimental studies on rodent diabetic models, Medarova *et al.*^[96] evaluated pancreatic vascular volume, microvascular flow, and permeability, that are common disease biomarkers for both T1D and T2D^[97-99].

Moreover, PET imaging studies reported a strong relation between peripheral artery atherosclerosis and increased regional ¹⁸F-FDG uptake (glycolytic metabolism) in subjects presenting impaired glucose tolerance and T2D^[100,101]. In addition, inflammatory condition associated with atheroma or atherosclerosis progression has been investigated by both single and

dual-modal imaging, using ^{18}F -FDG-PET/CT^[102], USPIO-MRI^[103-105], nanoparticle PET-CT^[106]. Finally, it is very promising, but mainly limited to the preclinical field, the use of nanoparticles appropriately functionalized with ligands or antibodies vs cell membrane molecules (VCAM-1, PECAM-1, E-selectin, P-selectin) to detect activated endothelial cells in different imaging modalities (OCT, MRI, enhanced US, PET)^[107-109], or even in the same acquisition session (hybrid MRI or PET-CT and MRI-PET scanners)^[110,111] in order to obtain a molecular contrast.

Imaging of ulcerations and diabetic foot

Lower extremity and particularly foot ulcers are among the most frequent complications of diabetes. It is estimated that 15%-25% of T1D and T2D patients are affected by skin ulcers in their lifetime^[112]. Factors as peripheral neuropathy and vascular disease contribute to the development of skin ulcerations. Some valuable information on vasculopathy can be provided by Doppler US examination in patients with diabetic foot^[113]. Moreover, in the last few years hyperspectral imaging (HIS) has been launched as a useful diagnostic tool to monitor microvascular changes and tissue perfusion impairment associated with diabetic ulcer formation and healing^[114]. By selecting proper wavelengths within the visible and very near infrared region (400-1000 nm) of the electromagnetic spectrum, HIS allows to acquire spatial maps of oxy- and deoxyhemoglobin and, thus, to quantify tissue oxygenation. In the management of diabetic foot ulcers, it represents a valuable tool in the assessment of wound healing potential and in guiding the proper therapy in order to prevent infections and amputations. If left untreated, a relevant cases of foot ulcers lead to infection, limited joint mobility, muscular alterations and deep-tissue necrosis^[112]. Bones may also be involved in two different clinical conditions associated with diabetic complications, such as osteomyelitis and Charcot osteoarthropathy^[115]. The former is mainly due to direct bone contamination from a soft-tissue ulcer and accounts for approximately one third of diabetic foot infections, whereas the latter is a chronic and progressive inflammatory disease affecting the bone and joints. Both osteomyelitis and Charcot foot are conditions with an increased risk of lower limb amputation from 25% to 50%. It has been suggested that about 50% of those amputations could be avoided by an early diagnosis and a multidisciplinary approach. The major diagnostic difficulty is in distinguishing osteomyelitis from non-infectious bony disorders as Charcot foot^[115].

X-ray planar radiographs are relatively inexpensive and readily available, but their sensitivity is quite poor and false negative results are not so rare, especially in the first stages of osteomyelitis. Bone biopsy is considered the technique of choice for detection of osteomyelitis, however conventional imaging (MRI, SPECT and hybrid SPECT/CT) are valuable

support in the early diagnosis of infections and their accurate anatomical localization^[116]. In addition, due to their non-invasive nature, imaging studies proved particularly useful in monitoring the progression of the disease and the efficiency of specific treatments. Valabhji *et al.*^[117] show the effective role of MRI also in guiding the time course of the antibiotic therapy in the management of diabetic foot complicated by osteomyelitis. Unfortunately, the major limitation of MRI imaging is its inability to accurately differentiate osteomyelitis from other inflammatory bone disease. Similarly, the use of SPECT imaging modality that combines technetium methyl-diphosphonate ($^{99\text{m}}\text{Tc}$ -MDP) bone scan with technetium hexamethylpropylene amine oxime ($^{99\text{m}}\text{Tc}$ -HMPAO)-labeled leukocytes scan is adequate for osteomyelitis diagnosis^[118], but is poor in the anatomical localization of the infection, due to the limited spatial resolution of nuclear imaging.

Recently, new hybrid imaging technologies combining SPECT localization of $^{99\text{m}}\text{Tc}$ -HMPAO-labeled leukocytes and high resolution X-ray CT have been introduced and provided effective in the differential diagnosing of osteomyelitis in patients with diabetes^[116]. The use of ^{18}F -FDG-PET has emerged as a possible alternative nuclear imaging modality combined with CT in the diagnosis of bone infection secondary to diabetic ulcerations. However, at present, the data on the role of PET and PET/CT in the evaluation of diabetic foot infections are limited and the results reported are rather inconsistent, especially in the absence of an appropriate reference standard^[119].

Imaging of diabetic cardiomyopathy

Accumulating data from experimental, pathological, epidemiological, and clinical studies have shown that diabetes mellitus results in cardiac functional and structural changes, independent of hypertension, coronary artery disease or any other known cardiac disease, which support the existence of diabetic cardiomyopathy. The pathophysiology of diabetic heart disease is likely multifactorial, involving altered myocardial metabolism, endothelial dysfunction and vascular disease, autonomic neuropathy, and increased myocardial fibrosis. Most of the conventional non-invasive imaging modalities can provide valuable insights into the disease process and can be useful for monitoring disease progression and evaluating the effectiveness of medical interventions.

Ventricular function and perfusion: Conventional diagnostic imaging modalities currently aids in non-invasive assessment of both systolic and diastolic dysfunction in diabetic patients. Pulsed wave Doppler studies measuring transmitral inflow, deceleration time and isovolumic relaxation time are the gold standard to diagnose ventricular diastolic dysfunction^[120,121]. Moreover, tissue Doppler imaging and strain rate imaging are considered more sensitive for detection

of LV dysfunction than conventional trans-thoracic echocardiography, especially in the early stages of diabetes in which the sole sub-endocardial dysfunction is overt. Cardiac MRI has recently emerged as a very good imaging tool for the diagnosis of structural and functional disorders of the myocardium. Gadolinium-enhanced cardiac MRI has been found to be useful in the prediction of major adverse cardiac events in diabetic patients without previous history of ischemic heart disease^[95,122]. Cardiac MRI is also useful to detect diastolic dysfunction and myocardial steatosis^[95]. Among the available imaging modalities, only PET allows quantitative assessment of myocardial blood flow using radiotracers kinetics^[95,123,124]. The combined images by MRI and PET provide a high spatial resolution detection of myocardial metabolic abnormalities and currently represent the more valuable imaging analysis in the diagnosis and prognosis of diabetic disease. Unfortunately, many diabetic patients with advanced stages of cardiomyopathy have had mechanical interventions that have inserted metallic devices into the heart (e.g., defibrillators, left ventricular assist devices) that preclude the possibility to use MRI. Under these circumstances, the low anatomical (spatial) resolution of PET can be compensated by the combined PET/CT imaging. This recent hybrid modality is also particularly indicated in the case of diabetic patients with or at risk of coronary artery disease, since CT is currently considered very reliable in evaluating coronary artery calcium plaque burden and, with the aid of contrast agents, it provides an accurate coronary angiogram^[125,126].

Cardiac autonomic neuropathy in diabetes: It is one of the diabetic complications that increase the risk of myocardial infarction and sudden death in diabetic patients. The need of an early diagnosis of cardiac autonomic neuropathy (CAN) for clinical decision-making of these patients is evident considering that it was estimated that the 5-year mortality rate is 5 times higher in diabetic patients with CAN compared with patients without evidence of CAN^[127]. CAN detection requires several indirect tests to assess the activity of both the parasympathetic and the sympathetic branches of the autonomic system. However, the only direct method to assess cardiac autonomic activity is by using nuclear imaging. Currently, either SPECT and PET clinical imaging is limited to assess sympathetic activity and innervation, with parasympathetic imaging limited mostly to preclinical and translational studies^[95]. Cardiac sympathetic imaging is focused on synaptic junction, and in particular on the pre-synaptic endings, where the norepinephrine transporter (NET) protein, also known as uptake 1, is localized^[128]. NET is responsible for the most part of the re-uptake of synaptic norepinephrine that is released following sympathetic nerve endings stimulation. Several studies used radiolabeled analogs of norepinephrine

to evaluate the cardiac neuronal activity and function. The most commonly used is metaiodobenzylguanidine (MIBG), a molecule that is taken up by NET protein but that is not catabolized by monoamine oxidase or catechol-o-methyltransferase, thus allowing to accumulate into the sympathetic synaptic endings^[129]. MIBG can be easily labeled with the radionuclide ¹²³I, a γ photon emitter and, thus, imaged by SPECT scanner. Besides, some PET tracers have also been developed based on molecules sharing similarities with norepinephrine, including ¹¹C-meta hydroxyephedrine (¹¹C-HED), ¹¹C-epinephrine (¹¹C-EPI) and ¹¹C-guanyl-meta-octopamine (¹¹C-GMO). Both clinical and experimental studies with these tracers have provided significant information on cardiac sympathetic dysfunction in many diseases, diabetes included^[130-132].

More recently, experimental and pre-clinical studies tested a new ¹⁸F-labeled NET substrate (namely, ¹⁸F-LMI1195) designed to allow PET cardiac neuronal imaging with high sensitivity and resolution^[133].

Altered myocardial metabolism: It is commonly accepted that one of the mechanism leading to diabetic cardiomyopathy is the accumulation of fatty acids in myocardial tissue (myocardial steatosis). When the fatty acid uptake oversteps the oxidative capability of myocyte, the exceeding fatty acids are stored in the cell cytoplasm as triglycerides. Intracellular triglycerides are inert *per se*. However, a proportional part of them are transformed in toxic intermediates through non-oxidative pathways. At present, myocardial triglycerides can be quantified (thus having an estimate of their toxic metabolites) by means of ¹H-MRS scanners with field strength ≥ 1.5 Tesla. Several experimental and clinical studies have used ¹H-MRS to correlate the increased myocardial triglyceride content and ventricular dysfunction in diabetes^[95]. Magnetic resonance spectroscopy is also suitable to monitor the effect of pharmacological treatment of diabetes on intra-myocardial triglyceride accumulation^[134,135].

Measuring of visceral and liver fat

Excessive body fat is a major risk factor for several diseases, including insulin resistance, T2D, and cardiovascular disease. In addition, fat accumulation in specific body tissues and/or organs (named, ectopic fat) such as visceral, intrahepatic and intramuscular lipid stores, pericardial, perivascular and perirenal fat depots, is considered an important predictor of cardiometabolic and vascular risk^[136,137]. Therefore, regional fat distribution might be a more predictive factor for specific risks than obesity itself and an accurate measurement of fat accumulation might represent an additional prognostic value in the risk assessment of patients. Dual energy X-ray absorptiometry (DEXA) is considered the reference choice to evaluate body composition^[138]. It measures three different compartments: fat mass,

non-bone lean mass and bone mineral content. DEXA is accurate, time and cost effective, widely available, and has low radiation exposure but is, on the whole, unable to discriminate among fat depots, except for a software that has been recently proposed to quantify the visceral fat compartment^[139]. For this reason, MRI and CT are currently considered the gold standard methods to measure adiposity and accurately distinguish between subcutaneous and ectopic visceral fat. However, the use of CT for fat distribution analysis is discouraged, especially in children, due to the high levels of radiation exposure. More recently, several studies have reported grey-scale and/or contrast-enhanced US as a promising technique to measure subcutaneous adipose tissue thickness and abdominal visceral fat^[140,141]. Moreover, a strong correlation between US and CT assessment of fat depots was found^[140]. If adequately validated, US might represent the clinical standard methodology for longitudinal studies on fat content and distribution in response to treatment^[142]. In addition, contrast-enhanced US showed a good sensitivity (> 95%) and specificity (> 90%) even in revealing fatty liver^[143,144]. However, as hepatic fat quantification is concerned, also CT, dual-energy CT (80 and 140 kVp), MRI and ¹H-MRS perform very well, with high specificity, and are considered the front runners in the non invasive diagnosis and quantification of moderate to severe liver steatosis^[145-147].

CONCLUSION

Considering the current trends in medicine, it can be expected that diagnostic non-invasive imaging techniques, particularly multimodal hybrid devices, will become increasingly available in the clinical arena and assume an always more important role in supplementing the clinical evaluation of the diabetic patient.

The challenge is to combine the diagnostic utility of imaging tools with therapeutic entities ("theranostics") in order to improve risk stratification and personalized therapy for diabetes management. It poses both scientific and technical problems: molecular and cellular biology and pathology on one side, and physical and chemical methodologies on the other. As for the contribution of medicinal chemistry, it is required the use of nanometer-scale materials to provide molecular imaging with simultaneous treatment. The nanomaterial platforms have to integrate molecular targeting ligands, therapeutic moieties and complementary imaging (multi)-modalities. Recently, Arifin *et al.*^[148] have introduced a biohybrid theranostic agent composed by human pancreatic islets encapsulated in a porous matrix together with functionalized Gd-gold nanoparticles which could serve as a contrast agent for three complementary imaging modalities (MRI, CT and US). They found that

microcapsules containing islet cells were able to restore normoglycemia in a mouse diabetic model and could be tracked by trimodal non-invasive imaging. Analogously, Barnett *et al.*^[149] reported the theranostic capabilities of functionalized magneto-capsules containing human pancreatic islet β -cells in mouse and swine pre-clinical models. Moreover, dextran-coated iron oxide nanoprobe, suitable for MRI, have been functionalized with small interfering RNA^[150,151] to silencing specific genes of choice. Although important in proving new principles, at present these contributions are still in an exploratory, preclinical stage. Future studies should be performed in models endowed with increased power of predicting human efficacy and safety, thus warranting clinical translation and development in a demanding regulatory environment.

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Gut-brain connection: The neuroprotective effects of the anti-diabetic drug liraglutide

Emanuel Monteiro Candeias, Inês Carolina Sebastião, Susana Maria Cardoso, Sónia Catarina Correia, Cristina Isabel Carvalho, Ana Isabel Plácido, Maria Sancha Santos, Catarina Resende Oliveira, Paula Isabel Moreira, Ana Isabel Duarte

Emanuel Monteiro Candeias, Inês Carolina Sebastião, Susana Maria Cardoso, Sónia Catarina Correia, Cristina Isabel Carvalho, Ana Isabel Plácido, Maria Sancha Santos, Catarina Resende Oliveira, Paula Isabel Moreira, Ana Isabel Duarte, CNC - Center for Neuroscience and Cell Biology, Rua Larga, Faculty of Medicine (1st Floor), University of Coimbra, 3004-517 Coimbra, Portugal

Emanuel Monteiro Candeias, Inês Carolina Sebastião, Susana Maria Cardoso, Sónia Catarina Correia, Cristina Isabel Carvalho, Ana Isabel Duarte, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Casa Costa Alemão - Pólo II, Rua D Francisco de Lemos, 3030-789 Coimbra, Portugal
Ana Isabel Plácido, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal

Maria Sancha Santos, Life Sciences Department, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal

Catarina Resende Oliveira, Institute of Biochemistry, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal
Paula Isabel Moreira, Institute of Physiology, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal

Author contributions: Candeias EM and Sebastião IC performed the literature search, wrote the text and draw Table 1; Cardoso SM, Correia SC, Carvalho CI, Plácido AI and Santos MS draw the figures; Oliveira CR, Moreira PI and Duarte AI suggested the theme to be reviewed, designed the text structure and made the several critical corrections and revisions until the submitted version was achieved.

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Correspondence to: Dr. Ana Isabel Duarte, CNC - Center for Neuroscience and Cell Biology, Rua Larga, Faculty of Medicine (1st Floor), University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal. anaimduarte@gmail.com
Telephone: +351-239-820190
Fax: +351-239-822776

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Abstract

Long-acting glucagon-like peptide-1 (GLP-1) analogues marketed for type 2 diabetes (T2D) treatment have been showing positive and protective effects in several different tissues, including pancreas, heart or even brain. This gut secreted hormone plays a potent insulinotropic activity and an important role in maintaining glucose homeostasis. Furthermore, growing evidences suggest the occurrence of several commonalities between T2D and neurodegenerative diseases, insulin resistance being pointed as a main cause for cognitive decline and increased risk to develop dementia. In this regard, it

has also been suggested that stimulation of brain insulin signaling may have a protective role against cognitive deficits. As GLP-1 receptors (GLP-1R) are expressed throughout the central nervous system and GLP-1 may cross the blood-brain-barrier, an emerging hypothesis suggests that they may be promising therapeutic targets against brain dysfunctional insulin signaling-related pathologies. Importantly, GLP-1 actions depend not only on the direct effect mediated by its receptor activation, but also on the gut-brain axis involving an exchange of signals between both tissues *via* the vagal nerve, thereby regulating numerous physiological functions (*e.g.*, energy homeostasis, glucose-dependent insulin secretion, as well as appetite and weight control). Amongst the incretin/GLP-1 mimetics class of anti-T2D drugs with an increasingly described neuroprotective potential, the already marketed liraglutide emerged as a GLP-1R agonist highly resistant to dipeptidyl peptidase-4 degradation (thereby having an increased half-life) and whose systemic GLP-1R activity is comparable to that of native GLP-1. Importantly, several preclinical studies showed anti-apoptotic, anti-inflammatory, anti-oxidant and neuroprotective effects of liraglutide against T2D, stroke and Alzheimer disease (AD), whereas several clinical trials, demonstrated some surprising benefits of liraglutide on weight loss, microglia inhibition, behavior and cognition, and in AD biomarkers. Herein, we discuss the GLP-1 action through the gut-brain axis, the hormone's regulation of some autonomic functions and liraglutide's neuroprotective potential.

Key words: Type 2 diabetes; Glucagon-like peptide-1; Gut; Brain; Insulin; Liraglutide; Alzheimer disease; Neuroprotection

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Core tip: Glucagon-like peptide-1 (GLP-1) physiological responses are dependent on a gut-brain axis and receptor (GLP-1R) activation. GLP-1Rs are widely expressed throughout the body, including several brain areas. GLP-1 may readily diffuse across the blood-brain-barrier, activating neuroprotective pathways. Given the native GLP-1 short half-life, liraglutide has been developed with a highly increased half-life, allowing its use to treat type 2 diabetes (T2D). Given T2D patients increased risk for obesity and dementia [*e.g.*, Alzheimer disease (AD)], and evidence from preclinical studies, whereby liraglutide showed impressive neuroprotective effects, clinical studies are underway to test the role of liraglutide on weight control and AD.

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INTRODUCTION

The incretin effect was first discovered when experiments were conducted to evaluate the possibility that food ingestion would lead to hormone secretion by the gut into the bloodstream to modulate pancreatic insulin secretion and lower blood glucose levels^[1-3]. Shortly after, glucose-dependent insulinotropic polypeptide (GIP) and gastrointestinal glucagon-like peptide-1 (GLP-1) were described as the incretin hormones secreted by intestinal cells that were responsible for a potent insulinotropic activity upon elevated plasma glucose (70% of the postprandial insulin secretion)^[4-7]. More recently, in an increased number of studies directed to the analysis of incretins effects in patients with type 2 diabetes (T2D), impaired interactions between mediators such as insulin, glucagon and incretin hormones have been increasingly suggested to underlie the development of T2D^[8].

In this review, we will focus primarily on the complex interaction between gut and central nervous system (CNS), particularly in the regulation of appetite and body weight. More specifically, we will briefly overview the role of the increasingly used anti-T2D drug from the incretin/GLP-1 mimetics class liraglutide in brain, with a special emphasis on its anorectic and potential neuroprotective effects on T2D-associated neurodegeneration.

INCRETIN HORMONES IN BRAIN: A PIVOTAL ROLE FOR GLP-1

Despite the known GIP action in inhibiting gastric acid secretion and gastrointestinal motility, as well as in the stimulation of insulin release^[6,7], it was observed that not only GLP-1 is more insulinotropic in hyperglycemic conditions than GIP^[9,10], but also that GIP insulinotropic activity was diminished in T2D patients^[11,12]. Thus, as the GIP secretion was maintained normal or even increased, such apparently reduced β -cell response to GIP might result from a down-regulation of GIP receptor expression/activity^[13,14]. This was further reinforced by the observation that, despite no changes in GLP-1-related insulinotropic activity in diabetic patients, their impaired insulin secretion could be associated with a decreased incretin effect^[15-17]. Importantly, in addition to its insulinotropic effects, GLP-1 has been also involved in the suppression of postprandial glucagon secretion, delaying gastric emptying, promoting early satiety (and the subsequent decrement in food intake), slowing the rate of endogenous glucose production and, ultimately, promoting weight loss, particularly in diabetic conditions^[4,18,19]. Moreover, it has been reported that, by stimulating cell proliferation and protecting against apoptosis, GLP-1 also enhanced pancreatic β -cell mass^[20,21]. In this perspective, it has been suggested that the combination of these effects may contribute to the normalization of blood glucose levels in T2D patients^[19,22], thus rendering the GLP-1

hormone (instead of GIP) a very attractive target for the treatment of T2D.

The gut-to-brain GLP-1-dependent axis: GLP-1 synthesis and secretion

As previously referred, GLP-1 is primarily synthesized and secreted from the intestine (ileum and colon) enteroendocrine L cells and, to a lesser extent, from pancreatic α -cells and from neurons located at the nuclei of brainstem [solitary tract nucleus (NTS), caudal brainstem and area postrema (AP)]^[23-25].

This hormone arises from the post-translational cleavage of proglucagon (catalyzed by the prohormone convertase) and, depending on the tissue, proglucagon may originate different products^[26]. For instance, in pancreas the major products are glucagon, glycentin related polypeptide and a major proglucagon fragment, containing the GLP-1 and GLP-2 sequences, whilst in brain and gut proglucagon processing liberates GLP-1, GLP-2 (which is not an incretin, as it is deprived from insulinotropic and glucose lowering properties), IP-2, glicentin, and oxyntomodulin^[23,26]. Additionally, recent studies showed that multiple forms of GLP-1 are secreted by humans, including GLP-1 (1-37) and GLP-1 (1-36) amides (synthesized as immature forms), as well as the bioactive forms glycine-extended form GLP-1 (7-37)-amide and the GLP-1 (7-36)-amide (this being the predominant form in plasma and brain)^[23,27].

Most GLP-1 is secreted postprandially, particularly after fat- and carbohydrate-rich meals. Interestingly, this secretion is proportional to the size of the meal and may reach 10-30 pM^[4,19]. Individual nutrients, including glucose and other sugars, fatty acids, essential amino acids and dietary fibers also stimulate GLP-1 release^[28]. Amongst these, the glucose and fructose mechanism of stimulation have been the more explored and it has been shown that, in humans, oral (but not intravenous) glucose administration stimulates GLP-1 secretion^[4,26]. Moreover, basal secretion of GLP-1 may even occur as a product of glucagon secretion in fasting state and reach 5-10 pM, being essential for maintaining glucose homeostasis^[4,25]. Interestingly, plasma GLP-1 (7-36)-amide increases rapidly (within just a few minutes) through a biphasic pattern of secretion and release after oral glucose absorption, composed by an early phase within 10-15 min followed by a prolonged second phase at 30-60 min^[28,29].

Although the majority of secreting L-cells are located in the distal small intestine, they can be found also throughout the entire length of the small intestine^[30,31]. Interestingly, these cells may contact with different regions, being stimulated by a variety of mediators. For instance, L-cells can contact directly with nutrients at their luminal surface and with vascular tissue through their basolateral surface, as well as with the enteric and the CNS *via* the vagus nerve^[31,32]. Hence, evidence suggests that early and late phases of GLP-1 secretion may be generated either through

(1) the direct nutrient stimuli to L-cells (particularly those located in more proximal regions of the small intestine, being at least partially responsible to induce the first phase of GLP-1 secretion); or (2) *via* the indirect action of neural and endocrine factors^[19,30,32]. More specifically, it has been hypothesized that the early GLP-1 secretion in rodents and humans may be indirectly regulated by the autonomic nervous system and neurotransmitters and peptides [e.g., gastrin-releasing peptide, acetylcholine, γ -aminobutyric acid (GABA), calcitonin gene-related peptide and GIP], with the vagus nerve playing an essential role herein^[31,33]. Additionally, others proposed that non-nutrient factors, as leptin and insulin, could also contribute to the rapid release of GLP-1^[33,34].

Intracellular signaling pathways underlying brain GLP-1 synthesis, secretion and action

Importantly, brain GLP-1 can be peripherally originated (from the intestine), reaching the CNS through leaks in the blood-brain barrier (BBB) (at the level of area postrema and subfornical organs), whereby it may readily diffuse across the BBB (GLP-1 is a relatively small molecule), and may also influence the activity of afferent vagal neurons^[35]. However, as previously referred, GLP-1 synthesis can also occur locally in brain, in a process dependent on the complex brainstem-hypothalamic-preproglucagon system. More specifically, in preproglucagon neurons from the CNS, proglucagon is processed to GLP-1 in neuronal cell bodies^[36]. First evidence showed that the largest population of GLP-1 immunoreactive innervations occurred in the dorsomedial and paraventricular nuclei of the hypothalamus, and to a lesser extent in the cortex and hindbrain^[37,38]. Then, others reported that preproglucagon neurons are primarily located in the lower brainstem, particularly in the caudal NTS and AP, some cell bodies being also found in the dorsomedial part of the medullary reticular nucleus^[37,39,40]. Besides this, both the NTS and AP appear to receive visceral sensory inputs generated by the vagal nerves that innervate the gastroduodenal tract^[41]. Indeed, it has been described that sensors in the hepatic portal vein may activate the vagus nerve, initiating a neural signal to the NTS/AP in the brainstem, which in turn transmits the information through axons to the hypothalamic nuclei^[42]. Intracellularly, GLP-1 secretion may be mediated by several signaling pathways. In general, these include the activation of protein kinases A (PKA) and C (PKC) and mitogen-activated protein kinase (MAPK), as well as an increase in intracellular calcium (Ca^{2+})^[43]. More specifically, upon a meal, the increase in blood glucose is accompanied by its uptake into the cells (namely *via* sodium/glucose transporters) and subsequent metabolism. As a result, the increment in ATP levels may lead to the closure of ATP-linked potassium channels and, ultimately, GLP-1 secretion^[33,43]. Conversely, inhibition of GLP-1 secretion

in gut has been described to involve a negative feedback, probably *via* GLP-1-mediated stimulation of somatostatin secretion^[34,44]. Interestingly, the neuropeptide galanin has been also identified as an inhibitor of GLP-1 secretion from intestinal L-cells, both *in vitro* and *in vivo*^[26,34].

Physiological responses of GLP-1 are elicited upon binding of the hormone to its receptors belonging to the class B family of 7-transmembrane heterotrimeric expressed G-protein-coupled receptors, a family that also includes receptors for glucagon, GLP-2, and GIP^[45,46]. Increasing evidence points towards an ubiquitous expression of GLP-1 receptor (GLP-1R), in tissues ranging from pancreas (α , β , and δ cells), lung, heart, kidney, stomach, intestine, pituitary, skin and ganglion neurons of the vagus nerve, to multiple regions of CNS (including brainstem, hypothalamus, hippocampus and cortex)^[26,30]. Importantly, GLP-1R expression was detected in mammalian brain neurons, astrocytes, microglia and endothelial cells^[47] and, even more strikingly, GLP-1Rs have been identified in lipid rafts, where they interact with caveolin-1, thereby regulating receptor subcellular localization, trafficking, and signaling^[48].

Interestingly, rat and human GLP-1Rs are polypeptide chains with 463 amino acids and share 90% sequence homology^[19]. Structurally, GLP-1R possesses a long N-terminal extracellular region responsible for peptide recognition and binding, and a cytoplasmic C-terminal containing the components for specific G protein coupling, thus having a major influence in signaling specificity and transmission^[49,50]. Once activated, GLP-1R stimulates the adenylyl cyclase system, increasing intracellular cyclic adenosine monophosphate (cAMP) levels and, subsequently, activating the downstream PKA and exchange protein activated by cAMP-2 (Epac2) pathways^[4,29]. Alternatively, GLP-1R activation may also increase intracellular Ca^{2+} and phospholipase C levels, or stimulate other signal transduction pathways, depending on the activated tissues, including phosphoinositide 3-kinase (PI3K), insulin receptor substrate-2, epidermal growth factor receptor transactivation, PKC, MAPK, cyclic AMP response element binding protein (CREB), pancreatic duodenal homeobox-1, and glucose transporter-2^[26,51,52].

Importantly, some authors suggested that at least part of GLP-1-associated endocrine effects (e.g., GLP-1R-dependent insulin secretion) may be indirectly mediated by neural mechanisms^[42]. This appears to be supported by the increasing notion that GLP-1R activation may generate new signals to guide the energetic flux towards tissues (*via* the autonomic nervous system) and ultimately regulating a diverse array of homeostatic functions (Figure 1)^[23,53,54].

The short-half life of GLP-1: Inactivation by dipeptidyl peptidase-4

Concerning the use of incretin-based anti-T2D therapy, we must bear in mind that a continuous GLP-1 ad-

ministration would be required to effectively maintain glucose homeostasis. In fact, given the native GLP-1 short half-life of less than 2 min [the hormone is rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4)]^[55,56], this would render its therapeutic use unfeasible, as we will discuss later.

DPP-4 is a ubiquitous and multifunctional enzyme that can be found either solubilized in blood or membrane-anchored in many cell types^[57]. This glycoprotein is widely expressed in multiple tissues, including kidney, lung, adrenal gland, pancreas, liver, thymus, lymph node, uterus, placenta, prostate and on the surface of lymphocytes, macrophages and endothelial cells^[58,59]. More relevant herein, DPP-4 appears to be also expressed in several brain areas (e.g., hypothalamus, hippocampus, circumventricular organs, choroid plexus, and leptomeninges)^[60,61]. And besides its well known role in GLP-1 inactivation, DPP-4 has been also implicated in numerous pleiotropic cellular processes involving cell cycle regulation, proliferation, adhesion, immunomodulation and apoptosis^[62-64].

Molecularly, DPP-4 is able to specifically cleave different dipeptides possessing an alanine, proline or hydroxyproline in the penultimate N-terminal position. These substrates include fibronectin, substance P, chemokines, neuropeptide Y (NPY), peptide YY (PYY), and the best validated *in vivo* substrates: GLP-1, GLP-2 and GIP^[62,63]. The resulting GLP-1 (7-36)-amide is metabolized to GLP-1 (9-37) or GLP-1 (9-36)-amide, which has a 1000-fold reduced affinity for GLP-1R and thus completely blunts its insulin-releasing activity^[56,57]. Besides DPP-4, another relevant step in GLP-1 inactivation process can be catalyzed by the neutral endopeptidase (NEP), a membrane-bound zinc metallopeptidase expressed in both the periphery and CNS, that is responsible for GLP-1 (7-36)-amide hydrolysis into smaller peptides^[65,66]. Therefore, as most of GLP-1 passing the portal circulation has been already degraded by DPP-4, GLP-1 (9-37) and GLP-1 (9-36)-amide constitute the major circulating forms of the hormone (with an estimated half-life of 8-10 min, as a result of renal clearance)^[25]. Apparently, this suggests that, after GLP-1 secretion and release by intestinal L-cells, DPP4 starts to continuously degrade the incretin hormone, thus accounting for 50% of GLP-1 inactivation^[30,67]. Then, after its passage through the liver, another large amount of the remaining intact bioactive form of the peptide is further inactivated, thus culminating in less than 10% of active GLP-1 reaching the blood circulation^[30].

INCRETIN-BASED THERAPIES: THE FUTURE OF ANTI-T2D AND NEURODEGENERATIVE DISEASES?

The management of T2D through a patient's lifetime is often difficult and frequently renders the achievement of therapeutic goals unsuccessful. However, this

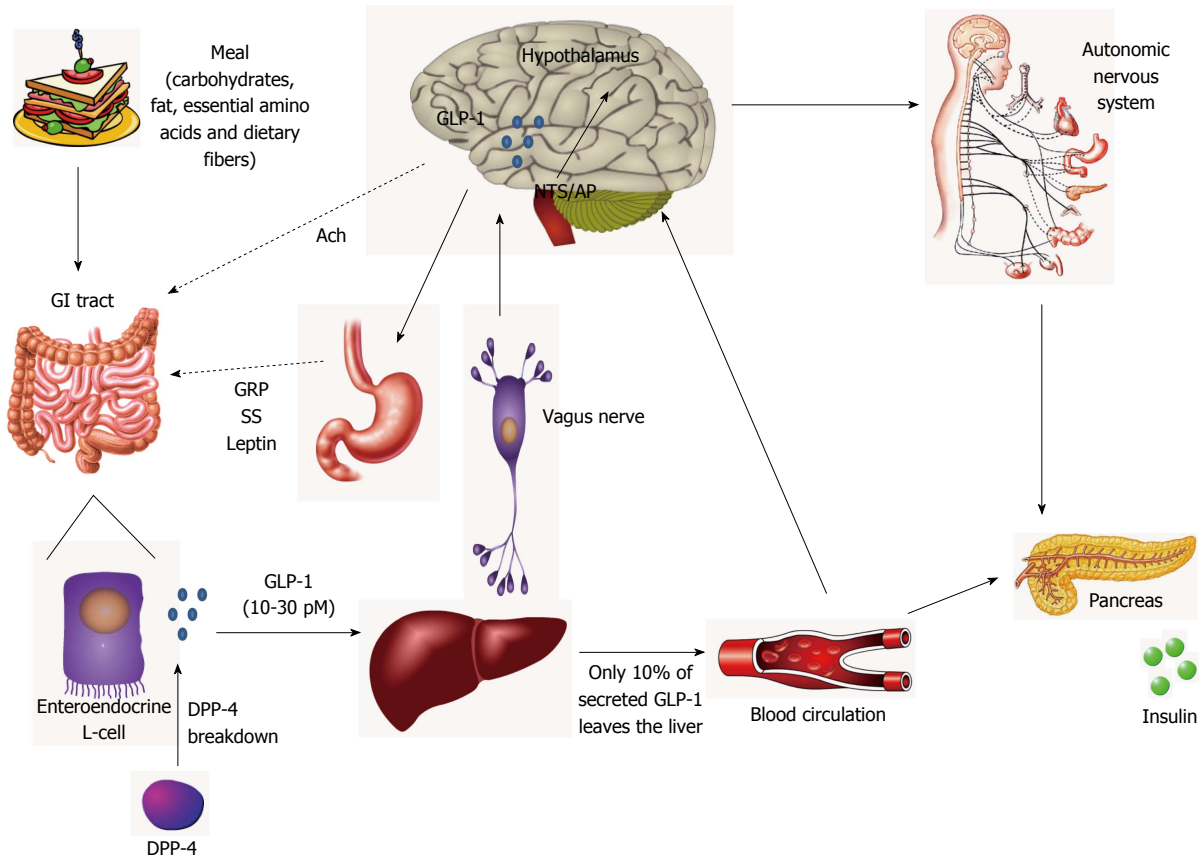


Figure 1 The gut-brain axis for the actions of glucagon-like peptide-1. After a meal ingestion, gastrointestinal (GI) tract is rapidly stimulated and glucagon-like peptide-1 (GLP-1) is secreted in the gut lumen by enteroendocrine L-cells. Besides the direct interaction of nutrients with L-cells, neural (acetylcholine) and endocrine (gastrin-releasing peptide, somatostatin and leptin) mechanisms are also involved in the control of GLP-1 secretion after food intake. Bioactive GLP-1 diffuses into the capillaries, immediately beginning to be degraded by dipeptidyl peptidase-4, so that more than 50% of the hormone is inactivated before reaching the portal circulation. In the liver, a further large amount is truncated, thus only 10% of the secreted GLP-1 leaves the liver and enters the systemic circulation and may reach the pancreas, the brain and other tissues via the endocrine pathway. However, the passage of GLP-1 through the hepatoportal vein activates vagal afferents nerves that initiate a neural signal towards the brain. In the central nervous system, the metabolic information is received by the solitary tract nucleus and the AP in the brainstem, which synthesize and project the GLP-1 to the hypothalamus. The GLP-1 receptor signaling is involved in the central control of energy homeostasis and food intake, and several autonomous functions, such as glucose-dependent stimulation of insulin secretion and inhibition of glucagon secretion in the pancreas, cardiovascular effects, regulation of gastric emptying and of endogenous glucose production in liver and glucose uptake and storage in muscle and adipose tissue. GRP: Gastrin-releasing peptide; ACh: Acetylcholine; SS: Somatostatin; DPP-4: Dipeptidyl peptidase-4; AP: Area postrema.

scenario has been increasingly challenged in the recent years, due not only to the promising results obtained with GLP-1-related therapy in T2D, but also to its widespread beneficial effects on body weight and metabolic parameters where other promising anti-T2D approaches failed^[68]. Altogether, this rendered the GLP-1R-mediated intracellular signaling one of the most appealing targets in the development of therapies for diabetes management. Importantly, and despite GLP-1's pharmaceutical promise, the first crucial step was the need to overcome its rapid degradation by DPP-4, thereby enhancing the hormone's action time^[69-71]. In this regard, two novel classes of glucose lowering agents, the DPP-4-inhibitors and the GLP-1R agonists (GLP-1RAs) have been intensively developed over the last years^[72].

DPP-4-inhibitors and GLP-1R agonists: The future therapeutic dream team?

DPP-4-inhibitors are orally-given small molecules that

compete with DPP-4 substrates for the active site of the enzyme, thus avoiding the inactivation of native bioactive GLP-1 and, therefore, extending its half-time and increasing its levels in circulation^[73,74]. Currently, there are three inhibitors approved for treatment of T2D in the United States and Europe: sitagliptin, saxagliptin and linagliptin^[74]. Besides these, vildagliptin is another DPP-4 inhibitor also available only in Europe^[73].

On the other hand, given the peptidic nature of GLP-1RAs, it is necessary to administer them by subcutaneous injection^[75]. These molecules have been developed based on the effects of native GLP-1 (binding to GLP-1R and activating similar glucoregulatory effects), but with a high resistance to DPP-4 degradation, thereby increasing the systemic GLP-1 activity^[73]. Currently, three GLP-1RAs are commercially available: exenatide twice daily (EBID), liraglutide once daily, and exenatide once weekly (EQW)^[74]. Importantly, most of these incretin-based therapies

have been approved as a second line therapy, in dual or triple combination with other anti-diabetic therapies, including metformin, sulphonylureas (SU) and thiazolidinediones (TZD)^[75].

Since the endogenous levels of incretin hormones appear to be reduced in T2D patients, increasing evidence points towards a higher effectiveness of GLP-1RAs-based therapies in glycemic control than the use of DPP-4-inhibitors, particularly in reducing glycated hemoglobin A_{1c} (HbA_{1c}) and postprandial glucose levels^[8,11]. Furthermore, contrary to GLP-1RAs, DPP-4-inhibitors appear to be weight neutral, with no effect in gastric emptying, and with less described positive cardiovascular effects^[76,77]. And although some adverse events have been reported in both therapeutic subclasses [namely an increased risk for infections with DPP-4 inhibitors and common gastrointestinal side effects (predominantly nausea) with GLP-1RAs], the overall tolerability was comparable and general positive results were described for both classes of incretin-related therapy, with no episodes of major hypoglycemia documented in patients on either therapy^[8,78]. Thus, it is not surprising that, in the last years, the incretin-based therapies (and particularly GLP-1RAs) have been increasingly faced as the potential “dream team” not only for the treatment of T2D and its associated complications, but also for other disorders involving changes in glucose homeostasis and metabolism [e.g., neurodegenerative diseases, as Alzheimer disease (AD)].

GLP-1R agonists: Exenatide and liraglutide

As previously referred, the main advantage of GLP-1RAs over DPP-4 in terms of T2D treatment appears to be the fact that these patients often present lower circulating incretins' levels^[8,11]. Although comparative clinical efficacy data are still limited to support the use of one GLP-1RA molecule over another, some comparative studies have already shown some differences between the two main GLP-1RAs, exenatide and liraglutide. First, liraglutide has been considered a true GLP-1 agonist, sharing 97% sequence identity with human GLP-1, while exenatide is a mimetic isolated from the saliva of the Gila monster (*Heloderma suspectum*) that shares only 53% structural similarity with native GLP-1^[79-83]. Additionally, the long-acting GLP-1 analog liraglutide, Arg³⁴,Lys²⁶-{N-ε-[γ-Glu(N-α-hexadecanoyl)]}-GLP-1 (7-37), has a substitution of a lysine residue with arginine at position 34 and a 16-carbon fatty acid chain *via* a glutamic acid spacer attached to lysine at position 26, thereby promoting the noncovalent binding of liraglutide to serum albumin that not only confers its DPP-4 enzyme resistance and protection from renal clearance, but also allows liraglutide molecules to form heptamers, slowing its absorption rate from injection site and increasing its half-life in plasma to 13 h (in contrast with the 2 h half-life of exenatide)^[84-86]. And although the efficacy of peptide injection into the organism may be partially

compromised by the formation of antibodies against GLP-1RAs, given its protein sequence differences liraglutide appears to be less immunogenic than exenatide^[75,87,88]. Indeed, the Liraglutide Effect and Action in Diabetes-6 (LEAD-6) study reported that 61% of T2D patients treated with exenatide developed antibodies compared to only 2.6% of patients given liraglutide^[89,90]. Importantly, this trial also showed that, during the 26-wk study, liraglutide was significantly more efficient than exenatide BID in reducing HbA_{1c} levels (1.12% vs 0.79% respectively) and in improving HOMA-β (homeostasis model assessment of β-cell function) index in T2D patients, thereby suggesting that liraglutide may also induce a better improvement in β-cell function^[89,90]. But, to us, the most striking point from the LEAD-6 clinical trial was that, in a 14-wk extension, patients who started and responded well to exenatide BID treatment could even further ameliorate some parameters when switching to liraglutide. For instance, these patients further reduced HbA_{1c} levels by 0.32%, body weight by 0.9 kg and systolic blood pressure by 3.8 mmHg^[89,90]. In another comparative study between liraglutide and exenatide QW - the DURATION-6 trial -, the first was shown to decrease HbA_{1c} levels by 1.48% in T2D patients compared to the 1.28% lowering achieved with exenatide QW^[91]. Additionally, in this study more patients submitted to liraglutide therapy were able to achieve HbA_{1c} < 7% than with exenatide QW^[91]. And, as in LEAD-6, weight loss was greater among patients receiving liraglutide (-3.58 kg vs -2.68 kg). Interestingly, 94% of the liraglutide-treated T2D patients from the DURATION-6 trial were satisfied with their treatment compared with 86% of those receiving exenatide^[91]. To further complete this comparative overview on both drugs, we must refer that, since the primary route of exenatide clearance from the body is through renal excretion, this may pose some risk of accumulation in patients with renal disease, and, thus, exenatide is not recommended for patients with hepatic impairment^[79,92,93]. Conversely, liraglutide (as GLP-1) is almost exclusively enzymatically degraded by DPP-4 and NEP, and therefore, renal impairment should not affect liraglutide efficacy^[94].

The actions of liraglutide in peripheral diseases:

T2D is mainly characterized by hyperglycemia and an impaired insulin action. However, T2D most dangerous and devastating consequences may arise from the development of long-term complications (e.g., retinopathy, nephropathy, cardiovascular disease, stroke, neuropathy, cerebrovascular disease), which in most cases are already installed by the time of diagnosis^[95].

As previously discussed, the first characteristic that turned GLP-1 into an ideal candidate for T2D treatment was its property to enhance glucose- induced insulin release and overcome insulin desensitization^[6,7]. However, over time GLP-1 has been shown to exert

Table 1 Studies assessing the effects of liraglutide in different organs and/or conditions

Liraglutide effects	Study	Ref.
Pancreas	Review	Davies <i>et al.</i> ^[99]
	Preclinical	Shao <i>et al.</i> ^[97]
		Yosida <i>et al.</i> ^[98]
Heart	Review	Davies <i>et al.</i> ^[99]
		Martín-Timón <i>et al.</i> ^[100]
		VilSBøll <i>et al.</i> ^[101]
		Seufert and Gallwitz ^[104]
	Preclinical	Liu <i>et al.</i> ^[103]
		Noyan-Ashraf <i>et al.</i> ^[106]
Kidney	Clinical	Shiraki <i>et al.</i> ^[107]
		Russell-Jones <i>et al.</i> ^[102]
		Marso <i>et al.</i> ^[105]
	Preclinical	Fujita <i>et al.</i> ^[114]
		Armstrong <i>et al.</i> ^[110]
Liver	Clinical	Davidson <i>et al.</i> ^[111]
		Eguchi <i>et al.</i> ^[116]
Muscle	Preclinical	Armstrong <i>et al.</i> ^[117]
		Ji <i>et al.</i> ^[109]
Weight and appetite	Preclinical	Li <i>et al.</i> ^[118]
		Davies <i>et al.</i> ^[99]
		Seufert and Gallwitz ^[104]
		Rigato and Fadini ^[119]
	Clinical	Sjoholm ^[121]
		van Bloemendaal <i>et al.</i> ^[123]
		Ng and Wilding ^[135]
		Toft-Nielsen <i>et al.</i> ^[17]
		Horowitz <i>et al.</i> ^[133]
		Wadden <i>et al.</i> ^[136]
		Wadden <i>et al.</i> ^[137]
		Senda <i>et al.</i> ^[138]
Neuroprotection/ T2D brain	Review	[139]
		Astrup <i>et al.</i> ^[140]
		Rosenstock <i>et al.</i> ^[141]
	Preclinical	Hölscher ^[205]
		Duarte <i>et al.</i> ^[206]
		Hölscher ^[210]
AD	Preclinical	Hou <i>et al.</i> ^[47]
		Hunter <i>et al.</i> ^[204]
		Hamilton <i>et al.</i> ^[207]
		Agrawal <i>et al.</i> ^[208]
		Cummings <i>et al.</i> ^[209]
		Parthasarathy <i>et al.</i> ^[212]
		McClean <i>et al.</i> ^[213]
		Han <i>et al.</i> ^[214]
	Clinical	McClean <i>et al.</i> ^[215]
		Long-Smith <i>et al.</i> ^[216]
Stroke	Preclinical	McClean <i>et al.</i> ^[217]
		Parthasarathy <i>et al.</i> ^[218]
		Yang <i>et al.</i> ^[219]

T2D: Type 2 diabetes; AD: Alzheimer disease.

many other interesting (and potentially therapeutically relevant) effects in the organism^[96] and, in this perspective, the GLP-1RA liraglutide may have also a significant impact (rather than the “mere” GLP-1R activation), not only in periphery, but also in CNS and in other pathologies besides T2D (Table 1), as we will further discuss.

Regarding the role of liraglutide in the periphery

and the progressive loss and dysfunction of pancreatic β -cells that constitutes one of the key features of T2D, several recent studies have shown that the drug was able not only to protect (through anti-apoptotic effects), preserve and enhance β -cell mass, but also to improve β -cell function [seen in the *db/db* and in the transient receptor potential melastatin 2 (TRPM2)-deficient mice models], thereby improving glucose control, and insulin secretion and sensitivity^[97-99].

It is well known that chronic hyperglycemia and poor blood glucose control may also underlie cardiovascular dysfunction and development of cardiovascular disease, thus rendering T2D a risk factor for cardiovascular disease^[100]. Therefore, it is not surprising that emerging data from animal and human studies also point towards a liraglutide-mediated reduction in systolic blood pressure and improvement in lipid profiles (decreased low-density lipoprotein cholesterol and triglycerides levels, and increased high-density lipoprotein cholesterol)^[99,101,102]. Moreover, Liu *et al.*^[103] demonstrated that liraglutide improves cardiac function in diabetic rats, probably due to a decreased expression of proteins involved in the endoplasmic reticulum stress pathway. Accordingly, recent studies also associated liraglutide treatment with a significant decrease in several cardiovascular risk biomarkers, such as plasminogen activator inhibitor-1, B-type natriuretic peptide^[104-106], interleukin-6 (IL-6) and tumor necrosis factor- α ^[107,108]. Besides cardiovascular function, liraglutide was also shown to reduce endothelial cell dysfunction in a process probably mediated by its anti-oxidant and anti-inflammatory effects^[107]. Moreover, other tissues can be directly or indirectly affected by GLP-1RA therapies, including muscle, liver and kidney^[109-111], thus rendering them highly promising against other pathologies than T2D. However, some caution must be used and further clarification is needed as, *e.g.*, was recently reported that, albeit rare the aggravation of an existing nephropathy in T2D patients submitted to GLP-1RA treatment may be due to external factors (*e.g.*, other medications), but if related with this therapy it may probably arise from gastrointestinal problems^[112,113]. Nevertheless, recent data also showed that liraglutide was able to counteract renal impairment in a mouse model that also displayed chronic hyperglycemia and increased renal oxidative stress^[114]. According to these authors, such protection was due to liraglutide-mediated inhibition of NAD(P)H oxidase and activation of cAMP-PKA pathway, thus suppressing the progression of renal failure^[114].

Concerning the effects of GLP-1 analogues in hepatic impairment, there is currently an increasing interest mostly due to the lack of effective therapies against hepatic diseases. Amongst them, the non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver diseases, being strongly related with insulin resistance, metabolic syndrome, cardiovascular disease, cerebral vessel

disease and T2D^[115]. In a recent pilot study involving patients diagnosed with diabetes complicated by NAFLD/non-alcoholic steatohepatitis (NASH), liraglutide (0.9 mg/body per day) was given for 24 wk^[116]. Data suggested that liraglutide significantly improved liver function (as given by the decreased serum levels of alanine aminotransferase, aspartate aminotransferase, ferritin and C-reactive protein) and histological changes in NASH patients^[116]. Besides this, data from LEAN-2 study demonstrated that liraglutide combined with metformin also exerted positive effects in liver enzymes and hepatic steatosis in T2D patients^[117].

Regarding the impact of liraglutide on muscle, Li *et al.*^[118] showed recently that this drug increases cAMP and AMP-activated protein kinase (AMPK) signaling pathways, which may in turn induce GLUT4 translocation in mouse skeletal muscle cells.

The role of liraglutide in appetite and weight loss: A centrally-regulated peripheral anorectic effect:

Unhealthy lifestyle, weight gain and obesity are intimately related with the increased prevalence of T2D worldwide, being estimated that 60%-90% of T2D patients are overweight^[119-121]. Importantly, weight gain has been also described as a common side-effect of standard anti-T2D therapies. Therefore, weight management should be an important issue herein, as it not only may interfere with treatment, but may also increase the risk for the development of long-term complications (*e.g.*, cardiovascular disease)^[120,122]. However, the control of body weight and energy balance is a complex process, involving different tissues and pathways, such as gut, adipose tissue, pancreas and brain^[30,123]. For instance, gut-derived hormones (*e.g.*, GLP-1, PYY, ghrelin, cholecystokinin and oxyntomodulin) are known to play a major role in feeding regulation, at least partially by relaying to the CNS information on nutritional status^[123]. On the other hand, GLP-1's well known anorectic properties have been suggested to arise from the combination of both central and peripheral effects, *via* another gut-brain connection^[23,124]. More specifically, GLP-1-mediated satiating effect requires primarily the activation of peripheral GLP-1Rs located on vagal sensory afferents in the gut and in the hepatportal region of the liver; after the transmission of the metabolic signals into the brain c-fos expression is increased in NTS neurons^[124,125]. Once activated, these neurons act on brain neuronal circuits from the brainstem and several other areas involved in appetite control, where GLP-1R are also expressed. These include the ventral tegmental area (VTA), nucleus accumbens (NAc) and hypothalamus^[37,39,123]. In the hypothalamus, GLP-1R are highly expressed in the paraventricular nucleus, dorsomedial hypothalamus and the arcuate nucleus (ARC) [where they overlap with the pro-opiomelanocortin (POMC) neurons (anorexigenic neurons)], being present at a lesser extent in agouti-related peptide (AgRP)/NPY neurons (orexigenic

neurons)^[23,126,127]. At this respect, Seo *et al.*^[128] demonstrated that intracerebroventricular (icv) infusion of GLP-1 stimulates the synthesis of anorexigenic peptides (POMC and cocaine- and amphetamine-regulated transcript), and simultaneously decreased the synthesis of NPY and AgRP in rodents. Accordingly, while effects on hypothalamic and brainstem circuits may regulate food intake, the inhibitory effect of GLP-1 on the rewarding value of food appears to be regulated by the regions of VTA and NAc (mesolimbic reward system)^[123,124]. This hypothesis was supported by the finding that the administration of the GLP-1R antagonist, exendin 9-39, into the NAc promoted hyperphagia in rats^[129]. After hypothalamic and brainstem (dorsal motor nuclei) stimulation, efferent impulses depart from these brain areas to regulate the gastrointestinal tract, pancreas and other peripheral organs, by slowing gastric emptying and acid secretion, and decreasing gut motility, thereby further controlling the feeding behavior and glucose metabolism^[39,124]. Although such slowed gastric emptying may also arise from a direct activation of gastric inhibitory GLP-1Rs, this appears to be more visible with long-acting GLP-1RAs, particularly liraglutide (whose potent gastric emptying capacity has been increasingly described)^[104]. Importantly, the rate of gastric emptying may also influence stomach distension, thereby stimulating gastric mechanoreceptors, with the subsequent activation of the nodose ganglion (inferior ganglion of vagus nerve) that may *per se* culminate in the activation of NTS neurons to induce satiety^[123]. Besides this, a direct central action of GLP-1 in satiation signaling was also demonstrated by an inhibition of food intake by pathways independent of the vagal afferents^[130-132].

As T2D-associated postprandial hyperglycemia may be further aggravated by an accelerated gastric emptying, it is not surprising that GLP-1RAs drugs have been successful in attenuating postprandial blood glucose levels also by slowing gastric emptying^[133]. And although this is not considered the main mechanism by which these drugs regulate appetite and weight loss, it has been widely described that the incidence of gastrointestinal adverse events (such as nausea and vomiting, which albeit transient may affect nearly 50% of treated patients) is often accompanied by a decrease in food intake and body weight. Despite the limited knowledge on the mechanisms involved, it is plausible that a direct effect on gastrointestinal system and a central action producing conditioned taste aversion may play a role herein^[104,123,134].

In recent clinical trials, T2D patients treated with liraglutide (either as a monotherapy or in combination therapies) have shown a weight loss, in contrast with the weight gain associated with SU, TZD and insulin^[119,121]. More strikingly, both people with or without T2D, treated with clinically relevant doses of liraglutide for 20 to 30-wk presented significant reductions in body weight (from 1 to 3 kg)^[99,135].

Importantly herein, despite the increased GLP-1 levels in response to the amount of nutrient intake, obese people were shown to have lower basal GLP-1 levels^[136]. Accordingly, a negative relation was also established between body mass index (BMI) and GLP-1 oral stimulation^[17]. Moreover, the SCALE Maintenance study reported a loss of 5.9 kg in obese patients treated with 3.0 mg liraglutide for 56-wk vs placebo-treated ones^[137]. Interestingly, even a study case involving the Prader-Willi syndrome (PWS) (a rare genetic disorder characterized by an extreme and insatiable appetite that ultimately leads to morbid obesity), described that a 25-year-old female hyperglycemic PWS patient submitted to liraglutide therapy, not only improved her glycemic control, but was also able to control hyperphagia and to decrease plasma ghrelin levels and her BMI^[138].

From the above, such consistent weight loss observed with liraglutide and other GLP-1RAs, not only in T2D patients, but also in non-T2D people has aroused the interest for the use of GLP-1RAs as promising pharmacotherapies for weight management. To further potentiate this interest, we must bear in mind that, to date, only one medical therapy against obesity is approved in Europe, with most current anti-obesity pharmacological drugs having serious undesirable side effects^[135]. In this regard, the use of these drugs in obese people is currently being tested in several clinical trials^[139-141].

The neuroprotection in type 2 diabetic and degenerative brain: Insulin and liraglutide: (1) Pathological commonalities between T2D and neurodegenerative diseases: neuroinflammation and brain dysfunctional insulin/ insulin-like growth factor-1 (IGF-1).

Obesity, hypertension, dyslipidemia and T2D all constitute risk factors, particularly vascular, for cognitive dysfunction^[142]. Numerous experimental data indicated a decrease in cognitive function in T2D patients, which is often accompanied by impairments in memory, attention, intelligence, processing speed and executive functions, as well as by brain atrophy (particularly in cortical, subcortical and hippocampal areas) and white matter abnormalities^[143,144]. Interestingly, the well described cell loss that occurs in T2D pancreas appears to be accompanied by cell loss also in the CNS upon disease progression^[145,146]. Indeed, the pathological progression of T2D and, more specifically, its associated glucose toxicity, insufficient insulin action, neuroinflammation and general aging, amongst others, can slowly lead to nervous damage and may ultimately result in diabetic neuropathy and diabetic encephalopathy, thus increasing the risk for dementia in diabetes^[147-149]. In line with this, chronic inflammatory response in brain has been suggested to play a pivotal role in propagating T2D-mediated injury. This may probably occur *via* activation of microglia and other immune cells, with the subsequent

release of neurotoxic amounts of proinflammatory cytokines and free radicals and ultimately leading to neurodegeneration and brain disease upon T2D progression^[150-152]. Additionally, similarly to desensitization of IR from peripheral tissues of T2D patients, increasing evidence also point towards the impairment of brain insulin signaling in AD^[153,154]. As a consequence, it has been increasingly suggested that brain insulin resistance could be a potential link between T2D and AD^[95,151]. This hypothesis has been supported by numerous evidences, *e.g.*, (1) a decrease in IR expression in brains from AD patients; (2) an inverse correlation found between AD Braak stage and the levels of such receptors; (3) the accumulation of hyperphosphorylated tau (a neuropathological hallmark of AD) upon insulin signaling impairment and the consequent inhibition of GSK-3 β phosphorylation and of tau protein phosphatase 2A^[155,156]; (4) the increased A β accumulation and decreased insulin degrading enzyme (IDE) levels in AD patients displaying also brain insulin resistance^[157]; and (5) the memory enhancement and protection against A β toxicity in AD patients submitted to insulin therapy^[158]. These and other *in vivo* and *in vitro* findings have been also corroborated and extended by epidemiological studies showing an increased risk for T2D patients to develop AD and *vice versa*. According to a very recent study, T2D patients have a 65% increased risk to develop AD later in their life^[155], whereas others found that 85% of AD patients had either T2D or increased fasting glucose levels^[159].

Parkinson disease (PD) is the second most common neurodegenerative disorder after AD, the most common form of parkinsonism (motor syndrome) and, pathophysiologically, is the predominant form of synucleinopathies, which are characterized by an abnormal accumulation of α -synuclein (α -syn) protein^[160]. PD is characterized by the neuronal accumulation of Lewy bodies (containing deposits of α -syn) and the progressive loss of dopaminergic neurons (particularly in substantia nigra) that may culminate in multiple motor (tremor, rigidity, slowness of movement, and postural instability) and non-motor (autonomic dysfunction and neuropsychiatric problems) symptoms occurring throughout the course of the disease^[161]. Nowadays, an increasing amount of evidence points towards several similar biochemical changes between PD and T2D, leading to the idea that dysfunctional insulin signaling might be at the center of those alterations^[162]. Amongst such commonalities are, *e.g.*, the increased serum IGF-1 levels in PD patients^[163,164], both the insulin and IGF-1 resistance found in basal ganglia and substantia nigra from PD^[165,166], the impaired dopaminergic signaling observed in a rat model of T2D^[167], and the observation that activation of PI3K/Akt pathway was able to rescue α -syn toxicity *in vitro*^[168].

Stroke (or cerebrovascular accidents) is considered the third most common cause of death in

developed countries and may develop as a long-term complication in comorbid diseases, such as T2D^[169,170]. Indeed, epidemiological studies point towards a 2- to 6-fold increased risk of T2D patients to development of stroke, whereas stroke victims often present impaired glucose tolerance^[171,172]. It is well known that this disease arises from a disturbance in the blood supply to the brain (due to ischemia or hemorrhage), thus resulting in neurological deficits and a loss of brain function^[173]. Interestingly, stroke has been regularly associated with increased neuroinflammation markers^[174], thereby rendering GLP-1 analogues an appealing therapeutic strategy, as we will discuss later.

Strikingly, such correlation between T2D, AD, PD and stroke appears to be also applicable to a wide range of other neurodegenerative diseases, including vascular dementia or Huntington disease (HD)^[95,170], thereby suggesting that T2D is a risk factor for at least some of these brain pathologies.

(2) Brain insulin/IGF-1 as metabolic and body weight regulators.

As it is well known, the primary regulators of glucose homeostasis and metabolism involve insulin/IGF-1 signaling pathways responsible for the control of both peripheral signals and CNS effects^[155]. Although most of the brain insulin is primarily secreted by the pancreas (whereas IGF-1 comes from the liver), being then transported by cerebrospinal fluid (CSF) and crossing the BBB into the brain, numerous evidence also points towards their local synthesis (particularly in brain cortex, olfactory bulb, hippocampus, hypothalamus, and amygdala)^[175]. Once bound to their receptors, the insulin and IGF-1 receptors (IR and IGF-1R, respectively) that are ubiquitously expressed in brain (particularly neurons), many physiological functions are activated, such as neuronal outgrowth and survival, enhancement of attention, memory formation and cognition, food intake and weight maintenance, synaptic protection and sexual regulation^[170,176,177]. Of these, food intake and energy homeostasis are the most directly related also with GLP-1R-mediated signaling, being mediated by a specialized group of hypothalamic glucosensing neurons that appear to be able to detect and respond to even small variations in glycemia^[130]. Such anorexigenic effect of insulin signaling depends on a strict control of the hypothalamic PI3K/protein kinase B (Akt) signaling pathway, on the inhibition of NPY and AgRP expression and on the induction of POMC neurons^[178,179].

From the above, it is not surprising that changes in body weight, hyperinsulinemia and insulin resistance may arise from alterations in the homeostatic balance, thus culminating in injurious effects on the organism^[180,181]. Indeed, hyperinsulinemia has been widely associated to a downregulation of insulin transport across the BBB, thus decreasing its uptake and levels in the brain, as well as the subsequent IR activity, ultimately leading to a brain insulin resistance^[182,183]. Moreover, insulin resistance and

subsequent impairment in glucose supply, transport and utilization may lead to glucose dysmetabolism that may be also accompanied by a decrease in cerebral flow and damaging effects to intracellular organelles, including mitochondria^[184-186]. As brain mitochondria are particularly susceptible to metabolic impairment, situations like brain insulin resistance may lead to dysfunctional respiratory chain and phosphorylation system, and alterations in mitochondrial dynamics and biogenesis that may significantly compromise ATP production and mitochondrial membrane potential. As a result, mitochondrial permeability transition pore may open and ultimately activate apoptotic cell death^[184]. Thus, defects in brain insulin/IGF-1 signaling may raise a deleterious vicious cycle involving metabolic impairment and mitochondrial dysfunction, that in turn generate a wide range of harmful effects (e.g., increased oxidative stress, advanced glycation end-products formation, inflammatory response, excitatory neurotransmitter release)^[175,187]. Altogether, these alterations can be responsible for a decrease in neuronal function and survival, and impaired synaptic activity and plasticity, that may be also accompanied by a decrease in neurogenesis, leading to impaired memory formation and storage, and learning potential, culminating in cognitive decline^[188]. To further intricate this subject, as we detailed above, both T2D and neurodegenerative diseases share several common features that together with the neuroprotective effects demonstrated by many of the already marketed anti-T2D therapeutic agents have potentiated the recent investigations worldwide on the potential beneficial role of such drugs also in the context of neurodegeneration^[96,189].

(3) Restoration of insulin/IGF-1 action as a potential therapeutic target in neurodegenerative diseases: what can we count on in a near future?

Preclinical studies involving insulin and IGF-1 to treat animal models of neurodegenerative diseases revealed promising results. In this regard, our group showed that insulin was able to protect against oxidative stress and a decline in mitochondrial oxidative phosphorylation efficiency induced by the amyloid β -peptide (A β , one of the main players in AD pathology) in streptozotocin (STZ)-induced type 1 diabetic rats^[190]. Similarly, Quesada *et al.*^[191] reported that IGF-1 treatment significantly increases tyrosine hydroxylase (TH) positive neurons and improves motor performance of the medial forebrain bundle 6-hydroxydopamine (6-OHDA) lesion rat model of PD, in a PI-3K/Akt-dependent manner. Additionally, we and others showed that by increasing blood insulin and brain IGF-1 levels, the *in vivo* peripheral administration of IGF-1 was not only able to protect against peripheral glucose intolerance^[192], but also to rescue motor deficits and brain glucose dysmetabolism upon HD^[193]. Molecularly, these observations were further supported and extended by a recent study using an *in vitro* model of human HD, in which insulin- and IGF-1-mediated

PI3K/Akt activation was able to improve mitochondrial function and decrease mitochondrial reactive oxygen species (ROS) formation^[194].

Even though insulin therapy can be very useful to prevent or slow the progression of long-term complications of diabetes and others pathologies, as diseases progress not only the hormone's beneficial effects may be lost (including glycemic control), but also it may increase the risk for severe hypoglycemic episodes, which have been also associated with increased brain damage. As a result this may further increase neuronal and cognitive dysfunction^[195]. Alternatively, the use of insulin sensitizers (another commonly prescribed class of anti-T2D drugs, whose most widely used member is metformin, a first line pharmacological approach against the pathology) could provide a reliable therapeutic alternative also in the context of neurodegenerative diseases^[154,196]. Indeed, metformin was demonstrated to protect the brains of the non-obese T2D Goto-Kakizaki rat models against oxidative imbalance, by decreasing oxidative stress markers and increasing antioxidant defenses^[197]. Additionally, Gupta *et al.*^[198] reported that metformin ameliorates neuronal insulin resistance and AD-associated characteristics in an *in vitro* model of the so-called "type 3 diabetes". Nevertheless, we must bear in mind that metformin presents several controversial side effects, and some authors suggested that metformin therapy and subsequent AMPK activation may even exacerbate previously existing impairments^[199-201].

From all the above, and given also the previously mentioned actions in the brain, the native GLP-1 hormone would be considered the "almost perfect" alternative to restore insulin action in CNS, either in T2D, neurodegenerative diseases or simply in normal aging. And this has been widely corroborated by its rapid capacity to cross the BBB when injected peripherally, acting as a growth factor in brain, whereby the hormone has been shown to stimulate the metabolism, the expression of genes associated with cell growth, repair and replacement and to protect against oxidative injury, neuroinflammatory response and apoptosis, thus exerting beneficial effects in neuronal health and cognition^[155]. However, given the previously described limited half-life of the native GLP-1, recent research interests have been mostly focused on the neuroprotective potential of DPP-4 inhibitors and GLP-1RAs.

Regarding DPP-4 inhibitors, their beneficial roles in CNS have been intensively analyzed and appear to constitute promising candidates for the treatment of CNS disorders. In this regard, sitagliptin (the first DPP-4 inhibitor approved for T2D treatment), has been recently shown to positively affect working and reference memories, and to increase the hypothalamic acetylcholine content and adiponectin receptor 1 expression in T2D Sprague-Dawley rats^[202]. Additionally, vildagliptin (another DPP-4 inhibitor)-

mediated decrease in A β , tau protein phosphorylation and neuroinflammatory markers levels were also accompanied by an amelioration in memory retention capacity in a STZ-induced AD rat model^[203].

On the other hand, and similarly to the native GLP-1, liraglutide and the other synthetic GLP-1RAs are able to readily cross the BBB, reaching the brain almost intact and thereby exerting neuroprotective effects^[204]. Therefore, it is not surprising that most of the preclinical studies that reported such protective effects were performed in T2D, AD, PD and also in stroke models^[205]. For instance, liraglutide has been increasingly suggested to prevent or attenuate T2D-associated neuronal and cognitive deficits^[206]. Although the underlying mechanisms remain unclear, Hamilton *et al.*^[207] proposed that such protection could rely on the activation of stem cells and neuronal progenitor cells to counteract T2D-induced neurodegeneration. Indeed, these authors reported that liraglutide was able to promote neurogenesis [as given by the increase in the number of 5'-bromo-2'-deoxyuridine (BrdU)-positive cells dividing progenitor cells and doublecortin-positive young neurons] in the dentate gyrus of three different types of obese T2D mouse models. Additionally, other authors observed that liraglutide administration in 2 mo-old pre-diabetic UCD-T2D rats (a polygenic obese model of T2D) reduced energy intake and body weight, improved insulin sensitivity and reduced triglycerides, thus delaying diabetes onset by about 4 mo compared to controls^[208,209]. Moreover, these authors reported that liraglutide normalized brain metabolic homeostasis (as given by TFAM, SIRT1, and AMPK phosphorylation), decreased hippocampal lipid oxidation (upon determination of 4-hydroxynonenal levels), improved brain mitochondrial regulation (*via* PGC-1 α) and preserved synaptic plasticity (as given by the BDNF-TrkB signaling) in the UCD-T2D rats over the disease progression^[208,209]. These results appear to be in line with previous evidences that GLP-1R activation may be involved in memory and learning^[210]. In fact, During *et al.*^[211] demonstrated that GLP-1R activation was correlated with an enhancement of associative and spatial learning, as GLP-1R-deficient mice exhibited a learning deficit phenotype. Strikingly, liraglutide (25 nmol/kg, once daily *i.p.*, for 30 d) has been also demonstrating potent anti-inflammatory effects in brains from a mouse model irradiated with 6Gy (X-ray, a model of chronic inflammation), as given by a reduction in microglia activation in cortex and dentate gyrus, and a decrease in mean astrocyte load after irradiation (by massively reducing the total GFAP load), in pro-inflammatory cytokines (IL-6, IL-12p70, IL-1 β) and in total nitrite levels^[212]. Moreover, liraglutide was able to decrease cerebral edema, and to ameliorate both neurobehavioral deficits (in modified Garcia test and wire hanging test) and inflammatory parameters (given by an increased brain phosphorylated AMPK and reduced neutrophil infiltration) in an intracerebral hemorrhage-induced

brain injury mouse model (strongly associated with inflammatory mechanisms)^[47].

Therefore, given also the pathological commonalities between T2D and AD, it is plausible that a therapeutic approach involving incretin analogues might be beneficial against the cognitive deficits occurring in AD.

The neuroprotective potential of liraglutide in AD has been increasingly analyzed. In one of the first studies, McClean *et al.*^[213] reported that liraglutide significantly affects brain neurotransmission and modulates synaptic plasticity by enhancing long-term potentiation (LTP) mechanisms. More recently, Han *et al.*^[214] observed that pre-treatment with liraglutide dose-dependently protected against the impairment in learning and memory (as given by the dysfunctional spatial memory and hippocampal late-phase LTP) induced by a bilateral intrahippocampal injection of A β in adult male Sprague-Dawley rats, suggesting a possible preventive strategy against the development of AD in T2D patients. Strikingly, McClean *et al.*^[215], Long-Smith *et al.*^[216] and McClean *et al.*^[217] described that once daily intraperitoneally (*i.p.*)-injected liraglutide for 8 wk was able to reduce brain amyloid plaque formation by 30%-50%, as well as the levels of soluble amyloid oligomers (by 25%) and amyloid precursor protein (APP) in the APP/PS1 AD mouse model (which expresses the human Swedish mutated form of APP and a mutated human form of presenilin-1). Additionally, these authors observed that chronic liraglutide administration blunted the A β -associated changes in neuronal IR localization and IRS-1 phosphorylation at serine 616 (a key marker of insulin resistance), increased IDE levels, reduced microglia activation by up 50%, decreased the A β -associated astrocytic activation, enhanced LTP and synaptophysin levels, and increased hippocampal neuronal progenitor cells, thus indicating a protection against neuroinflammation, synapse loss and deterioration of synaptic plasticity that may culminate in the restoration of memory function, particularly in object recognition and water maze tasks. Interestingly, Parthasarathy *et al.*^[218] also reported that chronic treatment with liraglutide-mediated increase in neurogenesis in an AD mouse model was accompanied by an increased differentiation of newly generated cells into mature neurons. Concerning abnormal tau protein phosphorylation (another neuropathological hallmark of AD), liraglutide treatment has been able to reduce both brain tau protein phosphorylation at different residues (Ser199, Ser202, and Ser396) and phospho-tau immunoreactivity in T2D rats, which also displayed a reduction of HOMA-IR close to control levels and a normalization of brain insulin signaling (as given by the activation of Akt and subsequent inhibition of GSK-3 β at Ser9)^[219]. Altogether, these results strongly suggest that liraglutide may be able to prevent and/or reverse the major pathological hallmarks of AD. In line with this, two clinical trials are

underway to assess the effects of liraglutide in AD. In a small randomized clinical trial at the University of Aarhus (Denmark)^[220], 17 early-onset AD patients were treated with liraglutide (1.8 mg, once a day, 26 wk) and the already available results point towards an effect of liraglutide on cerebral amyloid deposits in the brain (assessed by Pittsburgh compound B PET scan), but more novelties are expected soon. The second trial, at the Imperial College of London and launched in June 2013^[221], is a large-scale Phase 2 clinical trial involving 206 early AD patients treated with liraglutide (1.8 mg/d, for 12 mo), that aims to use fluorodeoxyglucose-PET scan to detect changes in cerebral glucose metabolic rate, microglia activation, CSF markers, and amyloid and tau levels, to correlate with eventual Alzheimer Disease Assessment Scale Executive (ADAS) and Magnetic resonance imaging (MRI) changes. Interestingly, another ongoing, Phase 2, randomized, double-blind clinical trial sponsored by the National Institute on Aging and aiming at analyzing the action of the long-acting GLP-1RA exendin-4 (Ex-4) in AD^[222] will end by December 2015 and involves the evaluation of patients' performance in the Clinical Dementia Rating scale sum-of-boxes, ADAS (cognitive sub-scale), behavior and cognition, changes on structural and functional MRI and magnetic resonance spectroscopy, hormonal and metabolic changes, as well as alterations in CSF and plasma AD biomarkers.

Regarding the protective potential of GLP-1 analogues against PD, evidences are still relatively scarce and mostly relying on preclinical assessing the role Ex-4 in PD. Upon a 3 wk, twice daily *i.p.* administration of Ex-4, it was observed a significant increase in the number of TH-immunoreactive neurons and of dopamine levels that, together with a reduction of amphetamine-induced rotations in the 6-OHDA rat model of PD, suggest that Ex-4 may have beneficial cellular and functional properties in this model of PD^[223,224]. Similar effects were also reported in another rat model of PD involving a lipopolysaccharide-induced lesion to substantia nigra^[224]. Moreover, Li *et al.*^[225] observed that Ex-4 protected against the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, another PD-like phenotype inducer)-associated loss of nigral neurons and striatal dopaminergic fibers, preserved dopamine levels and improved motor function. With all this in mind, in a recent randomized Phase 2 clinical trial, Ex-4 safety and efficacy was evaluated in 21 PD patients, which received 5 mg b.i.d for 1 mo or 10 mg b.i.d for 11 mo^[226]. The main observations herein included a good tolerability to Ex-4, improved motor scores [from the Unified Parkinson's Disease Rating Scale (MDS-UPDRS)] at 12 mo (a mean improvement of 2.7 vs a 2.2 point decline in control group) and cognitive efficiency (using the Mattis dementia rating scale 2) (a mean improvement of 2.8 vs a 3.5 point worsening in control group)^[226].

Given the previously mentioned increasing interest on the potential therapeutic use of GLP-1 analogues

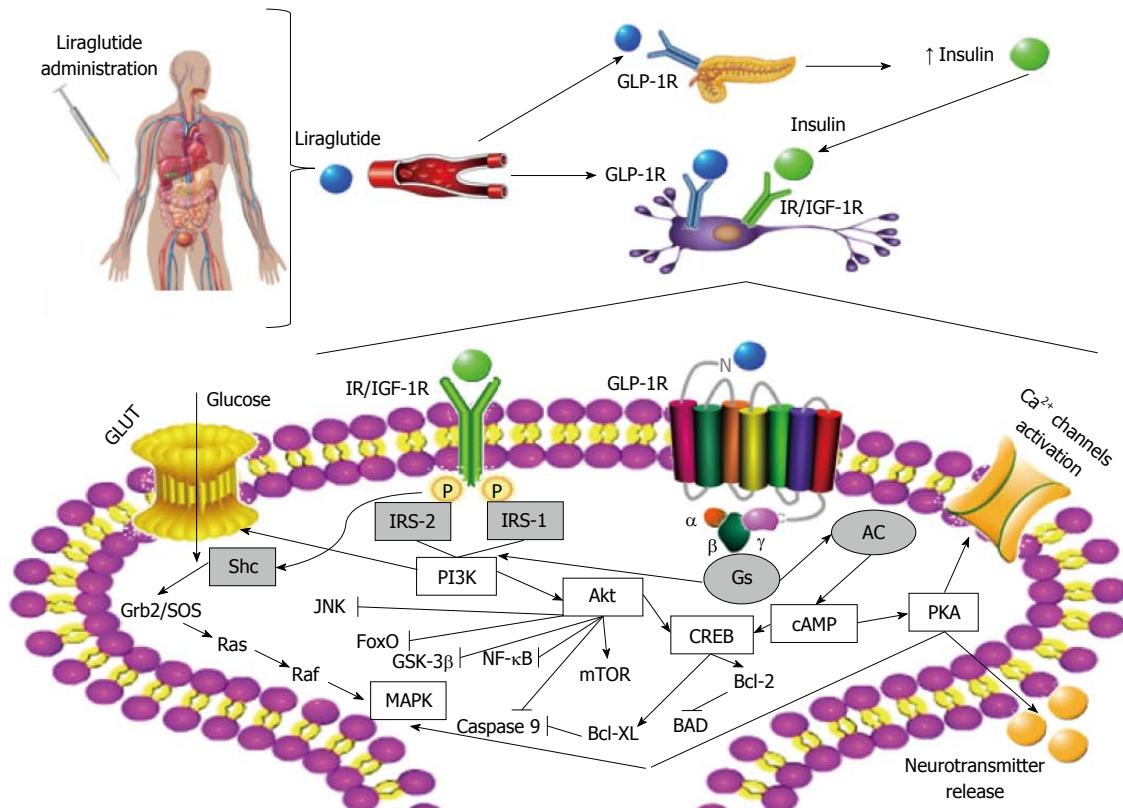


Figure 2 Overview of the main pathways induced by a peripheral administration of liraglutide in neurons. When liraglutide enters the body, it will move through the bloodstream and activate glucagon-like peptide-1 receptors (GLP-1R) widely expressed throughout tissues. In pancreas, GLP-1R activation exert insulinotropic effects, thus increasing insulin levels, which may migrate into the brain, crossing the blood-brain-barrier (BBB) and will activate insulin receptor (IR) or insulin-like growth factor-1 receptors (IGF-1R) expressed in neurons. Liraglutide by itself may also cross the BBB and activate GLP-1R present in the brain. Neuronal IR/IGF-1R and GLP-1R mediated intracellular signaling transduction pathways show several overlapping downstream targets. Activation of these pathways may mediate several biological responses in the central nervous system, such as control of cell metabolism and energy homeostasis, inhibition of apoptosis, reduction of inflammatory responses, modulation of synaptic neurotransmission, regulation of gene transcription, cell growth, synapse growth, cell repair and regeneration, facilitation of long-term potentiation and memory formation, among others. AC: Adenyl cyclase; Akt: Protein kinase B; Bcl-2: B-cell lymphoma 2; BAD: (Bcl-2) antagonist of death; Bcl-XL: B-cell lymphoma extra-large; Ca²⁺: Calcium; cAMP: Cyclic adenosine monophosphate; CREB: cAMP response element-binding protein; Foxo: Forkhead box O; GLUT: Glucose transporter; GRB2/SOS: Growth factor receptor-bound protein 2/son of sevenless; GSK-3 β : Glycogen synthase 3 beta; GTP: Guanosine triphosphate; IRS-1: Insulin receptor substrate 1; IRS-2: Insulin receptor substrate 2; JNK: C-Jun N-terminal kinase; MAPK: Mitogen associated protein kinase; mTOR: Mammalian target of rapamycin; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: Phosphoinositide 3-kinase; PKA: Protein kinase A; Shc: Src homology-2/ α -collagen-related protein.

for stroke treatment, in a recent study Sato *et al.*^[227] demonstrated that liraglutide was able to ameliorate behavioral scores, to reduce brain infarct volumes at 24 h, to decrease the levels of ROS derivatives and to upregulate vascular endothelial growth factor in brain cortex of a rat model for stroke (90 min transient middle cerebral artery occlusion and 1 h reperfusion). Similarly, neuroprotective effects upon stroke and ischemia were also reported for Ex-4^[225,228,229].

From the above, as exenatide and liraglutide are the currently marketed GLP-1RAs for the treatment of T2D, and since preclinical studies have demonstrated remarkable and consistent neuroprotective effects, some clinical trials are underway to assess the effect and efficacy of Ex-4 and liraglutide in AD and PD patients. And although the underlying molecular mechanisms (particularly those focused on the liraglutide's neuroprotective and anti-inflammatory potential) remain mostly unknown, a recent study on human neurons shed some light on this issue. Indeed,

Sharma *et al.*^[230] suggested that liraglutide-mediated neuroprotection may involve the PI3K/Akt pathway and its subsequent regulation of mammalian target of rapamycin; however, it is also plausible that GLP-1R activation under such circumstances may also activate the alternative extracellular signal-regulated kinase signaling. Traditionally, liraglutide-mediated activation of GLP-1R may further activate adenyl cyclase and increase cAMP content, leading to the subsequent activation of PKA and CREB, and ultimately contributing to cell survival, inhibition of apoptosis, activation of Ca²⁺ channels, cell growth, repair and regeneration, as well as regulation of translation/transcription processes in response to stress (Figure 2).

CONCLUSION

As the quote "the key to one's heart is through his stomach", the gut hormone GLP-1 (and, more specifically, its long-lasting synthetic analogs) may

hold the key for promising therapeutic effects against diseases affecting such different tissues, as the heart, pancreas, kidneys, liver, brain, and all the other tissues where we can find GLP-1Rs. It is plausible that this hormone, mainly secreted from L-cells, may exert its influence on the activities of several organs *via* complex axis involving the gut. And despite the still limited knowledge on this matter, we believe that the clarification of the molecular mechanisms involved herein might be of the outmost relevance in the context of the promising preventive/therapeutic potential of the long-acting GLP-1RAs against the development of long-term complications of several diseases. As a novel GLP-1RA and already marketed anti-T2D drug, and given the strong association between T2D and numerous neurodegenerative pathologies, liraglutide has been extensively investigated for such purpose in the recent years and has shown remarkable effects, not only peripherally, but also in CNS upon a wide range of diseases, including T2D, AD, PD and stroke. As liraglutide's positive effects in brain, we emphasize its promotion of neuronal survival, protection from apoptosis, regulation of neuroinflammatory response and modulation of stress response, thereby suggesting a strong neuroprotective potential. And this might be of a pivotal relevance, in our ever increasingly aged world and societies, characterized by an exponential increase in age-related diseases that still lack effective (or at least with a minimum of serious side-effects) preventive or therapeutic approaches, thereby posing an enormous socio-economic burden and urging the seek for an effective cure or delay in such diseases. Thus, the limited preclinical and clinical findings already available strongly suggest that liraglutide may emerge as a potential mono- or combined therapy against most of the common diseases afflicting the brain.

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Enterovirus and type 1 diabetes: What is the matter?

Carla Sanchez Bergamin, Sergio Atala Dib

Carla Sanchez Bergamin, Sergio Atala Dib, Department of Medicine, São Paulo Federal University, Rua Pedro de Toledo, São Paulo SP-04039001, Brazil

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Correspondence to: Sergio Atala Dib, MD, PhD, Department of Medicine, São Paulo Federal University, Rua Pedro de Toledo, 781 - 12 andar, Vila Clementino, São Paulo SP-04039001, Brazil. sergio.dib@unifesp.br
 Telephone: +55-11-55764744
 Fax: +55-11-55796636

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Abstract

A complex interaction of genetic and environmental factors can trigger the immune-mediated mechanism responsible for type 1 diabetes mellitus (T1DM) establishment. Environmental factors may initiate and possibly sustain, accelerate, or retard damage to β -cells. The role of environmental factors in this process has been exhaustively studied and viruses are among the most probable ones, especially enteroviruses. Improvements in *enterovirus* detection methods

and randomized studies with patient follow-up have confirmed the importance of *human enterovirus* in the pathogenesis of T1DM. The genetic risk of T1DM and particular innate and acquired immune responses to enterovirus infection contribute to a tolerance to T1DM-related autoantigens. However, the frequency, mechanisms, and pathways of virally induced autoimmunity and β -cell destruction in T1DM remain to be determined. It is difficult to investigate the role of enterovirus infection in T1DM because of several concomitant mechanisms by which the virus damages pancreatic β -cells, which, consequently, may lead to T1DM establishment. Advances in molecular and genomic studies may facilitate the identification of pathways at earlier stages of autoimmunity when preventive and therapeutic approaches may be more effective.

Key words: Virus; *Enterovirus*; *Coxsackievirus*; Type 1 diabetes mellitus; Auto-immune diabetes; Pathogenesis

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Core tip: A complex interaction of genetic and environmental factors can trigger the immune-mediated mechanism responsible for type 1 diabetes mellitus (T1DM) establishment. The role of environmental factors in this process has been exhaustively studied and viruses are among the most probable ones, especially enteroviruses. Improvements in enterovirus detection methods and randomized studies with patient follow-up have confirmed the importance of these viruses in the pathogenesis of T1DM. However the frequency of viruses induces autoimmunity or β -cell destruction and the mechanisms and pathways how they increment the autoimmunity in T1DM still to be determined. Here, we review these mechanisms and all evolution in enterovirus studies and T1DM. Advances in molecular and genomic studies may facilitate the identification of pathways at earlier stages of autoimmunity when preventive and therapeutic approaches may be more effective.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic endocrine disorder that is caused by the progressive destruction of pancreatic β -cells, which results in insulin deficiency. A complex interaction between genetic and environmental factors may trigger this immune-mediated mechanism^[1]. The most important T1DM susceptibility genes are located in the *HLA-DR* and *DQ* loci^[2]. However, T1DM is not induced by genetic susceptibility alone, and environmental factors may initiate and possibly sustain, accelerate, or retard the damage to β -cells^[3,4]. The role of environmental factors in the development of T1DM has been suggested because of the seasonal variation in the incidence of T1DM^[5] and the conspicuous variation in the incidence of T1DM between different countries^[6,7]. Immigrants often acquire a level of risk for developing T1DM that is typical for their new home country^[8]. In addition, the incidence of T1DM has rapidly increased during the last decade^[9-11] despite the increased prevalence of protector genes for T1DM and a concomitant decrease in high-risk genes^[12,13]. Changes in the environment and how individuals respond to these variations have been indicated as being responsible for this increase in T1DM.

The following environmental factors have been suspected to contribute to the development of T1DM: dietary factors, such as cow's milk proteins^[14,15], vitamin D deficiency^[16,17] and gluten^[18]; pancreatic toxins^[19,20], such as streptozotocin and nitrites; psychological factors^[21]; and viral infection factors^[22]. Viruses are among the most probable environmental factors in the development of T1DM, including rubella virus^[23], rotavirus^[24], mumps virus, cytomegalovirus and enteroviruses^[25-27]. Recent studies using different approaches have suggested that the most promising candidates for viral triggers with clinically significant associations with T1DM development are enteroviruses^[28-31].

However, it has been difficult to establish viruses as the inducers of T1DM. First, the link between infections and autoimmunity is multifactorial^[32]. Several infections may act together or in an appropriate temporal sequence to trigger clinical autoimmunity. Furthermore, the particular virus that is involved in triggering T1DM may be hard to detect systemically or in the target organ after the initiation of the autoimmune response^[33]. Second, the long duration of time between the possible triggering effect and the onset of the clinical symptoms of diabetes makes it difficult to establish a direct relationship. Third, T1DM

patients and healthy individuals undergo multiple viral infections during their lifetime, and several of these viruses may even protect individuals from autoimmune disease^[34,35]. Fourth, the "fertile field hypothesis" suggests that viral infections render tissue a "fertile ground" for autoaggressive lymphocytes to invade and expand, which leads to T1DM^[36,37]. Therefore, the activation of the immune system may have a role in the pathogenesis of this disease^[38].

In this review, the potential mechanisms of enterovirus infections in the establishment of T1DM will be discussed.

ENTEROVIRUSES

The *Enterovirus* genus of the *Picornaviridae* family consists of small, non-enveloped, positive, single-strand RNA viruses, including *polio viruses* (PVs), *Coxsackie viruses* A and B (CVA and CVB), *echoviruses* (EVs) and new enteroviruses. Three different PVs (types 1-3) and more than 60 non-polio enteroviruses cause disease in humans. These human *enteroviruses* (HEVs) include 23 CVAs (types 1-24; type 23 does not exist), 6 CVBs (types 1-6), 28 EVs (types 1-33; types 10, 22, 23 and 28 do not exist) and 4 other enteroviruses (EV 68-71)^[39].

Enterovirus infections are transmitted from person to person by fecal-oral and, less commonly, respiratory routes, which indicates that these infections usually begin in the gastrointestinal or respiratory mucosa. After replicating in the mucosa, the virus spreads through the lymphatic system into the circulation after a brief viremic phase at secondary replication sites, which determines the types of symptoms^[40].

Most *enterovirus* infections are asymptomatic or produce subclinical or mild symptoms, such as nonspecific febrile disease, muscle pain, sore throat, gastrointestinal distress, headache and abdominal discomfort. However, a wide variety of symptoms that affect various organs may occur, such as hand, foot and mouth disease; acute hemorrhagic conjunctivitis; aseptic meningitis; myocarditis; severe neonatal sepsis-like disease; and acute flaccid paralysis^[41].

Independent of location and symptom intensity, viral replication is continuous in the lymphatic tissue. The incubation period varies from 2-30 d, and enteroviruses can be detected in various types of biological specimens from humans. Enteroviruses may be detected most readily in stools for up to 3-4 wk but rarely more than 2-3 mo. Serologic diagnoses in the acute phase are difficult because most cases are asymptomatic^[42].

Systemic *enterovirus* infection may lead to viral dissemination to other target organs, and *enterovirus* RNA and protein may be detected in intestinal, heart or pancreatic tissues by reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry or *in situ* hybridization^[43].

ASSOCIATION BETWEEN ENTEROVIRUSES AND T1DM: A HISTORICAL OVERVIEW

There are convincing experimental results for the role of *EV* infection in T1DM development using mouse models^[44-46], and some mechanisms of beta cell damage have been proposed based on experiments with non-obese diabetic (NOD) mice^[47].

In humans, *enterovirus* infection has been suspected to be involved in the pathogenesis of T1DM since the late 1960s, when Gamble *et al.*^[45] described a seasonal variation in the incidence of T1DM following *enterovirus* infection^[48] and demonstrated that the frequency of neutralizing antibodies against the *CVB4* serotype was increased in newly diagnosed T1DM patients^[49]. A *CVB4* virus was subsequently isolated from the pancreas of a child who died from diabetic ketoacidosis, and this virus strain caused diabetes in a susceptible mouse strain^[50]. However, subsequent studies failed to replicate this result.

In many serological studies, *enterovirus* antibodies have been more prevalent in diabetic patients than in healthy children^[51]. However, critics have questioned these data because control patients were not matched for HLA risk alleles, and the detection methods for *enterovirus* cannot differentiate between HEV types. Several reports have not presented the same outcome, and the role of *enterovirus* infection in the development of T1DM has remained controversial^[52].

Oikarinen *et al.*^[53] analyzed the role of past exposure to different *CVB* serotypes by measuring neutralizing antibodies specifically against each of the six serotypes in 249 children who were newly diagnosed with T1DM and in 249 control subjects from five European countries. Antibodies against *CVB1* were more frequently detected in diabetic children than in the control group. This study suggests that coxsackie virus B may include a diabetogenic virus group and indicates that *CVB1* may be a member of this group. However, because of the cross-sectional study design, the findings do not support a causality link between *enterovirus* infection and T1DM. Nevertheless, the same virus type has recently been observed to increase the risk of T1DM in the prospective Diabetes Prediction and Prevention (DIPP) study as a potential initiator of the β -cell-damaging process^[54].

Much attention has been paid to the possible immunological cross-reactivity that is induced by a homology sequence in the 2C non-structural *CVB* protein and a principal diabetes autoantigen glutamic acid decarboxylase (GAD65), which share a common amino acid sequence^[55,56]. GAD65 is an important target antigen in the pathogenic process of diabetes. In mice, the insulinitis establishment coincides with GAD65 specific reactivity, and tolerance induction to GAD65 can prevent the disease^[57,58]. Humoral and cellular responses have been detected against GAD65 before

the onset of clinical diabetes^[59], and auto antibodies are positive several years before diagnosis^[60]. The importance of this homology in T1DM pathogenesis is supported by data showing that T cells that respond to this sequence are present both in NOD mice and T1DM patients^[61,62]. This mechanism will be discussed below.

In more recent studies, RT-PCR has been used to detect *CVB*-specific RNA in the sera of newly diagnosed T1DM patients^[63]. Yeung *et al.*^[64] conducted a useful systematic review and meta-analysis of observational molecular studies on the detection of *enterovirus* in T1DM patients. Observational case-control studies measured *enterovirus* RNA or viral protein in the blood, stool or tissue of prediabetic and diabetic patients by molecular methods. The 24 selected papers and two abstracts demonstrated a clinically significant association between *enterovirus* infection and autoimmunity/T1DM (odds ratios ranging from 5.5 to 17.4).

Human studies on the relationship between *enterovirus* and T1DM have been retrospective and based on the detection of virus infections in newly diagnosed T1DM patients. However, this study design does not allow for the evaluation of possible causal associations. To overcome this issue, several prospective studies have been performed to assess the role of *CVB* and other *HEV* infections in the induction and acceleration of T1DM and islet autoimmunity. These studies include the Childhood Diabetes in Finland (DiMe) study^[65,66], the DIPP study^[67,68], and the Trial to Reduce T1DM in the Genetically at Risk (TRIGR)^[69], which were conducted in Finland; the BABYDIAB^[70] and Babydiat^[71] studies in Germany; the Diabetes and Autoimmunity Study in the Young (DAISY)^[72,73] in Colorado, United States; and the Environmental Triggers of Type 1 Diabetes (MIDIA)^[74] in Norway. These studies included children with an increased risk of T1DM, which was defined as a first-degree family history of T1DM, HLA susceptibility genes or both. The sampling frequency and the method of *enterovirus* detection varied between these studies (Table 1).

A positive association between *EV* infections and a rapid progression from autoimmunity to clinical T1DM was observed both in the DiMe study as well as in the DAISY follow-up study (human longitudinal studies). However, there was no agreement in the studies' conclusions between *EV* infection and islet autoimmunity development.

The results of these prospective studies may be controversial due to heterogeneity in the study design, the small number of patients in each study and the low sensitivity of the methods used to detect *enterovirus* infection. Another important confounding factor is the frequency of sampling because *EV* RNA can rarely be found continuously in stool samples for more than 3 mo, and it is found for a shorter time in serum samples^[75]. The studies that indicated a positive association between *enterovirus* infection and T1DM used smaller sampling intervals and a wider panel

Table 1 Longitudinal studies evaluating the association between enterovirus infection and autoimmunity/type 1 diabetes mellitus

Study	Enterovirus infection and autoimmunity/T1DM	Cases/controls	Infection diagnose method	End point	Ref.
DiMe	+	22/110	Antibody assays	Diabetes	[65]
DiMe	+	49/105	EV RNA in serum	Diabetes	[66]
DIPP	+	21/104	Antibody assays; EV RNA in serum and stool-RT-PCR	Autoimmunity	[67]
DIPP	+	41/196	Antibody assays; EV RNA in serum-RT-PCR	Autoimmunity	[68]
TRIGR	+	19/84	Antibody assays; EV RNA in serum-RT-PCR	Autoimmunity	[69]
BABYDIAB	-	28/51	Antibody assays	Autoimmunity	[70]
Babydiet	-	22/82	EV RNA in stool	Autoimmunity	[71]
DAISY	-	26/39	EV RNA in serum, saliva and rectal swab-RT-PCR	Autoimmunity	[72]
DAISY	+	50/90	EV RNA in serum	Diabetes	[73]
MIDIA	-	27/53	EV RNA in stool-RT-PCR	Autoimmunity	[74]

EV RNA: *Enterovirus* RNA; RT-PCR: Reverse transcription-polymerase chain reaction; T1DM: Type 1 diabetes mellitus; DiMe: Diuretics In the Management of Essential Hypertension; DIPP: Department of industrial policy and promotion; TRIGR: Trial to Reduce IDDM in the Genetically at Risk; DAISY: Diabetes and Autoimmunity Study in the Young.

of *enterovirus* assays than the studies that indicated no association. Similarly, most *enterovirus* infections are asymptomatic, and a negative result for the virus at diagnosis does not mean that its contribution is meaningless.

The prevalence of *EV* infections varies in populations, and independent of this, the vast majority of people infected will not develop autoimmunity or T1DM, as illustrated by Sarmineto^[76]. This study showed that in Cubans that were exposed to an echovirus epidemic, a large number of patients seroconverted to islet autoantibody positivity, but T1DM prevalence has not increased. It remains to be determined how often enteroviruses induce β cell damage, autoimmunity development and clinical diabetes.

ENTEROVIRUSES AND THE DEVELOPMENT OF T1DM

Hygiene hypothesis

The hygiene hypothesis was first proposed by Strachan^[77] to explain the increasing rates of asthma in highly developed countries, suggesting that contacts with a high number of infections early in life could properly modulate the adaptive immune system, and the significant changes in human living standards and the improvement of sanitary conditions meant that people had less exposure to infection, favoring an impaired immune response to environmental triggers^[35,78]. This concept may be applied to many autoimmune diseases, but it does not explain all of these diseases, as there is a complex interplay between environmental exposure, the host, and other confounding variants^[78-80].

Exposure to HEV, which is typically transmitted through a fecal-oral pathway, becomes less common as individual age, and infection with *HEV* later in life could result in an unbalanced immune response. In other words, where *enterovirus* infections are frequent,

children develop an efficient immune response to these viruses, and when they are exposed in the future, the effects are not exacerbated or harmful. This may explain the rising worldwide incidence of T1DM over the last decade, mainly in developed societies where *enterovirus* infections are less prevalent^[81-83].

The age when individuals are first exposed to enteroviruses may be critical in determining how the virus interacts with the host immune system (as recently demonstrated in a NOD mouse model^[84]) and ultimately, whether T1DM develops. *Enterovirus* infections during the first year of life have been correlated with protection from the onset of T1DM^[85]. A study of *enterovirus*-specific cellular immunity in Estonian and Finnish children at 9 mo of age found that *enterovirus* infections were inversely correlated with T1DM risk^[86]. Estonian children who were immunized with live-attenuated *PV* at early ages had stronger T cell responses to *CVB4* and *PV* type 1 compared with Finnish children who were immunized with inactivated *PV*. This stronger T cell immunity led to higher cross-reactivity with other *enterovirus* serotypes. Despite the higher incidence of *enterovirus* infections in Estonia, the incidence of T1DM is five times lower than that in Finland^[87]. In addition, the frequency of T1DM is higher in the firstborns of multiplex families than in younger children, which could be explained by a lower exposure of firstborns than siblings to infections^[88]. Therefore, viral infections during childhood may protect individuals from developing T1DM or may delay disease onset.

Implication of HEV in T1DM

CVB infections may have two different roles in the etiology of T1DM in humans: protective or triggering^[89]. These proposed models are based on observations that *CVBs* do not replicate productively in healthy, non-inflamed pancreatic islets from NOD mice^[90]. In individuals who are genetically predisposed to T1DM but not to insulinitis, *CVB* infection may induce a protective

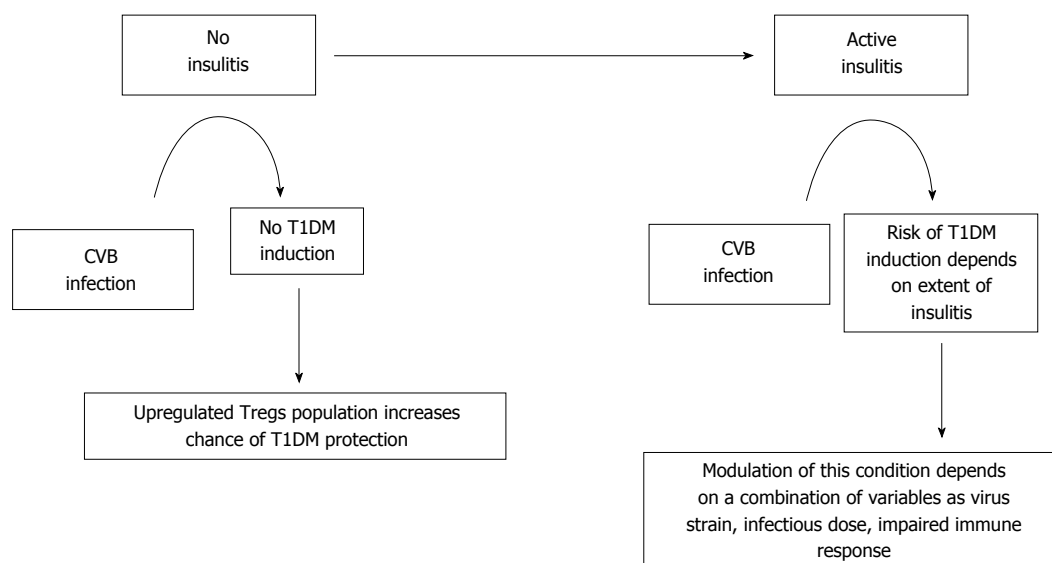


Figure 1 Enterovirus infection pathways on type 1 diabetes mellitus pathogenesis (adapted from Tracy *et al*^[47]). T1DM: Type 1 diabetes mellitus; CVB: Coxsackie viruses.

Treg population that prevents the development of pathogenic autoimmune islet-specific T cells, thereby reducing the risk of autoimmune T1DM. However, in the absence of a protective Treg population, the extent of insulinitis tends to increase with the depletion of β -cells and results in an elevated risk of developing autoimmune diabetes. The likelihood of developing T1DM requires a basic condition of genetic predisposal with the presence of anti-islet autoimmunity due to a particular virus strain and infectious dose in addition to extensive insulinitis at the time of infection. A significant number of β -cells must be destroyed before the adaptive immune system may be activated. Therefore, depending on the host environment during infection, the virus may either induce protection against autoimmune T1DM onset or, with significant insulinitis, may induce T1DM development (Figure 1)^[89].

Autoimmune islet inflammation may facilitate productive virus replication and induce β -cell damage^[47].

Roles of enteroviruses in β -cell injury mechanisms that lead to the development of T1DM

Several hypotheses have been proposed to explain how enteroviruses affect T1DM (Figure 2)^[28,40,89]. The mechanisms differ for each *enterovirus*; however, their coexistence is supported^[33]. Enteroviruses have a strong pancreotropism^[91]; human islets express coxsackie virus receptor^[92], and beta cells are susceptible to enteroviruses *in vitro*^[93]. During *enterovirus* infection, pancreatic islet cells may exhibit cytolysis, which exposes previously hidden self-components^[94,95]. Dotta *et al*^[96] detected *enterovirus* in three of six pancreatic tissue samples from patients with T1DM. Additionally, Coxsackie viruses lead to direct pancreatic injury. Elshebani *et al*^[97] demonstrated that enteroviruses that were isolated from patients who were newly diagnosed with T1DM

infected induced the destruction of human pancreatic islets *in vitro*.

Alternatively, β -cell damage may result from a virus-induced inflammatory reaction in the exocrine pancreas compartment. Viral infections often lead to the production of proinflammatory cytokines and the activation of antigen-presenting cells (APCs). In addition, these infections may cause tissue damage and may expose endogenous antigens that are presented by APCs. In individuals who are genetically predisposed to T1DM, viral infections may result in the impaired activation of self-reactive T cells through a mechanism that is independent of specific T-cell receptor (TCR) stimulation^[98,99]. This process, called "bystander activation", does not require specific TCR stimulation and was supported by a study of *CVB4* infection in transgenic mice that resulted in the activation of circulating naive islet-specific T cells and clinical diabetes development^[100].

Furthermore, the mechanism of cell destruction may be based on molecular mimicry^[101]. The activation of a T-cell population against an environmental antigen results in the development of autoimmune disease if the epitope recognized shows sequence or structural similarity with a self-protein. Although virus-specific T lymphocytes are activated during an infection, antibody responses are critical in the defense against enteroviruses and are responsible for the clearance of the infection. Neutralizing antibodies are directed against the capsid surface of *CVB* and nonstructural proteins. These proteins are produced exclusively during the replication of the virus and are released as a consequence of the lysis of the infected cells. Directly linked to T1DM triggering was an observation of the amino acid sequence similarity between *CVB4* nonstructural protein 2C and GAD65 (PEVEKEK), which suggests that the cellular anti-viral response

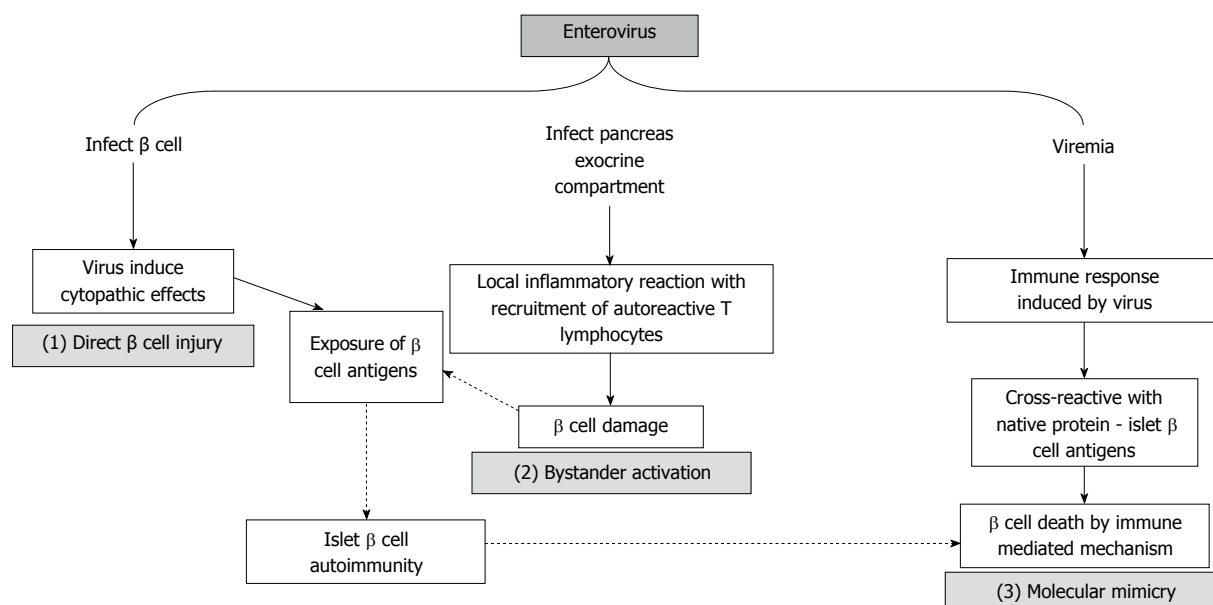


Figure 2 Schematic representation of possible injury mechanisms from enterovirus infection in type 1 diabetes mellitus development (adapted from Roivainen^[40]).

may cross-react with the native protein, inducing an autoimmune response^[102].

However, in several studies, the molecular mimicry hypothesis has been controversial because healthy control groups presented reactivity to the molecular section of GAD65^[103]. In addition, molecular mimicry is extremely common in nature, which suggests that an impaired immune response may be crucial for β -cell damage in contrast with molecular mimicry^[103].

All these mechanisms described may occur simultaneously. In fact, inflammatory conditions induced by virus infection will trigger autoimmunity resulting in T1DM only in susceptible individuals^[38]. This hypothesis, Fertile Field, postulates that following the inflammation caused by virus infection, autoreactive T cells may be generated by bystander activation or molecular mimicry or both. The damage of beta cells and its presentation to immune system lead to antigenic epitope spreading, which explains the broad autoreactive T-cell repertoire in T1DM patients. This hypothesis may be of interest because *enterovirus* infection activates a strong innate immune response^[104,105].

Innate and acquired immunity

Immune responses against infection by microbes are highly complex, and recent advances in the understanding of innate immunity components have elucidated the integration of innate immunity and acquired immunity. Innate immunity has limited specificity for microbes and works in a similar manner against most infectious agents. The main components of the innate immune system are physical and chemical barriers, blood proteins, including the complement system and other inflammatory mediators, such

as phagocytes (neutrophils and macrophages) and natural killer cells. In contrast with the innate immune system, the acquired immune system consists of defense mechanisms with remarkable specificity for distinguishing molecules. The acquired immune response increases in magnitude with successive exposure to a specific microbe. Acquired immunity develops as a response to infection. The components of the acquired immune system are lymphocytes and their products, which are known as antibodies.

The innate immune response provides the initial defense against infectious agents. Pathogen recognition by the innate immune system is usually mediated by receptors that recognize the molecular patterns of different organisms. Recent studies have demonstrated that this recognition is mainly mediated by Toll-like receptors (TLR)^[106,107]. TLR2 detects lipoproteins, lipoteichoic acid and zymosan. TLR3 recognizes dsRNA, and TLR4 recognizes lipopolysaccharide (LPS). Additionally, flagellin is detected by TLR5, ssRNA by TLR7/8 and CpG DNA by TLR9. TLRs are expressed in multiple tissues, predominantly in the cells of the immune system, particularly APCs^[108].

TLR signaling induces the expression of costimulatory molecules and the production of inflammatory cytokines, such as interferons (IFNs) and interleukins (ILs), which activate both the innate and acquired immune responses. An intact innate immune response is critical for host survival during *enterovirus* infection. The rapid induction of IFNs is important for protection against infection. A recent study found a high rate of mortality in mice that were unresponsive to type I IFNs or lacked IFN- β after CVB infection. In addition, mortality occurred early in these mice^[109].

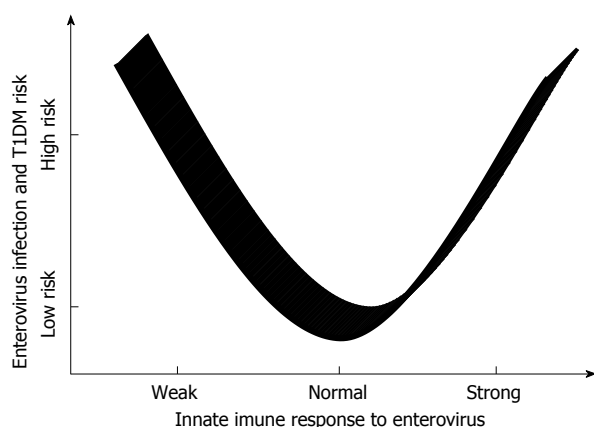


Figure 3 Hypothetic relationship between innate immune response to enterovirus and type 1 diabetes mellitus risk. T1DM: Type 1 diabetes mellitus.

Viral infection, innate immunity and T1DM

Viruses activate the innate immune response and induce the production of proinflammatory cytokines^[98,110,111]. Enteroviruses demonstrate a tropism for the pancreas. However, the mechanism of β -cell damage is unclear^[112].

CVB4 infection induces the production of pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β) and tumor necrosis factor α (TNF- α)^[113]. These cytokines are involved in the host defense response against infection; however, recent studies have suggested that pro-inflammatory cytokines that are activated in response to viral infections may play a role in the pathogenesis of T1DM^[114,115]. TNF- α may be involved in β -cell damage^[116].

Using RT-PCR, Wen *et al.*^[117] recently isolated pancreatic islet β -cells from different species of mice and detected TLR2, 3, 4 and 9 in the islet cells of normal mice but a higher expression of TLR2, 3 and 4. The same methodology was applied in a study of pancreatic β -cells from three healthy human donors, and a higher expression of TLR3 was found in these cells. However, after treatment with microbial stimuli, LPS from gram-negative bacteria and CpG oligonucleotide DNA increased the expression of TLR2, 4 and 9. Moreover, viral stimulation with poly (I:C), a synthetic dsRNA that is capable of triggering immune responses, TLR2, 3, 4 and 9 expression was observed. The same microbial stimuli were assessed *in vivo*, and only the viral stimulus poly (I:C) triggered diabetes mellitus.

In NOD mice, CVB can induce diabetes because of the framework that is established by insulinitis, which is caused by the increased expression of TLR4 and 8 by dendritic cells^[118]. A study in human pancreatic cells demonstrated that CVB4 leads to the production of inflammatory cytokines, particularly IL-6 and TNF α . TLR4 is necessary for triggering this immune response; however, this response is independent of CVB4 internalization and replication. Therefore, the interaction between TLR4 and CBV4 in pancreatic cells may induce an innate immune response^[119].

Studies in experimental models have demonstrated that the innate immune response is crucial for host survival during *enterovirus* infection. A weak innate immune response may allow unrestricted replication and the systemic spread of the virus. Tissue damage may ensue as a direct effect of the infecting virus, and an inefficient immune response may enable viral persistence. A robust innate immune response will limit early viral replication and diminish virally instigated damage, thereby allowing the host to mount an adaptive immune response. However, autoimmune pathologies may arise in the wake of a very potent immune response. In this scenario, the innate immune response to infection triggers the activation of self-reactive T cells^[120]. Many autoimmune diseases are associated with the excessive production of IFNs^[121]. Therefore, Lien *et al.*^[122] postulated that the relationship between viruses and TLRs in the development of diabetes in BioBreeding diabetes-resistant (BBDR) rats is a complex process that involves the modulation of auto-reactive T cells. In response to pathogenic microorganisms, the innate immune response that is mediated by TLR may lead to the bystander activation of auto-reactive T cells, the release of pro-inflammatory cytokines and the impairment of pancreatic islet cells^[123,124].

In contrast, a strong immune response is more likely to prevent the virus from productively infecting host cells and is more likely to hinder virus access to the pancreas. However, an inefficient response enhances the risk for systemic viral spread and the induction of an innate immune response in tissues that are targeted by the virus. Therefore, viral infection with a weak initial immune response may result in higher systemic levels of pro-inflammatory cytokines and the activation of auto-reactive T cells^[120] (Figure 3).

Interferon transcriptional signature

Two studies were recently conducted with children from the BABYDIET cohort^[125] and the DIPP study^[126] who were genetically predisposed to T1DM. Using targeted and genome-wide transcriptomics, the expression of IFN signatures during the onset of autoimmunity and the progression to clinical T1DM was investigated. An IFN signature was first characterized in systemic lupus erythematosus disease in which IFN was found to be correlated with disease severity.

In these two studies, both research groups identified an IFN signature in the children before the development of islet autoimmunity. Ferreira *et al.*^[125] found that the IFN signature was increased before seroconversion in predisposed patients and corresponded with two or more episodes of respiratory infection, suggesting that the IFN signature is related to viral infections. The expression was intermediate in samples that were collected postseroconversion and in children with clinical T1DM.

The increased expression of type 1 IFN is a normal

response to viral and bacterial infections in healthy individuals, but the pattern of expression and the presence of an IFN signature in peripheral blood may be a marker of a recent antiviral immune response. In individuals with a genetic risk for T1DM, an altered response to viral infection with unbalanced effector and regulatory cells may result in an overreaction. Importantly, the expression of an IFN signature is consistent with the activation of innate immune pathways during the initiation of islet autoimmunity and may be the first sign of this process.

CONCLUSION

Extended and cumulative discussions have been conducted on the role of *enterovirus* infection in the etiopathogenesis of T1DM, and substantial supporting evidence has been found. Improvements in *enterovirus* detection methods and randomized studies with patient follow-up have confirmed the importance of HEV in T1DM development and progression. However, the frequency, mechanisms, and pathways of virally induced autoimmunity and β -cell destruction in T1DM remain to be determined. In this way, the causal link between EV and T1DM involves a complex interplay between viruses, β -cells, innate and acquired immune systems in the particular genetic context of an individual. The influence of these several concomitant mechanisms by which the virus damages pancreatic β -cells, which, consequently, may lead to T1DM establishment makes investigating the role of *enterovirus* infection quite difficult. It will be extremely important to design prospective studies with large study populations, more frequent sampling of various specimens and a standardized methodology to detect *enterovirus* infection, linking it to islet autoimmunity or T1DM and controlling for confounding factors. Advances in molecular and genomic studies may facilitate the identification of pathways at the earlier stages of autoimmunity when preventive and therapeutic approaches may be more effective.

REVIEW CRITERIA

The databases that were searched in this study included PubMed and Embase. Papers that were published from 2004-2014 were included in the study, with a particular interest in papers that were published after 2009. The search terms were "virus", "*enterovirus*", "*coxsackievirus*", "type 1 diabetes mellitus", "auto-immune diabetes", and "insulin-dependent diabetes". The selected publications were full-text papers that were published in English. Additional references were selected from the reference lists of the selected article.

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Diabetes therapies in hemodialysis patients: Dipeptidase-4 inhibitors

Yuya Nakamura, Hitomi Hasegawa, Mayumi Tsuji, Yuko Udaka, Masatomo Mihara, Tatsuo Shimizu, Michiyasu Inoue, Yoshikazu Goto, Hiromichi Gotoh, Masahiro Inagaki, Katsuji Oguchi

Yuya Nakamura, Masatomo Mihara, Tatsuo Shimizu, Michiyasu Inoue, Yoshikazu Goto, Hiromichi Gotoh, Saiyu Soka Hospital, Kitaya, Soka-city Saitama-ken 340-0046, Japan
 Hitomi Hasegawa, Mayumi Tsuji, Yuko Udaka, Katsuji Oguchi, Department of Pharmacology, School of Medicine, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

Masahiro Inagaki, Department of Chemistry, College of Arts and Sciences, Showa University, Kamiyoshida, Fujiyoshida-city, Yamanashi-ken 403-0005, Japan

Author contributions: Nakamura Y devised the study concept and design; Nakamura Y searched the literature; Nakamura Y, Shimizu T, Goto Y and Gotoh H analyzed the literature; Nakamura Y, Hasegawa H, Udaka Y and Mihara M interpreted the literature; Nakamura Y drafted the article; Nakamura Y, Tsuji M and Inagaki M revised the article for important intellectual content; Oguchi K gave final approval for the article.

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Correspondence to: Yuya Nakamura, MD, Saiyu Soka Hospital, 1-21-37, Kitaya, Soka-city Saitama-ken 340-0046, Japan. y.nakamura@med.showa-u.ac.jp
 Telephone: +81-4-89446111
 Fax: +81-4-89448080

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Abstract

Although several previous studies have been published on the effects of dipeptidase-4 (DPP-4) inhibitors in diabetic hemodialysis (HD) patients, the findings have yet to be reviewed comprehensively. Eyesight failure caused by diabetic retinopathy and aging-related dementia make multiple daily insulin injections difficult for HD patients. Therefore, we reviewed the effects of DPP-4 inhibitors with a focus on oral antidiabetic drugs as a new treatment strategy in HD patients with diabetes. The following 7 DPP-4 inhibitors are available worldwide: sitagliptin, vildagliptin, alogliptin, linagliptin, teneligliptin, anagliptin, and saxagliptin. All of these are administered once daily with dose adjustments in HD patients. Four types of oral antidiabetic drugs can be administered for combination oral therapy with DPP-4 inhibitors, including sulfonylureas, meglitinide, thiazolidinediones, and alpha-glucosidase inhibitor. Nine studies examined the antidiabetic effects in HD patients. Treatments decreased hemoglobin A1c and glycated albumin levels by 0.3% to 1.3% and 1.7% to 4.9%, respectively. The efficacy of DPP-4 inhibitor treatment is high among HD patients, and no patients exhibited significant severe adverse effects such as hypoglycemia and liver dysfunction. DPP-4 inhibitors are key drugs in new treatment strategies for HD patients with diabetes and with limited choices for diabetes treatment.

Key words: Dipeptidase-4 inhibitors; Hemodialysis; Diabetes mellitus; Blood glucose-related factors; Anti-inflammatory effects

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Core tip: Until now, the effectiveness of dipeptidase-4 (DPP-4) inhibitors on diabetic hemodialysis (HD) patients has not been reviewed. All 7 DPP-4 inhibitors are available for HD patients; administration is once daily with dose adjustments. The effectiveness of DPP-4 inhibitor treatment in HD patients is high, and adverse events do not increase as a result. DPP-4 inhibitors may prevent inflammation and atherosclerosis, which are principal prognostic factors for HD patients. In summary, DPP-4 inhibitors are key drugs in new treatment strategies for HD patients with diabetes and limited choices for its treatment.

Nakamura Y, Hasegawa H, Tsuji M, Uda Y, Mihara M, Shimizu T, Inoue M, Goto Y, Gotoh H, Inagaki M, Oguchi K. Diabetes therapies in hemodialysis patients: Dipeptidase-4 inhibitors. *World J Diabetes* 2015; 6(6): 840-849 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i6/840.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i6.840>

INTRODUCTION

Diabetes is the biggest cause of renal failure worldwide^[1,2]. Diabetes treatment is an very important factor in the overall survival of hemodialysis (HD) patients^[3,4]. While insulin therapy is the primary treatment for HD patients, impaired eyesight caused by diabetic retinopathy and aging-related dementia make multiple daily insulin injections difficult for many patients^[5]. Moreover, in HD patients, many diabetes oral medicines cause serious side effects such as hypoglycemia and lactic acidosis. Hence, the development of new diabetes oral medicines with little or no side effects is needed for these patients.

Dipeptidase-4 (DPP-4) inhibitors are the most highly used diabetic drugs and show both a lower incidence of hypoglycemia and good safety^[6]. In addition, they induce an ingestion control effect and may also prevent atherosclerosis and reduce cardiovascular events^[7,8]. Therefore, these medications are strongly expected to improve the quality of life and prognosis of diabetic HD patients.

As a new class of diabetic medications, sodium-glucose co-transporter 2 (SGLT2) inhibitors both inhibit glucose reabsorption in renal tubules and increase glucose excretion, but cannot be administered to dialysis patients. G-protein-coupled receptor 40 (GPR40) agonist, GPR119 receptor agonist, and glucokinase activators are new antidiabetic medications currently in clinical trials and thus are not yet available. Therefore, DPP-4 inhibitors have been the mainstay drugs during the past several years for HD patients with diabetes. Accordingly, a comprehensive research of the pharmacokinetics and pharmacodynamics of DPP-4 inhibitors in HD patients is important. Some reports have investigated the effectiveness of DPP-4 inhibitors in HD patients^[9-18]. However, there has

been no review of new treatment strategies for HD patients who have diabetes and limited choices for its treatment. Therefore, this review evaluated the effects of DPP-4 inhibitors as a new therapeutic strategy for diabetic patients.

SEARCH STRATEGY

A MEDLINE search (1966 to July 2014) for published clinical studies and pertinent review articles published in English was conducted with the following keywords: "DPP-4 inhibitor", "hemodialysis", "end stage renal disease", "sitagliptin", "vildagliptin", "alogliptin", "linagliptin", "teneligliptin", "anagliptin", "saxagliptin", "glucagon-like peptide-1 (GLP-1)", "insulin", "glucagon", and "insulin resistance". References of identified articles were searched for additional relevant sources. Articles relevant to the efficacy, safety, and pharmacology of DPP-4 inhibitors in HD patients were also identified from the references cited in works obtained from the MEDLINE search results.

SEVEN DPP-4 INHIBITORS

At present, 7 DPP-4 inhibitors are available worldwide: sitagliptin, vildagliptin, alogliptin, linagliptin, teneligliptin, anagliptin, and saxagliptin. All DPP-4 inhibitors are available to HD patients, and administration is once daily. However, the dose adjustments are different for each DPP-4 inhibitor. Five DPP-4 inhibitors are excreted renally (*i.e.*, sitagliptin, vildagliptin, alogliptin, anagliptin, and saxagliptin). Meanwhile, both linagliptin and teneligliptin are excreted through bile; therefore, a reduction of the dose is unnecessary for HD patients. In particular, the renal excretion rate of linagliptin is 5%, which is the lowest among the DPP-4 inhibitors^[19]. Therefore, linagliptin is easy to use in patients with renal failure including HD patients. DPP-4 inhibitors interact with dipeptidase-4 in 2 different ways^[20]. The inhibition of DPP-4 by vildagliptin and saxagliptin is a 2-step process entailing the formation of a reversible covalent enzyme-inhibitor complex; this is characterized by slow rates of inhibitor binding and inhibitor dissociation, and results in the enzyme equilibrating slowly between its active and inactive forms^[21,22]. In contrast, the other DPP-4 inhibitors form noncovalent bonds (*i.e.*, hydrogen bonds) with residues present in the catalytic site^[23-25]. Some metabolites of DPP-4 inhibitors have drug activities. For example, 5-hydroxysaxagliptin is a metabolite of saxagliptin and has a half of the activity of the original drug^[26]. Therefore, unchanged substances are not representative of the effects of all drugs (Table 1).

Sitagliptin

The molecular weight of sitagliptin is 523.32 Da, and the administration therapeutic dosage is 25 mg once daily in HD patients. The bioavailability of this medicine is 87%^[27], and the protein-binding rate is 38%^[28]. Hepatic metabolism by CYP3A4 and CYP2C8 is low,

Table 1 Seven dipeptidase-4 inhibitors

Dose and pharmacokinetics	Sitagliptin	Vildagliptin	Alogliptin	Linagliptin	Teneligliptin	Anagliptin	Saxagliptin
Daily dose (mg)	25	50	6.25	5	20	100	2.5
Molecular weight (Da)	523.32	303.4	461.51	472.54	628.86	383.45	333.43
Bioavailability	87%	85%	100%	30%	Unknown	73.2%	Unknown
Protein binding	38.0%	9.3%	28.2%-38.4%	> 80%	77.6%-82.2%	37.1%-48.2%	Negligible
C _{max} (<i>vs</i> healthy volunteer)	1.4 fold	1.4 fold	3.2 fold	1.5 fold	1.0 fold	1.4 fold	Unknown
AUC (<i>vs</i> healthy volunteer)	4.5 fold	2.0 fold	3.8 fold	1.5 fold	1.5 fold	3.2 fold	2.1 fold
t _{1/2} (h) (<i>vs</i> healthy volunteer)	2.2	2	Unknown	Unknown	1.0 fold	0.9 fold	Unknown
Dialyzability	13.50%	3.00%	7.20%	Unknown	15.60%	Unknown	4.00%

C_{max}, AUC and t_{1/2} were measured under different dose conditions. AUC: Area under the curve.

and active metabolites are not produced. Therefore, most sitagliptin is excreted unchanged in the urine (87%) and feces (13%)^[29]. After the administration of sitagliptin 50 mg, compared to cohorts with normal renal function, HD patients had 1.4-fold higher observed plasma maximum concentration (C_{max}) levels, 4.5-fold higher area under the curve (AUC), and a 2.2-fold higher half-life (t_{1/2})^[28,30]. Furthermore, 13.5% of this drug is excreted by 4 h of HD^[31].

Vildagliptin

Vildagliptin has a molecular weight of 303.40 Da and is administered therapeutically at a dosage of 50 mg once daily in HD patients. The bioavailability and protein binding rate are 85%^[32,33] and 9.3%^[33,34], respectively. The mean elimination t_{1/2} after intravenous administration is short (about 2 h), and the amount of renal excretion of unchanged vildagliptin is 23% of the oral administration dose^[34,35]. Relative to cohorts with normal renal function, HD patients who received an administration of vildagliptin 50 mg had 1.4-fold and 2.0-fold higher C_{max} levels and AUC, respectively. Following the administration of vildagliptin 100 mg, HD patients had 2.0-fold higher t_{1/2} as compared to those with normal renal function. Additionally, 3% of this drug is removed by 4 h of HD^[34].

Alogliptin

At a molecular weight of 339.39 Da, alogliptin is administered at a therapeutic dosage of 6.25 mg once daily in HD patients. Its bioavailability is 100%, and the protein-binding rate is 28.2%-38.4%^[36]. In comparison to cohorts with normal renal function, HD patients had 3.2-fold and 3.8-fold higher C_{max} levels and AUC, respectively, following administration of alogliptin 50 mg. The t_{1/2} is unknown in HD patients. Furthermore, 7.2% of this drug is removed by 4 h of HD^[36,37].

Linagliptin

Linagliptin has a molecular weight of 472.54 Da, and the therapeutic dosage is 5 mg once daily in HD patients. Its bioavailability and protein binding rate are 30%^[38,39] and > 80%^[19], respectively. Relative to cohorts with normal renal function, the HD patients

showed 1.5-fold higher C_{max} levels and 1.5-fold higher AUC after the administration of linagliptin 5 mg. The t_{1/2} and dialyzability are unknown in HD patients^[40,41].

Teneligliptin

The molecular weight of teneligliptin is 628.86 Da, and the therapeutic dosage is 20 mg once daily in HD patients. Its bioavailability is unknown, and the protein-binding rate is 77.6%-82.2%^[42]. Compared to cohorts with normal renal function, HD patients had 4.5-fold higher AUC after teneligliptin 20 mg administration. The C_{max} levels and t_{1/2} in HD patients are the same as those in subjects with normal renal function after teneligliptin 20 mg administration. Furthermore, 15.6% of this drug is removed by 4 h of HD^[42].

Anagliptin

Anagliptin has a molecular weight of 383.45 Da, and is administered at a therapeutic dose of 100 mg once daily in HD patients. Its bioavailability and protein-binding rate are 73.2% and 37.1%-48.2%, respectively^[43]. Relative to cohorts with normal renal function, HD patients had 1.4-fold higher C_{max} levels, 3.2-fold higher AUC, and 0.9-fold higher t_{1/2} after anagliptin 400 mg administration^[43]. The dialyzability of this drug in HD patients is unknown.

Saxagliptin

At a molecular weight of 333.43 Da, saxagliptin is administered at a therapeutic dosage of 2.5 mg once daily in HD patients. Its protein-binding rate is negligible^[44]. Compared to cohorts with normal renal function, HD patients had a 2.1-fold higher AUC after saxagliptin 10 mg administration^[45]. The C_{max} levels and t_{1/2} are unknown in HD patients. Furthermore, 4.0% of this drug is removed by 4 h of HD^[45].

COMBINATION THERAPIES OF ORAL ANTIDIABETIC DRUGS WITH DPP-4 INHIBITORS IN HD PATIENTS

At present, 7 types of oral antidiabetic drugs are

Table 2 Combinations of oral antidiabetic drugs with dipeptidase-4 inhibitors

Class	Action mechanism	Glucose target	Drug	Daily dose (mg)
Sulfonylurea	Increases insulin secretion	Fasting and postprandial	Glipizide	2.5-10
			Gliclazide	40-240
			Gliquidone	45-60
Meglitinide	Increases insulin secretion	Postprandial	Repaglinide	0.5-12
Thiazolidinediones	Insulin sensitizer	Fasting and postprandial	Mitiglinide	7.5-15
			Rosiglitazone	4-8
			Pioglitazone	15-45
Alpha-glucosidase inhibitor	Delays carbohydrate absorption	Postprandial	Voglibose	0.6-0.9
			Acarbose	75-300
			Miglitol	150-225

available worldwide: sulfonylureas, meglitinides, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, SGLT2 inhibitors, and DPP-4 inhibitors. Beyond agreement on DPP-4 inhibitors, the guidelines differ with respect to the oral diabetes therapeutic drugs that can be administered to HD patients, and countries vary as to recommendations on oral antidiabetic medicines^[46]. According to the Kidney Disease Outcomes Quality Initiative (KDOQI), glipizide (sulfonylurea), gliclazide (sulfonylurea), repaglinide (meglitinide), and thiazolidinediones can be administered to HD patients. In the guideline of the Japanese Society for Dialysis Therapy, HD patients can take repaglinide (meglitinide), mitiglinide calcium hydrate (meglitinide), and alpha-glucosidase inhibitor. Some reports state that gliquidone can be administered to HD patients^[47]. Many oral diabetes therapeutic drugs induce serious side effects in HD patients. In particular, sulfonylureas, biguanides, and thiazolidinediones can induce hypoglycemia, lactic acidosis, and fluid retention, respectively. Only a few oral antidiabetic drugs can be administered to HD patients before the use of DPP-4 inhibitors. Therefore, the side effects of drugs administered concomitant with DPP-4 inhibitors must be examined in detail (Table 2).

Sulfonylureas

With progressive decreases in kidney function, the clearance of sulfonylureas and their active metabolites decrease, and the $t_{1/2}$ is prolonged^[48-51]. First-generation sulfonylureas should generally be avoided in HD patients because they depend on the kidney to eliminate both the original drug and active metabolites. Accordingly, the $t_{1/2}$ and risk of hypoglycemia increase. According to the KDOQI, glipizide and gliclazide are among the usable second-generation sulfonylureas because they do not produce active metabolites or increase the risk of hypoglycemia in patients with decreased renal function. Glipizide and gliclazide are administered at a dose of 2.5-10 mg/d^[47] and 40-240 mg/d^[47,52], respectively. There are also reports of the administration of gliquidone in HD patients at a more than twice daily dose of 45-60 mg^[47].

Meglitinides

According to the KDOQI and guideline of the Japanese

Society for Dialysis Therapy, repaglinide and mitiglinide calcium hydrate (mitiglinide) can be administered to HD patients. In those with an estimated glomerular filtration rate (eGFR) of < 30 mL/min, repaglinide results in a 4-fold increase in the $t_{1/2}$ after 1 wk following its administration, as well as increase in AUC, in comparison to subjects with no renal failure. However, no changes are seen in the maximal plasma concentration, suggesting chronic kidney disease (CKD) influences both the metabolism and hepatic clearance of this medicine rather than bioavailability^[47,53]. Furthermore, active metabolite concentrations do not increase with repaglinide^[1]. Therefore, there is no relationship between renal failure and risk of hypoglycemia for this medicine in treated patients^[54]. Repaglinide is usually taken preprandially at each meal at a dose of 0.5-12 mg/d^[47,55]. Mitiglinide acts on liver metabolism^[56]; hence, the metabolites of mitiglinide calcium hydrate have no antidiabetic effects. Therefore, the risk of hypoglycemia from this drug is low in HD patients. Accordingly, HD patients can take this drug preprandially at each meal at a dose of 7.5-15 mg/d.

Thiazolidinediones

As rosiglitazone is metabolized in the liver, it is not necessary to reduce its dose in patients with renal failure^[57], since it does not increase the risk of hypoglycemia in CKD patients. Meanwhile, pioglitazone shows similar pharmacokinetic properties between patients with or without CKD because of its high molecular weight, protein-binding competency, and hepatic metabolism, and there is no effect in HD patients^[58,59]. Therefore, it is not necessary to adjust the dose in CKD patients. Pioglitazone is administered once daily at a dose of 15-45 mg. There are no specific data regarding fluid retention in CKD patients. Nevertheless, there is the potential risk of congestive heart failure by fluid overload, particularly in those patients with both renal and cardiac failure.

Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors increase glucagon-like peptide-1 levels and reduce gastric inhibitory polypeptide responses after eating. Therefore, combination therapies

Table 3 Efficacies of dipeptidase-4 inhibitor monotherapies

Ref.	Study duration (mo)	n	DPP-4 inhibitor	Treatment dose (mg)	Parameter (%)	Pre-treatment	Post-treatment	Efficacy
Arjona Ferreira <i>et al</i> ^[9]	12	64	Sitagliptin	25	HbA1c	7.9	7.2	-0.7
Ito <i>et al</i> ^[10]	6	5	Vildagliptin	50	GA	Unknown	Unknown	Unknown
					HbA1c	6.0	5.5	-0.5
Kume <i>et al</i> ^[11]	6	26	Vildagliptin	50	GA	21.8	19.7	-2.1
					HbA1c	Unknown	Unknown	Unknown
Ito <i>et al</i> ^[12]	6	9	Vildagliptin	50 or 100	GA	23.8	21.2	-2.6
					HbA1c	6.7	6.0	-0.7
Nakamura <i>et al</i> ^[13]	24	16	Alogliptin	6.25	GA	24.7	20.1	-4.6
					HbA1c	7.1	5.8	-1.3
Nakamura <i>et al</i> ^[14]	6	21	Linagliptin	5	GA	22.5	19.6	-2.9
					HbA1c	Unknown	Unknown	Unknown
Otsuki <i>et al</i> ^[15]	6	14	Teneligliptin	20	GA	21.3	18.0	-2.3
					HbA1c	6.4	Unknown	-0.3 to -0.8
	7				GA	21.1	Unknown	-1.7 to -2.3

HbA1c: Hemoglobin A1c; GA: Glycated albumin.

with DPP-4 inhibitors may be more effective^[60].

The guideline of the Japanese Society for Dialysis Therapy states that all 3 types of alpha-glucosidase inhibitors can be administered in HD patients. Voglibose is not absorbed in the blood and is orally administered before each meal at 0.6-0.9 mg/d^[61]. The plasma levels of acarbose and its metabolites increase several fold in patients with renal failure. The peak plasma concentration and exposure of this drug in patients with severe renal impairment (eGFR < 25 mL/min) are 5- and 6-fold higher than in patients with normal renal function, respectively^[46,48]. However, only a small amount of acarbose is absorbed, since its bioavailability is very low^[62]. Additionally, its metabolites have very small antidiabetic effects. Acarbose is orally administered before each meal at 75-300 mg/d. Miglitol accumulates with a decrease in renal function. Those patients with an eGFR of < 25 mL/min and taking 75 mg miglitol (25 mg three times a day) have double the plasma exposure as subjects with an eGFR of > 60 mL/min^[46]. However, miglitol has no antidiabetic effects. The molecular weight of miglitol (383.45 Da) is low, and its protein binding rate is < 3.9%. Therefore, miglitol is eliminated by HD treatment. Miglitol is orally administered at 150-300 mg/d, usually before each meal^[47,63].

EFFICACIES OF DPP-4 INHIBITORS IN HD PATIENTS

The effectiveness of DPP-4 inhibitors is summarized in Table 3. DPP-4 inhibitor treatment decreases hemoglobin A1c (HbA1c) and glycated albumin (GA) levels by 0.3%-1.3% and 1.7%-4.9%, respectively. It is difficult to compare the effects of DPP-4 inhibitors, because the strength and selectivity of DPP-4 inhibition are related to their respective therapeutic effects. Moreover, the curative effects of these drugs might be related to ethnicity. For example, some studies report

greater effectiveness of DPP-4 inhibitor treatment in Japanese patients with diabetes based on the evaluation of GA levels^[64-66].

Monotherapy

Eight studies have investigated the diabetes therapeutic effects of DPP-4 inhibitors only in HD patients and not CKD patients. Of these, seven studied the antidiabetic effectiveness of only DPP-4 inhibitor monotherapy (*i.e.*, sitagliptin, vildagliptin, alogliptin, linagliptin, and teneligliptin) (Table 3). However, there have been no studies of anagliptin or saxagliptin monotherapy.

Arjona Ferreira *et al*^[9] investigated the efficacies of sitagliptin monotherapy in 64 diabetic HD patients. All patients were administered sitagliptin 25 mg once daily for the monotherapy research. Forty patients newly started sitagliptin therapy, and 24 switched from other medications. Mean HbA1c and fasting plasma glucose levels decreased from 7.95% to 7.2% and from 159 to 133 mg/dL, respectively, 12 mo after treatment initiation.

Three studies have evaluated vildagliptin monotherapy in HD patients. Of these, 2 studied only vildagliptin monotherapy, while the other was a sub-analysis study in which the patients were categorized into either vildagliptin monotherapy or combination therapy groups. Ito *et al*^[10] investigated the efficacies of vildagliptin monotherapy in 5 diabetic HD patients who were following diet and exercise regimens. All patients were administered vildagliptin 50 mg once daily for the monotherapy research. At 6 mo after treatment, HbA1c and GA levels had decreased from 6.0% ± 0.3% and 21.8% ± 2.6% to 5.5% ± 0.6% and 19.7% ± 3.3%, respectively. Kume *et al*^[11] investigated the efficacies of vildagliptin monotherapy in 26 diabetic HD patients. Sixteen patients newly started sitagliptin therapy, and 7 patients switched from other oral antidiabetic drugs (3 patients were

Table 4 Efficacies of both monotherapies and combination therapies with dipeptidase-4 inhibitors

Ref.	Study duration (mo)	<i>n</i>	DPP-4 inhibitor	Treatment dose (mg)	Combination therapy	Parameter (%)	Pre-treatment	Post-treatment	Efficacy
Ito <i>et al</i> ^[12]	6	30	Vildagliptin	50 or 100	Mitiglinide and/or voglibose	HbA1c	6.7	6.1	-0.6
						GA	24.5	20.5	-4.0
Fujii <i>et al</i> ^[16]	12	30	Alogliptin	6.25	Mitiglinide and/or voglibose	HbA1c	7.2	6.3	-0.9
						GA	25.6	20.7	-4.9
Nowicki <i>et al</i> ^[17]	12	19	Saxagliptin	2.5	Unknown	HbA1c	8.7	7.5	-1.2
						GA	Unknown	Unknown	Unknown

HbA1c: Hemoglobin A1c; GA: Glycated albumin.

unknown). All patients were administered vildagliptin 50 mg once daily for the monotherapy research. Mean GA and postprandial plasma glucose (PPG) levels had decreased from 23.8% to 21.2% and 204 to 157 mg/dL respectively, 6 mo after treatment initiation. In the subanalysis study, all 9 patients were administered an initial dose of vildagliptin 50 mg once daily. Thereafter, if 8 wk of continuous vildagliptin administration did not result in the target HbA1c value (< 7.0%) or GA value (< 21.0%) being achieved, the vildagliptin dose was increased to 100 mg daily from week 8. The HbA1c, GA, and PPG levels showed mean changes of -0.7%, -4.6%, and -54 mg/dL in the monotherapy group^[12].

Nakamura *et al*^[13] investigated the diabetes therapeutic effects of alogliptin and linagliptin monotherapy in HD patients in 2 studies. In the study of alogliptin monotherapy, 16 diabetic HD patients were eligible based on diet and exercise regimens. All patients were administered alogliptin 6.25 mg once daily. At 2 years after treatment initiation, the HbA1c and GA levels had decreased from 7.1% ± 0.2% to 5.8% ± 1.6% and from 22.5% ± 0.7% to 19.6% ± 0.6%^[13]. In the study of linagliptin monotherapy, 21 diabetic HD patients were eligible based on diet and exercise regimens. All patients were administered linagliptin 5 mg once daily. GA levels decreased from 21.3% ± 0.6% to 18.0% ± 0.6% 6 mo after treatment initiation^[14].

Otsuki *et al*^[15] investigated the efficacies of teneligliptin monotherapy in 14 diabetic HD patients. All patients were administered teneligliptin 20 mg once daily. Seven patients newly started teneligliptin therapy, and 7 patients switched from other medications. The mean changes in HbA1c and GA were -0.3% to -0.8% and -1.7% to -2.3% after treatment^[15].

Monotherapy and combination therapies

Two studies have evaluated the combined efficacies of both monotherapy and combination oral diabetic therapy with DPP-4 inhibitors in HD patients. In the evaluation of combination therapy with vildagliptin, 30 HD patients with diabetes were eligible to participate. All patients were administered vildagliptin 50 mg once daily as an initial dose. Nine patients newly started vildagliptin therapy, and 21 patients switched from other medications. Thereafter, if 8 wk of continuous

vildagliptin administration did not result in the target HbA1c value (< 7.0%) or GA value (< 21.0%) being achieved, the vildagliptin dose was increased to 100 mg daily from week 8; this was done in 19 patients. Another 11 patients were administered 50 mg daily. Mean HbA1c, GA, and PPG decreased from 6.7% to 6.1%, 24.5% to 20.5%, and 186 to 140 mg/dL, respectively, 6 mo after treatment initiation^[12].

In the study of combination therapy with alogliptin, 30 HD patients with diabetes were eligible. All patients were administered alogliptin 6.25 mg once daily. Fifteen patients newly started sitagliptin therapy, and 15 patients switched from other medications. Mean HbA1c, GA, and PPG decreased from 7.1% to 6.3%, 25.6% to 20.7%, and 212 to 156 mg/dL, respectively, 12 mo after treatment initiation. When patients were divided into the alogliptin monotherapy and combination (alogliptin plus mitiglinide and/or voglibose) therapy groups (*n* = 15 each), the mean changes in GA and PPG were greater in the monotherapy group (the specific decreases are unclear)^[16].

There is one subanalysis study of DPP-4 inhibitors that included only HD patients from among those with CKD. A total of 19 HD patients with diabetes were eligible. All patients were administered saxagliptin 2.5 mg once daily. The diabetes treatments before saxagliptin therapy were unknown. Mean HbA1c and PPG decreased from 8.75% to 7.5% and 177 to 138 mg/dL, respectively, 12 mo after treatment initiation (the details of monotherapy and combination therapies are unclear)^[17,18] (Table 4).

Glycemic control parameters

Blood glucose is a principal parameter for assessing the effects of diabetes therapy. Blood is collected at 3 time points: PPG, fasting plasma glucose, and the start of HD treatment. Therefore, it is difficult to compare blood glucose levels among studies. GA may more accurately reflect glycemic control, because HbA1c, the more general available parameter, is falsely low in HD patients^[67-69]. This probably results from the shortened survival time of erythrocytes in CKD patients, as well as the reduced time for the glucose-hemoglobin chemical reaction to occur^[70]. Another reason underlying the falsely low HbA1c levels in HD patients is related to the erythropoietin injections and

resultant increase in younger erythrocytes^[71]. GA is a predictor of death, hospitalization, and cardiovascular events in HD patients with diabetes^[72,73].

IMPACTS OF DPP-4 INHIBITORS ON BLOOD GLUCOSE-RELATED FACTORS IN HD PATIENTS

Some studies have investigated the impacts of DPP-4 inhibitors on blood glucose-related factors (*i.e.*, insulin, glucagon, and insulin resistance) in HD patients. In the study of sitagliptin, HOMA-IR increased 12 mo after sitagliptin treatment, while fasting insulin, proinsulin, proinsulin/insulin ratio, and HOMA-IR showed no changes from baseline^[9]. Meanwhile, the study of vildagliptin investigated insulin and C-peptide but observed no significant differences for these parameters from baseline after 6 mo^[11]. The study of alogliptin investigated insulin, C-peptide, and glucagon monthly for 3 mo. However, they were not significantly different before and after alogliptin treatment^[13]. In the study of teneligliptin, C-peptide level at baseline was 4.94 ng/mL and increased significantly to 5.96 ng/mL after 5 mo of treatment^[15]. Three studies assessed active GLP-1 levels before and after DPP-4 inhibitor treatment in HD patients. Samples were taken before the start of HD treatment, and active GLP-1 levels increased 2-3 fold after DPP-4 inhibitor therapy^[11,13,14].

ANTI-INFLAMMATORY EFFECTIVENESS OF DPP-4 INHIBITORS IN HD PATIENTS

Inflammation is an important prognostic factor in HD patients^[74]. Therefore, if DPP-4 inhibitors prevent inflammation and atherosclerosis, they may improve the prognosis of HD patients. However, almost no research has investigated the anti-inflammatory or anti-atherosclerosis efficacies of DPP-4 inhibitors in HD patients. Only 2 reports have assessed the anti-inflammatory efficacies of DPP-4 inhibitors in HD patients. In the study of vildagliptin, interleukin-6 levels decreased after 6 mo, although not significantly^[11]. In the study of linagliptin, PGE2 and interleukin-6 levels decreased significantly^[14]. Four mechanisms have been proposed to underlie the anti-inflammation properties of linagliptin: increased GLP-1^[75,76], DPP-4 (CD26) suppression^[77,78], xanthine-related skeletal structure, and a diabetic therapy effect^[79,80]. Increased GLP-1, DPP-4 suppression, and an antidiabetic effect are the effects that are common among all DPP-4 inhibitors. However, among the 7 DPP-4 inhibitors, linagliptin is the only one with xanthine-related skeletal structure effects. The pharmacological anti-inflammatory mechanisms of xanthine-related skeletal structure are unknown. The meta-analysis of the cardiovascular events with DPP-4 inhibitors examined 73678 patients in a total of 82 randomized controlled

trials including the SAVOR-TIMI 53 and EXAMINE trials^[81-83]. Only linagliptin reduced major adverse cardiovascular events compared to placebo/alternative diabetes therapy. This suggests the anti-inflammatory effectiveness of linagliptin may be involved in its anti-atherosclerotic effects.

SAFETY/TOLERABILITY

Hypoglycemia is the most serious general side effect of diabetes treatment. In clinical trial data, the incidence of hypoglycemic events due to DPP-4 inhibitors ranges from < 0.1%-5%. Meta-analysis of data from clinical trials indicated few hypoglycemic events due to vildagliptin and sitagliptin^[6]. Adverse events other than hypoglycemia due to DPP-4 inhibitors include rash, hives, abdominal fullness, pancreatitis, constipation, headache, giddiness, nasopharyngitis, headache, and upper respiratory tract infection. However, the occurrence of these side reactions is low^[84].

One study reports higher incidences of cellulitis and headache (6.3%) with sitagliptin compared to glipizide^[9]. One patient experienced a drug-related rash^[13], and another experienced constipation^[15]. However, in 8 studies that evaluated the diabetes therapeutic effects of DPP-4 inhibitors in HD patients, no patients showed severe side effects (*e.g.*, hypoglycemia and liver dysfunction). Therefore, adverse events resulting from DPP-4 inhibitor treatments do not occur at a higher incidence than in HD patients.

CONCLUSION

Treating HD patients with DPP-4 inhibitors does not result in an increased incidence of adverse events. Furthermore, DPP-4 inhibitors are strongly anticipated to be effective in HD patients with diabetes. Moreover, drugs with anti-inflammatory and anti-atherosclerotic effects are attractive options for HD patients, whose prognosis is associated with inflammation and atherosclerosis. DPP-4 inhibitors are key drugs that are part of new treatment strategies for HD patients with diabetes, whose choices for diabetes treatment are limited. A once-weekly oral DPP-4 inhibitor, SYR-472^[85], which could reduce the number of required administrations, might be approved in the future. Therefore, the number of treatment options for HD for diabetic patients is anticipated to increase.

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Diabetes mellitus: The epidemic of the century

Akram T Kharroubi, Hisham M Darwish

Akram T Kharroubi, Department of Medical Laboratory Sciences, Faculty of Health Professions, Al-Quds University, Jerusalem 91000, Palestine

Hisham M Darwish, Department of Biochemistry, Faculty of Medicine, Al-Quds University, Jerusalem 91000, Palestine

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Correspondence to: Akram T Kharroubi, PhD, Associate Professor of Biochemistry and Endocrinology, **Dean** of Faculty of Health Professions, Department of Medical Laboratory Sciences, Faculty of Health Professions, Al-Quds University, P.O. Box 51000, Abed Elhamaid Shoman Street, Beit Hanina-Jerusalem, Jerusalem 91000, Palestine. akram.kharroubi@gmail.com
Telephone: +972-2-2791243
Fax: +972-2-2791243

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Abstract

The epidemic nature of diabetes mellitus in different regions is reviewed. The Middle East and North Africa

region has the highest prevalence of diabetes in adults (10.9%) whereas, the Western Pacific region has the highest number of adults diagnosed with diabetes and has countries with the highest prevalence of diabetes (37.5%). Different classes of diabetes mellitus, type 1, type 2, gestational diabetes and other types of diabetes mellitus are compared in terms of diagnostic criteria, etiology and genetics. The molecular genetics of diabetes received extensive attention in recent years by many prominent investigators and research groups in the biomedical field. A large array of mutations and single nucleotide polymorphisms in genes that play a role in the various steps and pathways involved in glucose metabolism and the development, control and function of pancreatic cells at various levels are reviewed. The major advances in the molecular understanding of diabetes in relation to the different types of diabetes in comparison to the previous understanding in this field are briefly reviewed here. Despite the accumulation of extensive data at the molecular and cellular levels, the mechanism of diabetes development and complications are still not fully understood. Definitely, more extensive research is needed in this field that will eventually reflect on the ultimate objective to improve diagnoses, therapy and minimize the chance of chronic complications development.

Key words: Diabetes; Classification of diabetes; Type 1 diabetes; Type 2 diabetes; Gestational diabetes; Diagnosis; Etiology; Genetics

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Core tip: Diabetes mellitus is rising to an alarming epidemic level. Early diagnosis of diabetes and prediabetes is essential using recommended hemoglobin A1c criteria for different types except for gestational diabetes. Screening for diabetes especially in underdeveloped countries is essential to reduce late diagnosis. Diabetes development involves the interaction between genetic and non-genetic factors. Biomedical research continues

to provide new insights in our understanding of the mechanism of diabetes development that is reviewed here. Recent studies may provide tools for the use of several genes as targets for risk assessment, therapeutic strategies and prediction of complications.

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DEFINITION OF DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome^[1-3].

CLASSIFICATION OF DIABETES MELLITUS

Although classification of diabetes is important and has implications for the treatment strategies, this is not an easy task and many patients do not easily fit into a single class especially younger adults^[1,4-6] and 10% of those initially classified may require revision^[7]. The classical classification of diabetes as proposed by the American Diabetes Association (ADA) in 1997 as type 1, type 2, other types, and gestational diabetes mellitus (GDM) is still the most accepted classification and adopted by ADA^[1]. Wilkin^[8] proposed the accelerator hypothesis that argues "type 1 and type 2 diabetes are the same disorder of insulin resistance set against different genetic backgrounds"^[9]. The difference between the two types relies on the tempo, the faster tempo reflecting the more susceptible genotype and earlier presentation in which obesity, and therefore, insulin resistance, is the center of the hypothesis. Other

predictors of type 1 diabetes include increased height growth velocity^[10,11] and impaired glucose sensitivity of β cells^[12]. The implications of increased free radicals, oxidative stress, and many metabolic stressors in the development, pathogenesis and complications of diabetes mellitus^[13-18] are very strong and well documented despite the inconsistency of the clinical trials using antioxidants in the treatment regimens of diabetes^[19-21]. The female hormone 17- β estradiol acting through the estrogen receptor- α (ER- α) is essential for the development and preservation of pancreatic β cell function since it was clearly demonstrated that induced oxidative stress leads to β -cell destruction in ER- α knockout mouse. The ER- α receptor activity protects pancreatic islets against glucolipotoxicity and therefore prevents β -cell dysfunction^[22].

TYPE 1 DIABETES MELLITUS

Autoimmune type 1 diabetes

This type of diabetes constitutes 5%-10% of subjects diagnosed with diabetes^[23] and is due to destruction of β cells of the pancreas^[24,25]. Type 1 diabetes accounts for 80%-90% of diabetes in children and adolescents^[2,26]. According to International Diabetes Federation (IDF), the number of youth (0-14 years) diagnosed with type 1 diabetes worldwide in 2013 was 497100 (Table 1) and the number of newly diagnosed cases per year was 78900^[27]. These figures do not represent the total number of type 1 diabetes patients because of the high prevalence of type 1 diabetes in adolescence and adults above 14 years of age. One reported estimate of type 1 diabetes in the United States in 2010 was 3 million^[28,29]. The number of youth in the United States younger than 20 years with type 1 diabetes was estimated to be 166984 in the year 2009^[30]. The prevalence of type 1 diabetes in the world is not known but in the United States in youth younger than 20 years was 1.93 per 1000 in 2009 (0.35-2.55 in different ethnic groups) with 2.6%-2.7% relative annual increase^[26,31]. Type 1 diabetes is mainly due to an autoimmune destruction of the pancreatic β cells through T-cell mediated inflammatory response (insulinitis) as well as a humoral (B cell) response^[25]. The presence of autoantibodies against the pancreatic islet cells is the hallmark of type 1 diabetes, even though the role of these antibodies in the pathogenesis of the disease is not clear. These autoantibodies include islet cell autoantibodies, and autoantibodies to insulin (IAA), glutamic acid decarboxylase (GAD, GAD65), protein tyrosine phosphatase (IA2 and IA2 β) and zinc transporter protein (ZnT8A)^[32]. These pancreatic autoantibodies are characteristics of type 1 diabetes and could be detected in the serum of these patients months or years before the onset of the disease^[33]. Autoimmune type 1 diabetes has strong HLA associations, with linkage to *DR* and *DQ* genes. HLA-DR/DQ alleles can be either predisposing or protective^[1]. This autoimmune type 1 diabetes is

Table 1 Number of subjects with type 1 diabetes in children (0-14 years), with diabetes in adults (20-79 years) and with hyperglycemia (type 2 or gestational diabetes) in pregnancy (20-49 years)

Region	Type 1 diabetes in children (0-14 yr)		Diabetes in adults (20-79 yr)				Hyperglycemia in pregnancy (20-49 yr)	
	2013		2013		2035		2013	
	Number in thousands	Newly diagnosed in thousands	Number in millions	Comparative prevalence	Number in millions	Comparative prevalence	Cases in live births in millions	Comparative prevalence
Africa	39.1	6.4	19.8	5.7%	41.5	6.0%	4.6	14.4%
Europe	129.4	20.0	56.3	6.8%	68.9	7.1%	1.7	12.6%
Middle East and North Africa	64.0	10.7	34.6	10.9%	67.9	11.3%	3.4	17.5%
North America and Caribbean	108.6	16.7	36.8	9.6%	50.4	9.9%	0.9	10.4%
South and Central America	45.6	7.3	24.1	8.2%	38.5	8.2%	0.9	11.4%
South East Asia	77.9	12.5	72.1	8.7%	123.0	9.4%	6.3	25.0%
Western Pacific	32.5	5.3	138.2	8.1%	201.8	8.4%	3.7	11.9%
World	497.1	78.9	381.8	8.3%	592.0	8.8%	21.4	14.8%

Data extracted from International Diabetes Federation Diabetes Atlas, 6th ed, 2013.

characterized by the absence of insulin secretion and is more dominant in children and adolescents.

In addition to the importance of genetic predisposition in type 1 diabetes, several environmental factors have been implicated in the etiology of the disease^[9,33]. Viral factors include congenital rubella^[34,35], viral infection with enterovirus, rotavirus, herpes virus, cytomegalovirus, endogenous retrovirus^[36,37] and Ljungan virus. Other factors include low vitamin D levels^[38], prenatal exposure to pollutants, improved hygiene and living conditions decreased childhood infections in countries with high socioeconomic status leading to increased autoimmune diseases (hygiene hypothesis), early infant nutrition such as using cow's milk formula instead of breast feeding^[39] in addition to insulin resistance in early childhood due to obesity or increased height growth velocity. The role of environmental factors remains controversial^[40]. Recent evidence supported the causative effect of viral infections in diabetes^[41-43].

Type 1 diabetes often develops suddenly and can produce symptoms such as polydipsia, polyuria, enuresis, lack of energy, extreme tiredness, polyphagia, sudden weight loss, slow-healing wounds, recurrent infections and blurred vision^[27] with severe dehydration and diabetic ketoacidosis in children and adolescents. The symptoms are more severe in children compared to adults. These autoimmune type 1 diabetes patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia^[1]. The complete dependence on insulin of type 1 diabetes patients may be interrupted by a honeymoon phase which lasts weeks to months or in some cases 2-3 years. In some children, the requirement for insulin therapy may drop to a point where insulin therapy could be withdrawn temporarily without detectable hyperglycemia^[44].

Idiopathic type 1 diabetes

A rare form of type 1 diabetes of unknown origin (idiopathic), less severe than autoimmune type 1 diabetes and is not due to autoimmunity has been reported. Most patients with this type are of African or Asian descent and suffer from varying degrees of insulin deficiency and episodic ketoacidosis^[45].

Fulminant type 1 diabetes

This is a distinct form of type 1 diabetes, first described in the year 2000, and has some common features with idiopathic type 1 diabetes being non-immune mediated^[46,47]. It is characterized by ketoacidosis soon after the onset of hyperglycemia, high glucose levels (≥ 288 mg/dL) with undetectable levels of serum C-peptide, an indicator of endogenous insulin secretion^[48]. It has been described mainly in East Asian countries and accounted for approximately 20% of acute-onset type 1 diabetes patients in Japan (5000-7000 cases) with an extremely rapid and almost complete beta-cell destruction resulting in nearly no residual insulin secretion^[48,49]. Both genetic and environmental factors, especially viral infection, have been implicated in the disease. Anti-viral immune response may trigger the destruction of pancreatic beta cells through the accelerated immune reaction with no detectable autoantibodies against pancreatic beta cells^[48,50]. Association of fulminant type 1 diabetes with pregnancy has also been reported^[51].

TYPE 2 DIABETES MELLITUS

The global prevalence of diabetes in adults (20-79 years old) according to a report published in 2013 by the IDF was 8.3% (382 million people), with 14 million more men than women (198 million men vs 184 million women), the majority between the ages 40 and 59 years and the number is expected to rise beyond 592 million by 2035 with a 10.1% global prevalence.

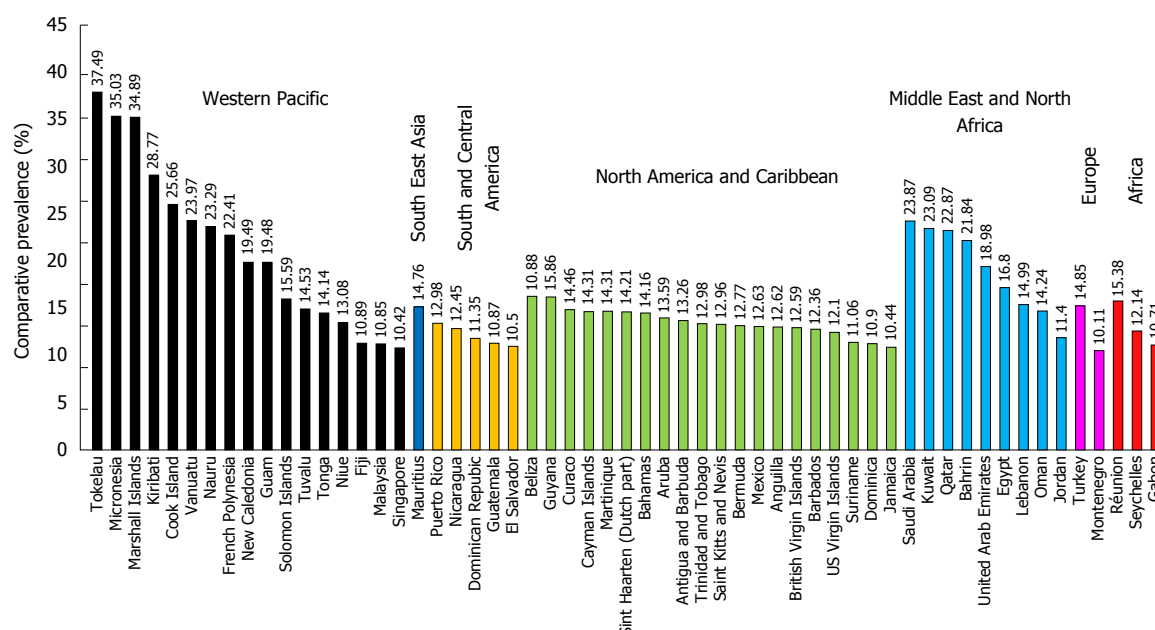


Figure 1 Comparative prevalence of diabetes in adults (20-79 years) in countries with high prevalence ($\geq 10\%$). Data extracted from International Diabetes Federation Diabetes Atlas, 6th ed, 2013.

With 175 million cases still undiagnosed, the number of people currently suffering from diabetes exceeds half a billion. An additional 21 million women are diagnosed with hyperglycemia during pregnancy. The Middle East and North Africa region has the highest prevalence of diabetes (10.9%), however, Western Pacific region has the highest number of adults diagnosed with diabetes (138.2 millions) and has also countries with the highest prevalence (Figure 1)^[27]. Low- and middle-income countries encompass 80% of the cases, “where the epidemic is gathering pace at alarming rates”^[27]. Despite the fact that adult diabetes patients are mainly type 2 patients, it is not clear whether the reported 382 million adults diagnosed with diabetes also include type 1 diabetes patients.

More than 90%-95% of diabetes patients belong to this type and most of these patients are adults. The number of youth (less than 20 years) with type 2 diabetes in the United States in the year 2009 was 0.46 in 1000 and accounted for approximately 20% of type 2 diabetes in youth^[26]. The increased incidence of type 2 diabetes in youth is mainly due to the change in the lifestyle of the children in terms of more sedentary life and less healthy food. Obesity is the major reason behind insulin resistance which is mainly responsible for type 2 diabetes^[52-54]. The ADA recommends screening of overweight children and adolescence to detect type 2 diabetes^[55,56]. The prevalence of obesity in children is on the rise^[6] which is probably the main reason for the increased incidence of type 2 diabetes in the young (30.3% overall increase in type 2 diabetes in children and adolescence between 2001 and 2009)^[26].

Insulin resistance in type 2 diabetes patients increases the demand for insulin in insulin-target

tissues. In addition to insulin resistance, the increased demand for insulin could not be met by the pancreatic β cells due to defects in the function of these cells^[18]. On the contrary, insulin secretion decreases with the increased demand for insulin by time due to the gradual destruction of β cells^[57] that could transform some of type 2 diabetes patients from being independent to become dependent on insulin. Most type 2 diabetes patients are not dependent on insulin where insulin secretion continues and insulin depletion rarely occurs. Dependence on insulin is one of the major differences from type 1 diabetes. Other differences include the absence of ketoacidosis in most patients of type 2 diabetes and autoimmune destruction of β cells does not occur. Both type 1 and type 2 diabetes have genetic predisposition, however, it is stronger in type 2 but the genes are more characterized in type 1 (the *TCF7L2* gene is strongly associated with type 2 diabetes)^[58]. Due to the mild symptoms of type 2 diabetes in the beginning, its diagnosis is usually delayed for years especially in countries where regular checkup without symptoms is not part of the culture. This delay in diagnosis could increase the incidence of long-term complications in type 2 diabetes patients since hyperglycemia is not treated during this undiagnosed period.

In addition to diabetes, insulin resistance has many manifestations that include obesity, nephropathy, essential hypertension, dyslipidemia (hypertriglyceridemia, low HDL, decreased LDL particle diameter, enhanced postprandial lipemia and remnant lipoprotein accumulation), ovarian hyperandrogenism and premature adrenarche, non-alcoholic fatty liver disease and systemic inflammation^[6,54]. The presence of type 2 diabetes in children and adolescence who are

not obese^[59-61], the occasional severe dehydration and the presence of ketoacidosis in some pediatric patients with type 2 diabetes^[55] had led to the misclassification of type 2 to type 1 diabetes.

Some patients with many features of type 2 diabetes have some type 1 characteristics including the presence of islet cell autoantibodies or autoantibodies to GAD65 are classified as a distinct type of diabetes called latent autoimmune diabetes in adults (LADA)^[62]. People diagnosed with LADA do not require insulin treatment. In a recent study, Hawa *et al.*^[63] reported 7.1% of European patients with type 2 diabetes with a mean age of 62 years, tested positive for GAD autoantibodies and the prevalence of LADA was higher in patients diagnosed with diabetes at a younger age. This classification of LADA as a distinct type of diabetes is still controversial^[6,64-66].

Insulin resistance and signaling

Defects in the insulin-dependent substrate proteins IRS-1 and IRS-2 mediated signaling pathway are implicated in the development of metabolic disorders, mainly diabetes. This pathway mediates the cellular response to insulin and involves a large array of insulin-stimulated protein kinases including the serine/threonine kinase AKT and protein kinase C (PKC) that phosphorylate a large number of Ser/Thr residues in the insulin receptor substrate (IRS) proteins involved in the metabolic response to insulin^[67]. In addition, other non-insulin dependent kinases including the AMP-activated protein kinase, c-Jun N-terminal protein kinase and G protein-coupled receptor kinase 2 that are activated under various conditions can phosphorylate the two insulin responsive substrates^[67-71]. Disruption in the AKT and PKC kinases is central to the development of diabetes^[72] and is associated with all major features of the disease including hyperinsulinemia, dyslipidemia and insulin resistance^[73]. Replacing the wild type IRS-1 with a mutant version of the protein having alanine instead of tyrosine in three locations using genetic knock-in approach provided evidence to the central role of IRS-1 phosphorylation in the development of insulin resistance^[74]. Using a similar approach to generate IRS-1 mutant with a single mutation involving a specific tyrosine residue, confirmed the role of IRS-1 phosphorylation in the development of insulin resistance pathogenesis^[75]. The large cumulative evidence indicates a complex array of factors including environmental factors^[76] and a wide range of cellular disturbances in glucose and lipid metabolism in various tissues^[77] contribute to the development of insulin resistance. This condition generates complex cellular metabolic changes in a variety of tissues, mainly liver and muscles, that include the inability of the liver to transport and dispose glucose, control glucose production *via* gluconeogenesis, impaired storage of glucose as glycogen, *de novo* lipogenesis and

hypertriglyceridemia^[77]. Among the factors implicated in the development of insulin resistance, obesity is the most predominant risk factor leading to insulin insensitivity and diabetes which involves several mechanisms that participate in the pathogenesis of the disease^[78]. Obesity-induced insulin resistance is directly linked to increased nutrient flux and energy accumulation in tissues that directly affect cell responsiveness to insulin^[77]. However, it seems that other insulin-independent mechanisms are involved in the overall metabolic disturbances of glucose homeostasis and diabetes including activities in extra-hepatic tissues in addition to the central role of liver.

OTHER TYPES OF DIABETES MELLITUS

Monogenic diabetes

Characterization of the genetic etiology of diabetes enables more appropriate treatment, better prognosis, and counseling^[79]. Monogenic diabetes is due to a genetic defect in single genes in pancreatic β cells which results in disruption of β cell function or a reduction in the number of β cells. Conventionally, monogenic diabetes is classified according to the age of onset as neonatal diabetes before the age of six months or Maturity Onset Diabetes of the Young (MODY) before the age of 25 years. However, certain familial defects are manifested in neonatal diabetes, MODY or adult onset diabetes^[2,9,80]. Others believe that classification of diabetes as MODY and neonatal diabetes is obsolete and monogenic diabetes is currently used relating specific genetic etiologies with their specific treatment implications^[79]. Beta cell differentiation depends on the expression of the homeodomain transcription factor PDX1 where mutation in the gene results in early onset diabetes (MODY) and its expression decreases before the onset of diabetes^[81]. The angiopoietin-like protein 8 (ANGPTL8) may represent a potential "betatrophin" that acts to promote the proliferation of beta cells, however, studies using mice lacking the ANGPTL8 active gene or overexpressed protein indicated that it did not seem to play a role in beta cells proliferation^[82].

Mitochondrial diabetes is due to a point mutation in the mitochondrial DNA associated with deafness and maternal transmission of the mutant DNA can result in maternally-inherited diabetes^[1,83].

Mutations that result in mutant insulin or the inability to convert proinsulin to insulin result in glucose intolerance in some of these cases. Genetic defects in the insulin receptor or in the signal transduction pathway of insulin have been demonstrated to result in hyperinsulinemia and modest hyperglycemia to severe diabetes^[1].

Disease of the exocrine pancreas

Damage of the β cells of the pancreas due to diffused injury of the pancreas can cause diabetes. This damage

could be due to pancreatic carcinoma, pancreatitis, infection, pancreatectomy, and trauma^[1]. Atrophy of the exocrine pancreas leads to progressive loss of the β cells^[84]. Accumulation of fat in the pancreas or pancreatic steatosis could lead to diabetes due to decreased insulin secretion but may require a long time before the damage to β cells occurs^[85]. In most cases, extensive damage of the pancreas is required before diabetes occurs and the exocrine function of the pancreas is decreased in these patients^[86]. Cirrhosis in cystic fibrosis may contribute to insulin resistance and diabetes^[2].

Hormones and drugs

Diabetes has been found in patients with endocrine diseases that secrete excess hormones like growth hormone, glucocorticoids, glucagon and epinephrine in certain endocrinopathies like acromegaly, Cushing's syndrome, glucagonoma, and pheochromocytoma, respectively^[1]. Some of these hormones are used as drugs such as glucocorticoids to suppress the immune system and in chemotherapy and growth hormone to treat children with stunted growth.

Genetic syndromes

Diabetes has been detected in patients with various genetic syndromes such as Down syndrome, Klinefelter syndrome, Turner syndrome and Wolfram syndrome^[1].

PREDIABETES

Individuals with prediabetes do not meet the criteria of having diabetes but are at high risk to develop type 2 diabetes in the future. According to the ADA Expert Committee, individuals are defined to have prediabetes if they have either impaired fasting plasma glucose (IFG) levels between 100-125 mg/dL (5.6-6.9 mmol/L) or impaired glucose tolerance test (IGT) with 2-h plasma glucose levels in the oral glucose tolerance test (OGTT) of 140-199 mg/dL (7.8-11.0 mmol/L). The World Health Organization (WHO) still adopts the range for IFG from 110-125 mg/dL (6.1-6.9 mmol/L). Prediabetes has been shown to correlate with increased cardiovascular mortality^[87,88] and cancer^[89]. The definition of prediabetes with the indicated cut off values is misleading since lower levels of glucose in the normal range are still correlated with cardiovascular disease in a continuous glycemic risk perspective^[90]. In accordance with the recommendation of the ADA in 2009 to use hemoglobin A1c (HbA1c) to diagnose diabetes, ADA also recommended the use of an HbA1c (5.7%-6.4%) to diagnose prediabetes^[91]. The number of people with IGT according to IDF was 316 million in 2013 (global prevalence 6.9% in adults) and is expected to rise to 471 million in 2030^[27]. According to a report in 2014 by the Center for Disease Control and Prevention, 86 million Americans (1 out of 3) have prediabetes^[92]. Four of the top ten countries with the

highest prevalence of prediabetes are in the Middle East Arab States of the Gulf (Kuwait, Qatar, UAE and Bahrain with prevalence of 17.9%, 17.1%, 16.6% and 16.3%, respectively)^[27]. The number of people diagnosed with prediabetes is different according to the method and criteria used to diagnose prediabetes. The number of people with prediabetes defined by IFG 100-125 mg/dL is 4-5 folds higher than those diagnosed using the WHO criteria of 110-125 mg/dL^[93]. Diabetes and prediabetes diagnosed using an HbA1c criteria give different estimates compared to methods using FPG or OGTT. Higher percentages of prediabetes were diagnosed using HbA1c compared to FPG^[94-96]. Prediabetes is associated with metabolic syndrome and obesity (especially abdominal or visceral obesity), dyslipidemia with high triglycerides and/or low HDL cholesterol, and hypertension^[97]. Not all individuals with prediabetes develop diabetes in the future, exercise with a reduction of weight 5%-10% reduces the risk of developing diabetes considerably (40%-70%)^[98]. Individuals with an HbA1c of 6.0%-6.5% have twice the risk of developing diabetes (25%-50%) in five years compared to those with an HbA1c of 5.5%-6.0%^[99].

DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS

Diabetes mellitus is diagnosed using either the estimation of plasma glucose (FPG or OGTT) or HbA1c. Estimation of the cut off values for glucose and HbA1c is based on the association of FPG or HbA1c with retinopathy. Fasting plasma glucose of ≥ 126 mg/dL (7.0 mmol/L), plasma glucose after 2-h OGTT ≥ 200 mg/dL (11.1 mmol/L), HbA1c $\geq 6.5\%$ (48 mmol/mol) or a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) along with symptoms of hyperglycemia is diagnostic of diabetes mellitus. In addition to monitor the treatment of diabetes, HbA1c has been recommended to diagnose diabetes by the International Expert Committee in 2009^[100] and endorsed by ADA^[101], the Endocrine Society, the WHO^[102] and many scientists and related organizations all over the world. The advantages and disadvantages of the different tests used to diagnose diabetes have been reviewed by Sacks *et al.*^[103]. The advantages of using HbA1c over FPG to diagnose diabetes include greater convenience and preanalytical stability, lower CV (3.6%) compared to FPG (5.7%) and 2h OGTT (16.6%), stronger correlation with microvascular complications especially retinopathy, and a marker for glycemic control and glycation of proteins which is the direct link between diagnosis of diabetes and its complications^[104-109]. It is recommended to repeat the HbA1c test in asymptomatic patients within two weeks to reaffirm a single apparently diagnostic result^[110].

A cut off value for HbA1c of $\geq 6.5\%$ (48 mmol/mol) has been endorsed by many countries and dif-

ferent ethnic groups, yet ethnicity seems to affect the cut off values to diagnose diabetes^[111,112]. Cut-off values of 5.5% (37 mmol/mol)^[113] and 6.5% (48 mmol/mol)^[114] have been reported in a Japanese study, 6.0% (42 mmol/mol) in the National Health and Nutrition Examination Survey (NHANES III), 6.2% (44 mmol/mol) in a Pima Indian study, 6.3% (45 mmol/mol) in an Egyptian study as reported by Davidson^[105]; and three cut-off values for Chinese^[112]. The Australians recommended the use of two cut-off values: $\leq 5.5\%$ to “rule-out” and $\geq 7.0\%$ to “rule-in” diabetes^[115]. Variations in the prevalence of diabetes^[94,116-119] and prediabetes^[120] due to ethnicity have been documented. Most studies diagnosed less subjects with diabetes using HbA1c compared to FPG or OGTT^[121-123]. Yet, other studies reported more subjects diagnosed with diabetes using HbA1c^[96,124-126].

GESTATIONAL DIABETES

Hyperglycemia in pregnancy whether in the form of type 2 diabetes diagnosed before or during pregnancy or in the form gestational diabetes has an increased risk of adverse maternal, fetal and neonatal outcome. Mothers with gestational diabetes and babies born to such mothers have increased risk of developing diabetes later in life. Hyperglycemia in pregnancy is responsible for the increased risk for macrosomia (birth weight ≥ 4.5 kg), large for gestational age births, preeclampsia, preterm birth and cesarean delivery due to large babies^[127]. Risk factors for gestational diabetes include obesity, personal history of gestational diabetes, family history of diabetes, maternal age, polycystic ovary syndrome, sedentary life, and exposure to toxic factors^[3].

Diagnosis of type 2 diabetes before or during pregnancy is based on criteria mentioned before. Fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L) or 2-h plasma glucose ≥ 200 mg/dL (11.1 mmol/L) after a 75 g oral glucose load. However, gestational diabetes has been diagnosed at 24-28 wk of gestation in women not previously diagnosed with diabetes using two approaches: the first approach is based on the “one-step” International Association of the Diabetes and Pregnancy Study Groups (IADPSG) consensus^[128] and recently adopted by WHO^[129]. Gestational diabetes is diagnosed using this method by FPG ≥ 92 mg/dL (5.1 mmol/L), 1-h plasma glucose after a 75 g glucose load ≥ 180 mg/dL (10.0 mmol/L) or 2-h plasma glucose after a 75 g glucose load ≥ 153 mg/dL (8.5 mmol/L). This criteria is derived from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study^[127] even though the HAPO study showed a continuous relationship between hyperglycemia and adverse short-term pregnancy outcome with no threshold reported^[130]. The second approach is used in the United States and is based on the “two-step” NIH consensus^[131]. In the first step 1-h plasma glucose after a 50 g glucose load under nonfasting state \geq

140 mg/dL (7.8 mmol/L) is followed by a second step under fasting conditions after a 100 g glucose load for those who screened abnormal in the first step. The diagnosis of gestational diabetes is made when at least two of the four plasma glucose levels are met. The four plasma glucose levels according to Carpenter/Coustan criteria are: FPG ≥ 95 mg/dL (5.3 mmol/L); 1-h ≥ 180 mg/dL (10.0 mmol/L); 2-h ≥ 155 mg/dL (8.6 mmol/L); and 3-h ≥ 140 mg/dL (7.8 mmol/L)^[1].

The use IADPSG criteria in comparison with the Carpenter/Coustan criteria was associated with a 3.5-fold increase in GDM prevalence as well as significant improvements in pregnancy outcomes, and was cost-effective^[132]. In another retrospective cohort study of women diagnosed with gestational diabetes, Ethridge *et al*^[133] have shown that newborns of women diagnosed with gestational diabetes by IADPSG approach have greater measures of fetal overgrowth compared with Carpenter-Coustan “two-step” approach neonates. A strategy of using fasting plasma glucose as a screening test and to determine the need for OGTT is valid^[134,135]. According to Sacks^[136], correlation of glucose concentrations and the risk of subsequent complications will eventually lead to universal guidelines.

The use of ADA/WHO cut off value of HbA1c $\geq 6.5\%$ (48 mmol/mol) to diagnose gestational diabetes is not recommended by the “one step” IADPSG criteria or the “two-step” NIH criteria. Further investigation is required in light of recent reports on HbA1c in combination with OGTT and its usefulness to predict adverse effect of gestational diabetes or obviate the use OGTT in all women with gestational diabetes^[137-141].

DIABETES AND GENETICS

Diabetes is a complex disease that involves a wide range of genetic and environmental factors. Over the past several years, many studies have focused on the elucidation of the wide spectrum of genes that played a role in the molecular mechanism of diabetes development^[142-144]. However, despite the vast flow of genetic information including the identification of many gene mutations and a large array of single nucleotide polymorphisms (SNPs) in many genes involved in the metabolic pathways that affect blood glucose levels, the exact genetic mechanism of diabetes remains elusive^[145,146]. Evidently, a major complication is the fact that a single gene mutation or polymorphism will not impose the same effect among different individuals within a population or different populations. This variation is directly or indirectly affected by the overall genetic background at the individual, family or population levels that are potentially further complicated by interaction with highly variable environmental modifier factors^[147,148].

Molecular genetics and type 2 diabetes

One of the major focuses of biomedical research is to delineate the collective and broad genetic variants in the

human genome that are involved in the development of diabetes. This major effort will potentially provide the necessary information to understand the molecular genetics of the different forms of diabetes including type 1, type 2 and monogenic neonatal diabetes among individuals of all populations and ethnic groups. Despite the fact that linkage and association studies allowed the identification and characterization of many candidate genes that are associated with type 2 diabetes^[144,149,150], however, not all of these genes showed consistent and reproducible association with the disease^[151]. Genome wide association studies (GWAS) in various populations identified 70 loci associated with type 2 diabetes and revealed positive linkage of many mutations and SNPs that influence the expression and physiological impact of the related proteins and risk to develop type 2 diabetes. One study involved several thousand type 2 diabetes patients and control subjects from the United Kingdom allowed the identification of several diabetes putative loci positioned in and around the *CDKAL1*, *CDKN2A/B*, *HHEX/IDE* and *SLC30A8* genes in addition to the contribution of a large number of other genetic variants that are involved in the development of the disease^[152]. Two similar studies from the Finns and Swedish populations and the United States resulted in the identification of similar single nucleotide variants^[153] that are linked to the risk of acquiring type 2 diabetes^[154,155]. The study in the United States population included in addition to type 2 diabetes, the association of the identified SNPs with the level of triglycerides in the tested subjects^[155]. These SNPs are located near several candidate genes including *IGFBP2* and *CDKAL1* and other genes in addition to several other variants that are located near or in genes firmly associated with the risk of acquiring type 2 diabetes. Other GWAS analysis studies were performed in the Chinese, Malays, and Asian-Indian populations which are distinct from the European and United States populations in addition to meta-analysis of data from other populations in the region revealed relevant findings among patients with European ancestry^[156]. The results of the combined analysis showed significant association of SNPs in the *CDKAL1*, *CDKN2A/B*, *HHEX*, *KCNQ1* and *SLC30A8* genes after adjustment with gender and body mass index. More recently, meta-analysis of GWAS data involving African American type 2 diabetes patients identified similar loci to the previous studies with the addition of two novel loci, HLA-B and INS-IGF^[157]. These results provide strong evidence of common genetic determinants including common specific genes that are linked to diabetes. A small list of specific genetic markers seem strongly associated with the risk of developing type 2 diabetes including the *TCF7L2*^[158] and *CAPN10*^[159,160] genes which also play a significant role in the risk and pathogenesis of the disease^[158,159]. The association of *TCF7L2* gene variants with type 2 diabetes and its mechanism of action received special attention by several investigators^[161,162]. Over expression of

the protein was shown to decrease the sensitivity of beta islet cells to secrete insulin^[163,164] and was more precisely involved in the regulation of secretory granule fusion that constitute a late event in insulin secretion pathway^[165]. The role of *TCF7L2* in insulin secretion was partially clarified^[166] that involves modifying the effect of incretins on insulin secretion by lowering the sensitivity of beta cells to incretins. Several other genes have been found to be significantly associated with the risk of developing type 2 diabetes including a specific SNP in a hematopoietically-expressed homeobox (*HHEX*) gene^[167]. The islet zinc transporter protein (*SLC30A8*)^[168] showed positive correlation with the risk of developing type 2 diabetes where variant mutations in this gene seem protective against the disease which provides a potential tool for therapy^[169]. More recently, a low frequency variant of the *HNF1A* identified by whole exome sequencing was associated with the risk of developing type 2 diabetes among the Latino population and potentially may serve as a screening tool^[170]. Genetic variants and specific combined polymorphisms in the interleukin and related genes including interleukin-6 (*IL-6*), tumor necrosis factor- α and *IL-10* genes were found to be associated with greater risk of developing type 2 diabetes^[171], in addition to genetic variants in the genes for *IL12B*, *IL23R* and *IL23A* genes^[172]. In a study involving the hormone sensitive lipase responsible for lipolysis in adipose tissues, a deletion null mutation, which resulted in the absence of the protein from adipocytes, was reported to be associated with diabetes^[173]. Nine specific rare variants in the peroxisome proliferator-activated receptor gamma (*PPARG*) gene that resulted in loss of the function of the protein in adipocytes differentiation, were significantly associated with the risk of developing type 2 diabetes^[174]. In addition, certain SNPs in the alpha 2A adrenergic receptor (*ADRA2A*) gene, involved in the sympathetic nervous system control of insulin secretion and lipolysis, were found to be associated with obesity and type 2 diabetes^[175]. Link analysis between the melatonin MT2 receptor (*MTNR1B*) gene, a G-protein coupled receptor, identified 14 mutant variants from 40 known variants revealed by exome sequencing, to be positively linked with type 2 diabetes^[176]. The authors suggested that mutations in the *MT2* gene could provide a tool with other related genes in modifying therapy for type 2 diabetes patients based on their specific genetic background to formulate personalized therapies which potentially may ensures the optimum response. Interestingly, mutations in the clock^[177,178] and *Bmal1*^[179] transcription factor genes which are involved in beta cells biological clock affecting growth, survival and synaptic vesicle assembly in these cells, resulted in reduced insulin secretion and diabetes. Evidently, prominent metabolic functions involve the production of specific reactive metabolites, leading to oxidative stress, which affect lipids, proteins and other biological compounds leading to serious damage in

various tissues and organs. Mutations and SNPs in the antioxidant genes, including superoxide dismutase, catalase and glutathione peroxidase, that decrease their activity are implicated in the risk and pathogenesis of type 2 diabetes^[180]. The metabolic syndrome was shown to be associated with the development of type 2 diabetes in a population that is described as highly endogenous especially in individuals over 45 years of age^[181]. Since consanguinity marriages is high in this population, screening for this syndrome among families could provide an informative marker on the risk of developing type 2 diabetes^[181].

Molecular genetics of type 1 diabetes

Even though type 1 diabetes is basically described as an autoimmune disease that results in the destruction of pancreatic beta cells, however, single gene mutations and SNPs have been found to be associated with the susceptibility to this type of diabetes. Initially, two gene mutations were linked to the development of type 1 diabetes including the autoimmune regulator (*AIRE*) gene which affect the immune tolerance to self antigens leading to autoimmunity^[182] and the *FOXP3* gene which results in defective regulatory T cells^[183]. In addition, a mutation in the histone deacetylase *SIRT1* gene predominantly expressed in beta cells involved in the regulation of insulin secretion^[184] and played a role in modulating the sensitivity of peripheral tissues to insulin^[185] was detected in type 1 diabetes patients^[186]. Recently, additional mutations and SNPs in the *CTLA-4 +49A/G* and *HLA-DQB1* and *INS* gene VNTR alleles were found to be associated with type 1 diabetes, which have the advantage of differentiating between Latent autoimmune type 1 diabetes and type 2 diabetes^[187]. The *HLA-DQB1*, in combination with *HLA-DR* alleles and a polymorphism in *PTPN22* gene seem to be associated with the age onset of late type 1 diabetes^[188,189]. Two specific polymorphisms in the promoter region of a transmembrane protein (*DC-SIGN*) gene expressed in macrophages and played an important role of T- cell activation and inflammation were found to be protective against type 1 diabetes^[190]. An innovative non-parametric SNP enrichment tool using summary GWAS DATA allowed the identification of association between several transcription factors and type 1 diabetes and are located in a type 1 diabetes susceptibility region^[191]. Nine SNP variants in several genes associated with type 1 diabetes, not including the major histocompatibility gene region, were identified using extensive GWAS analysis^[192]. Furthermore, several novel SNPs in a region in chromosome 16 located in the *CLEC16A* gene were shown to be associated with type 1 diabetes and seem to function through the reduced expression of *DEX1* in B lymphoblastoid cells^[193]. Since more than 40 regions in the human genome were identified to be associated with the susceptibility to type 1 diabetes^[194-196], a weighted risk model was developed utilizing selected

genes SNPs could be used for testing infants for these genetic markers that could provide insights in the susceptibility to type 1 diabetes development or safe prevention of the disease among young children^[197].

Molecular genetics of monogenic diabetes

A large array of genes were identified to be involved in the development of monogenic diabetes^[80] which represent about 2%-5% of diabetes patients. Monogenic diabetes results primarily from gene defects that lead to a decrease in beta cell number or function. Monogenic diabetes genes were identified using linkage studies or code for proteins that directly affected glucose homeostasis. The majority of genes responsible for monogenetic diabetes code for either transcription factors that participate in the control of nuclear gene expression or proteins that are located on the cell membrane, cytoplasm and endoplasmic reticulum, proteins involved in insulin synthesis and secretion, exocrine pancreatic proteins and autoimmune diabetes proteins^[80]. The collective function of these proteins is their participation in glucose metabolism at different levels. Evidently, the hierarchy of a specific gene in the overall glucose metabolism pathway determines the onset of diabetes in the patient and whether it is neonatally expressed or have late onset expression (adulthood). Consequently, molecular defects in the structure and function of these genes lead to the disturbance of plasma glucose level, the primary pathological sign of diabetes. The molecular mechanism of permanent neonatal diabetes mellitus (PNMP) in addition to *MODY* explains the observed phenotype of monogenetic diabetes that involves loss of function of the expressed mutant protein. The first gene implicated in monogenic diabetes was the glucokinase (*GCK*) gene^[198] which functions as a pancreatic sensor for blood glucose where more than 70 mutations in the gene were identified that affected its activity^[199]. A recent study on *GCK* gene mutations causing neonatal and childhood diabetes showed that the majority of mutations resulted in the loss of the enzyme function primarily due to protein instability^[148,150]. Two hepatocytes nuclear factor genes that code for the *HNF4A* and *HNF1A* transcription factors were closely associated with *MODY1* and *MODY2*^[148,149]. Definitely, a whole list of other genes involved in monogenic diabetes are either overlooked or included in the genetic determinants of type 1 and type 2 diabetes which will be identified and clarified through more careful future studies.

MOLECULAR GENETICS OF DIABETES COMPLICATIONS

In addition to the genetic determinants of diabetes, several gene mutations and polymorphisms have been associated with the clinical complications of diabetes. The cumulative data on diabetes patients with a

variety of micro- and macrovascular complications support the presence of strong genetic factors involved in the development of various complications^[200]. A list of genes have been reported that are associated with diabetes complications including *ACE* and *AKR1B1* in nephropathy, *VEGF* and *AKRB1* in retinopathy and *ADIPOQ* and *GLUL* in cardiovascular diseases^[200]. A study on Chinese patients revealed a single SNP in the promoter region of the smooth muscle actin (*ACTA2*) gene correlates with the degree of coronary artery stenosis in type 2 diabetes patients^[201]. Furthermore, the alpha kinase 1 gene (*ALPK1*) identified as a susceptibility gene for chronic kidney disease by GWAS^[202], was demonstrated in type 2 diabetes patients^[203]. Three additional genes have been strongly correlated with this risk of diabetic retinopathy (DR) including the vascular endothelial growth receptor, aldose reductase and the receptor for advanced glycation products genes^[204] where specific polymorphisms in these genes seem to increase the risk of DR development in diabetes patients^[204]. A significant differential proteome (involving 56 out of 252 proteins) is evident that characterizes vitreous samples obtained from diabetes patients with the complication in comparison to diabetes patients without the complication and control individuals^[205]. Interestingly, a large portion of these proteins (30 proteins) belong to the kallikrein-kinin, coagulation and complement systems including complement C3, complement factor 1, prothrombin, alpha-1-antitrypsin and antithrombin III that are elevated in diabetic patients with retinopathy^[205]. In addition, 2 single nucleotides polymorphisms in the human related *B7-I* gene seem to mediate podocyte injury in diabetic nephropathy^[206]. Furthermore, increased concentration of the ligand of B7-1 correlates with the progression of end-stage renal disease (ESRD) in diabetes patients^[206]. These results indicate that B7-I inhibition may serve as a potential target for diabetes nephropathy prevention and/or treatment. Recently, it was shown that direct correlation is evident between circulating levels of tumor necrosis factors 1 and 2 and increased risk of ESRD in American Indian patients^[207]. The link between diabetes and proper bone development and health is evident. Studies using animal models with major significant reduction in insulin receptor (IR) in osteoprogenitor cells resulted in thin and rod-like weak bones with high risk of fractures^[208]. Similar findings were observed in animal models with bone-specific IR knockdown animals which points to the central role of IR in the proper development of bones^[208]. Type 2 diabetes is also associated with mitochondrial dysfunction in adipose tissues. Using knockout animal models of specific mitochondrial genes led to significant reduction in key electron transport complexes expression and eventually adipocytes death^[209]. These animals exhibited Insulin resistance in addition to other complications that can potentially lead to cardiovascular disease^[209].

CONCLUSION

Diabetes mellitus is the epidemic of the century and without effective diagnostic methods at an early stage, diabetes will continue to rise. This review focuses on the types of diabetes and the effective diagnostic methods and criteria to be used for diagnosis of diabetes and prediabetes. Evidently, diabetes is a complex disease with a large pool of genes that are involved in its development. The precise identification of the genetic bases of diabetes potentially provides an essential tool to improve diagnoses, therapy (more towards individualized patient targeted therapy) and better effective genetic counseling. Furthermore, our advanced knowledge of the association between medical genetics and the chronic complications of diabetes, will provide an additional advantage to delay or eradicate these complications that impose an immense pressure on patient's quality of life and the significantly rising cost of health-care services.

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Effect of diabetes mellitus on sleep quality

Salim Surani, Veronica Brito, Asif Surani, Shekhar Ghamande

Salim Surani, Veronica Brito, Asif Surani, Shekhar Ghamande, Department of Medicine, Section of Pulmonary, Critical Care and Sleep Medicine, Texas AM University, TX 78336, United States

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Correspondence to: Salim Surani, MD, Associate Professor, Department of Medicine, Section of Pulmonary, Critical Care and Sleep Medicine, Texas AM University, 1177 West Wheeler Ave, Suite 1, TX 78336, United States. srsurani@hotmail.com
 Telephone: +1-361-8857722
 Fax: +1-361-8507562

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Abstract

Diabetes mellitus (DM) is a highly prevalent condition affecting about 347 million people worldwide. In addition to its numerous clinical implications, DM also exerts a negative effect on patient's sleep quality.

Impaired sleep quality disrupts the adequate glycemic control regarded as corner stone in DM management and also lead to many deleterious effects causing a profound impact on health related quality of life. This article outlines various factors leading to impaired sleep quality among diabetics and delineates how individual factor influences sleep. The article also discusses potential interventions and lifestyle changes to promote healthy sleep among diabetics.

Key words: Diabetes mellitus; Sleep quality; Quality of life; Obstructive sleep apnea; Nocturnal hypoglycemia

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Core tip: Diabetes mellitus (DM) is one of the common chronic medical conditions affecting approximately 347 million people worldwide. Studies have shown that up to one third of patients with DM suffer from concomitant sleep disorder. Factors associated with disrupted sleep among diabetic patient include nocturia, nocturnal hypoglycemia, peripheral neuropathy, restless leg syndrome and sleep disordered breathing. These conditions, when associated with diabetes can cause fragmented sleep and poor quality of life. It is imperative for the primary care physicians and health care providers to address this important issue among the diabetic patients during their visit.

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INTRODUCTION

Sleep is a prerequisite for healthy functioning of human mind and body. It is generated based on a circadian rhythm and homeostatic pressure that follows a period

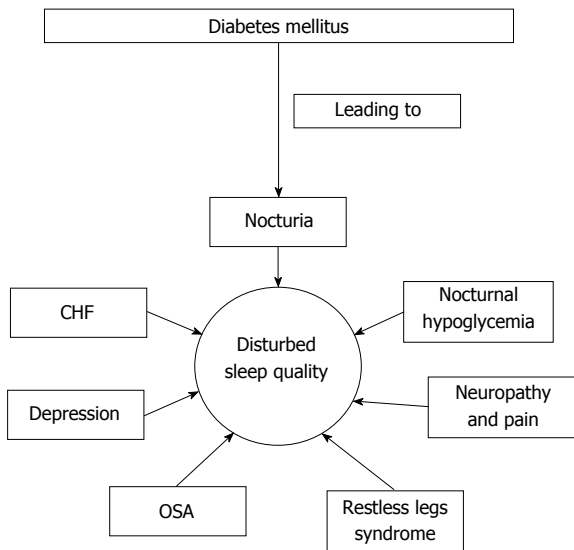


Figure 1 Illustration how different conditions associated with diabetes mellitus affects the sleep quality. OSA: Obstructive sleep apnea; CHF: Congestive heart failure.

of wakefulness. Poor or impaired sleep has not only being associated with various diseases and conditions but also led to poor performance and occupational accidents. Medical literature regarding sleep sciences reveals numerous studies that characterize sleep quality and determine various factors leading to impaired sleep among different patient populations^[1-6]. Based on findings from these studies, it is inferred that in addition to many behavioral factors including poor sleep habits and hygiene, various pathological conditions have been recognized that adversely affect the sleep. Most notable among these conditions are obstructive sleep apnea, chronic insomnia and restless legs syndrome.

Diabetes mellitus (DM) is being increasingly recognized as a significant health burden. Based on recent data published by World Health Organization (WHO), it is estimated that approximately 347 million people are suffering from diabetes worldwide, 90% of who have type 2 diabetes. Patients with DM, by virtue of its numerous clinical and associated implications, suffer a poor quality of life^[7,8]. It is not surprising that sleep quality among these patients are significantly impaired. Patients with DM can experience challenges to their sleep and wakefulness due to physiological imbalance and co-morbid sleep pathologies. In this review we attempted to present several factors, which directly affect the sleep quality among patients with DM (Figure 1).

DIABETES AND SLEEP

The significance of good sleep cannot be over emphasized when it comes to chronic medicinal condition like DM. Poor sleep quality, apart from its usual effect of daytime sleepiness, has ramifications that

affect every aspects of life. The pertinent ones are: exacerbation of seizures, short-term memory deficits, long-term cognitive effects and headache. These, when combine to “already worsened” quality of life in patients with chronic diseases, can have several deleterious consequences in an individual’s life.

Clinical research has shown that up to one third of patients with DM suffered from concomitant sleep disorders, as compared with 8.2% of controls without DM^[9]. In another study, more than half of the patients with type 2 DM are likely to report being “poor sleepers”, according to a research poll conducted at University of Pittsburgh. The patients with type 2 DM were more likely to have low Pittsburgh Sleep Quality index (PSQI)^[10]. (PSQI is a validated tool which measures sleep quality and pattern in older adults. It discriminate poor sleepers from normal by assessing seven components of sleep over a one month time interval)^[10]. Part of the components of these index’s metrics are common insomnia variables, such as sleep latency and efficiency. The same study also showed that the sleep quality correlated well with other diabetic quality of life scores^[11]. In general, patients with other chronic medical conditions are also more likely to experience insomnia. In a large community-base sample of 3282 adults, polysomnographic data was analyzed along with self-reported sleep habits and current health. The adjusted odds ratio for insomnia was 1.4 in patients with DM, compared with people without the disorder. Even when data obtained on polysomnography was adjusted for age, gender and apnea-hypopnea index, patients with diabetes mellitus and insomnia had lower sleep efficiency than those without the disease^[12]. Sleep deprivation and sleep fragmentation has been shown to correlate with insulin resistance in obese individuals^[13]. Poor sleep quality has implications on diabetes self-management. Adult diabetic patients, who reported poor quality sleep underwent completion of questionnaires measuring diabetes control, sleep quality and daytime sleepiness. The patients with poor sleep were found to have difficulty with diabetes control, other demographic factors such as education, age, gender, were measured and these were not found to be associated with diabetes control problems^[14]. Studies have linked poor sleep and insomnia to be associated with decrease in gamma-aminobutyric acid (GABA). A lower level of GABA is also seen in the patients with depression^[15]. GABA is produced in significant levels in pancreas. It has also been shown to be inhibiting apoptosis of the rodent beta cells. The glutamate decarboxylase the primary enzyme (GAD) involved in the synthesis of GABA has been linked with type 1 DM^[16,17]. It is possible that GABA is one of the neurotransmitter involved in the sleep quality among diabetics, when in low levels. Orexins also has been associated with sleep, arousal, energy balance and feeding. Orexins have been implicated in the metabolism of glucose.

Its expression is inhibited by obesity, obstructive sleep apnea and depression^[18]. The pathogenetic link between sleep deprivation and DM has been further elucidated in a recent cross over study involving fourteen healthy subjects who underwent 4 h sleep restriction. This sub-chronic sleep restriction led to a reduction in peripheral insulin sensitivity but interestingly the hepatic insulin sensitivity did not decrease. There was no significant reduction in slow wave sleep associated with it. There was a modest increase in cortisol and catecholamines. This increases lipolysis. Fasting non-esterified fatty acids and β -OH butyrate levels were elevated indicating an increase in lipolysis and hepatic fatty acid oxidation. The resting energy expenditure was unchanged but the respiratory quotient decreased consistent with fat oxidation. The elevated non-esterified fatty acids may modulate hepatic metabolism. Thus the insulin resistance is driven by extrahepatic tissue^[19].

NOCTURIA

Nocturia is defined by waking up at night to void and is considered clinically meaningful if it occurs two or more times per night. Patients report nocturia as a leading cause of sleep disturbance, affecting both sleep onset and maintenance^[20]. Polyuria, defined as 24-h urine output of more than 2800 mL (or above 40 mL/kg per 24 h)^[21]. Polyuria may cause nocturia through generally increased urine production wherein nocturnal urine output exceeds functional bladder capacity (FBC). However, polyuria and nocturnal polyuria are not mutually inclusive.

Nocturia in persons with DM can occur in association with polyuria, and the mechanism is solute diuresis, but can also be associated with frequently comorbid sleep-breathing disorder, such as obstructive sleep apnea. It has been postulated that in patients with obstructive sleep apnea, the negative intrathoracic pressure and stretching of the myocardium releases atrial natriuretic peptide (ANP). This, in turn, causes vasodilatation and inhibits aldosterone resulting in excess sodium and water excretion. When a sleep disorder is suspected, for example in patients with complaints of snoring, gasping for breath when sleeping, or with daytime sleepiness, a nocturnal polysomnography should be performed^[22]. In a study conducted in Australia with 74 patients with type 2 DM, nocturia was correlated with sleep maintenance difficulties^[23].

NOCTURNAL HYPOGLYCEMIA

Nocturnal hypoglycemia can lead to sleep disruption and can be regarded as one of the many factors leading to poor sleep quality among diabetics. Longer intervals between self-monitoring glycemia are generally seen during the night and this period is

associated with the highest sensitivity to insulin. Studies observing continuous glucose monitoring have shown that in patients with diabetes mellitus type 1 spent on average of 2.3 h per day with glucose levels below 70 mg/dL, and most of the hypoglycemic values occur at night^[24]. These findings were corroborated by another study with same principles of continuous monitoring, showing that in more than half of the nights studied, nocturnal hypoglycemia occurred^[25]. Moreover, various insulin regimens can also predispose a diabetic patient to nocturnal hypoglycemia. In a study comparing bedtime insulin NPH (neutral protamine hagedorn) and bedtime insulin glargine, the incidence of nocturnal hypoglycemia was statistically significant with bedtime insulin NPH (24% vs 9.9%)^[26].

RESTLESS LEGS SYNDROME

Restless legs syndrome is a sensorimotor-related sleep disorder and can be either primary or secondary, when related to other conditions such as anemia, pregnancy, or end-stage renal disease, among others. The International Restless Syndrome Study Group (IRLSSG) has published four essential criteria to diagnose patient with restless leg syndrome. It includes: Presence of an urge to move the legs the legs accompanied by an unpleasant sensation (creeping, crawling, painful, itching or jittering feeling in the legs), relieved by movement, aggravated by rest (*i.e.*, when patients lie down to attempt to fall asleep) and worsening of symptoms in the evening or at night^[27]. Other body parts such as the upper extremities can also be involved. Females suffer from RLS twice as much as their male counterparts across all different populations and ages^[28]. There is evidence linking an increased risk for RLS in patients with diabetes mellitus^[29,30]. Several diabetic patients with RLS also suffer from peripheral neuropathy^[29]. Diabetic patients who suffer from RLS are more likely to report worse sleep quality, have longer sleep latency and worse sleep efficiency, and experience more daytime dysfunction compared with diabetic controls without RLS^[31]. RLS can be treated with non-pharmacologic methods which include implementing lifestyle changes such as regular physical activity, restriction on all caffeinated food and drinks, using pneumatic compression devices applied to the thigh and leg during sleep, massage therapy, soaking the legs in warm water^[32]. Pharmacotherapy can be achieved with dopamine agonists, such as pramipexole and ropinirole, anticonvulsants, such as gabapentin, opiates, benzodiazepines, minerals and vitamin supplementation, particularly oral iron in patients with low serum ferritin levels below 50 μ g/L^[32]. Several pharmacological agents have been implicated in worsening symptoms of RLS. Selective serotonin receptor inhibitors, antihistamines, dopamine agonists and sympathomimetic agents are common drug associated with RLS^[33]. These include antihistamines,

dopamine antagonist, sympathomimetic agents, caffeine, nicotine and alcohol^[33]. Attempt should be made in avoiding the medications, which can worsen the RLS, and in turn impair the sleep quality in those patients.

SLEEP DISORDERED BREATHING

Obstructive sleep apnea (OSA) is a complex disorder characterized by recurrent episodes of pharyngeal obstruction during sleep, leading to repeated episodes of intermittent hypoxia, arousals from sleep, which in turn can lead to sleep fragmentation, decreased total sleep time and daytime hypersomnolence^[34]. Obesity is a risk factor for both obstructive sleep apnea and insulin resistance. Foster *et al* found that in a population comprising patients with obesity and type 2 DM in whom the impact of lifestyle changes were being studied, obstructive sleep apnea was highly prevalent, with only 13.4% of patients not having some degree of OSA on a home-based portable monitoring sleep test. Among obese adults with type 2 diabetes, half the patients had moderate or severe OSA (apnea-hypopnea index above 15 events per hour)^[35]. The interactions between obstructive sleep apnea and insulin resistance and glucose intolerance mellitus are being investigated. It has been postulated that there could be a causal effect for the development of insulin resistance in patients with chronic intermittent hypoxia (as it occurs during episodes of obstructive apneas and hypopneas). Possible mechanisms implicated include higher levels of cytokines in patients with OSA (plasma IL-6 and TNF- α), increase in sympathetic neural traffic and the release of gluco-regulatory neuroendocrine hormones such as cortisol^[36]. In a murine model of obese animals, chronic intermittent hypoxemia exacerbated fasting hyperglycemia, glucose intolerance and insulin resistance^[37]. A more recent study of patients with poorly controlled DM (HbA1C \geq 7%) revealed that intermittent hypoxia which is a consequence of sleep apnea, is frequent in these patients (37.2%). Furthermore, it was strongly associated with poor glycemic control (OR: 2.31, 95%CI: 1.06-5.04)^[38]. However, it is still unclear whether OSA may lead to the development of DM over time and more studies are needed to evaluate this possible causal relationship.

DIABETES AND HEART FAILURE

The lifetime risk of cardiovascular diseases in individuals with DM is high, ranging from 55%-79%. The risk is further accentuated with increasing adiposity^[39]. In a study conducted by Nicholas GA and colleagues, it was estimated that 11.8% subjects with baseline diabetes developed heart failure compared 7.7% without DM^[40]. Diabetic patients developing heart failure experiences disturbed sleep by several mechanisms including: Sleep disordered breathing

Cheyne Stokes breathing and Obstructive sleep apnea, effects of medicines, symptom of heart failure (dyspnea, orthopnea and paroxysmal nocturnal dyspnea) and health problems including pain and depression^[41].

The treatment of impaired sleep quality and sleep fragmentation should involve the education of the patients regarding the sleep hygiene techniques, behavioral modification, cognitive behavioral therapy and pharmacological therapies. The detail discussion of which is beyond the scope of this manuscript. In addition the specific etiologies, which are specific for the sleep fragmentation, poor sleep quality and insomnia among diabetics as nocturia, pain related to diabetic neuropathy, restless leg syndrome, obstructive sleep apnea, heart failure and nocturnal hypoglycemia should be addressed and treated.

CONCLUSION

DM is one of the most common diseases worldwide. DM, in addition to causing direct sleep disturbances as a result of nocturia, polyuria, diabetic neuropathy and neuropathy pain, has also been associated with several chronic illness as obstructive sleep apnea, cardiovascular complications, hypertension, cerebrovascular accidents and depression which can impair sleep and quality of life. The patient may not bring the sleep issues during their visit to healthcare providers, with acute issues taking precedence during their visit. It is important for the health care providers treating the patient with DM to address their sleep issues and the impaired quality of life due to inadequate and fragmented sleep, as it may be severely affect their recovery, control of diabetes as well as quality of life. Sleep education should also be considered an essential part in the diabetic management armamentarium.

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Alphabet Strategy for diabetes care: A multi-professional, evidence-based, outcome-directed approach to management

James D Lee, Ponnusamy Saravanan, Vinod Patel

James D Lee, Vinod Patel, Diabetes and Endocrinology Centre, George Eliot Hospital NHS Trust, CV10 7DJ Nuneaton, United Kingdom

Ponnusamy Saravanan, Vinod Patel, Warwick Medical School, University of Warwick, CV4 7AL Coventry, United Kingdom

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Correspondence to: Dr. Vinod Patel, Diabetes and Endocrinology Centre, George Eliot Hospital NHS Trust, College Street, CV10 7DJ Nuneaton, Warwickshire, United Kingdom. vinod.patel@warwick.ac.uk
Telephone: +44-24-76865212
Fax: +44-24-76865409

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Abstract

With the rising global prevalence in diabetes, healthcare

systems are facing a growing challenge to provide efficient and effective diabetes care management in the face of spiralling treatment costs. Diabetes is a major cause of premature mortality and associated with devastating complications especially if managed poorly. Although diabetes care is improving in England and Wales, recent audit data suggests care remains imperfect with wide geographical variations in quality. Diabetes care is expensive with a sizeable amount of available expenditure used for treating the complications of diabetes. A target driven, long-term, multifactorial intervention in patients with type 2 diabetes has been shown to reduce mortality and morbidity. The alphabet strategy is a novel approach to effective diabetes care provision, aiming to address patient education and empowerment, provide consistent comprehensive care delivered in a timely fashion, and allowing multidisciplinary team work.

Key words: Alphabet strategy; Diabetes care management; Checklist; Multifactorial intervention; Chronic disease management

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Core tip: The alphabet strategy is a novel approach to effective diabetes care provision, using a checklist approach to delivering multifactorial intervention. The aim is to address patient education and empowerment, provide consistent comprehensive care delivered in a timely fashion, and allow multidisciplinary team work. In this article, we demonstrate evidence for its clinical effectiveness.

Lee JD, Saravanan P, Patel V. Alphabet Strategy for diabetes care: A multi-professional, evidence-based, outcome-directed approach to management. *World J Diabetes* 2015; 6(6): 874-879 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i6/874.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i6.874>

INTRODUCTION

The increasing global prevalence of diabetes has been described by many as an current epidemic^[1]. The causes are complex but they are largely due to adverse lifestyle factors such as obesity and physical inactivity, as well as poverty. Although no country will be immune from the epidemic, most of the increase is expected to occur in low and middle income countries that are poorly set-up to manage the crisis^[2]. Diabetes care is expensive, with current direct costs estimated to be £9.8 billion in the United Kingdom, or approximately ten percent of the total healthcare expenditure. Treatment of complications account for 80% of this expenditure^[3].

No short-term cure exists for diabetes. Patients need to work closely with multidisciplinary teams to control risk factors in order to prevent or delay the advent of recognised complications. However diabetes care management remains imperfect with substantial variations in care quality. Data from the National Diabetes Audit from England and Wales found that on average under two-thirds of people received all eight recommended healthcare checks, with a range of performance from 18% to 78%^[4]. Such variation in routine healthcare performance also exists at a geographical level. For target care process achievement, only an average of 61% and 60% of subjects achieved their blood pressure and cholesterol goals respectively. Internationally, our own Global Alphabet Strategy Implementation Audit project across 45 single centres in 28 countries demonstrated considerable variations in care quality closely linked to each country's economic prosperity and healthcare spend^[5].

Our objective from the outset was to develop a diabetes strategy that would address the variation in care ensuring "simple things are done right all the time", promoting a consistent approach to management^[6]. It would involve the participation of patients in their own care, especially for their education and empowerment in disease management issues. The strategy had to be applicable in all clinical settings, allowing multidisciplinary teamwork across primary and secondary care interfaces. Finally, it had to be evidence-based, and simple to use and recall for both healthcare professionals and patients.

THE ALPHABET STRATEGY

Our framework is called the alphabet strategy, a mnemonic-based checklist incorporating the core components for comprehensive diabetes care^[7]. Its elements consist of: (1) Advice, specifically on avoidance of smoking, encouraging regular physical activity and judicious dietary choices leading to optimal weight attainment, and individualised recommendations such as influenza vaccination; (2) Blood pressure, with targets guided by co-morbidities; (3)

Cholesterol measurement, with targets determined by co-morbidities - Creatinine/microalbuminuria evaluation; (4) Diabetes glucose control, with target HbA1c individualised according to co-morbidities and aiming for avoidance of hypoglycaemia; (5) Eye exam, performed yearly, with prompt referral for intervention as clinically indicated; (6) Foot exam, conducted at least yearly, with prompt appropriate referral as indicated; and (7) Guardian drugs: opportune use of aspirin, ACE inhibitors or angiotensin receptor blockers, and statins protective against cardiovascular disease and other diabetes complications.

A substantial diabetes evidence base exists for each element of the checklist^[8-11]. Overall, the use of a multifactorial targeted intervention such as that used in the Steno-2 study resulted in significant reductions in macrovascular and microvascular complications as well as cardiovascular mortality^[12].

The aim of this paper is to outline the evidence-base and the potential for use of the alphabet strategy in clinical practice. We hope that this will lead to a reduction in diabetes complications and provide an education strategy for both patients and healthcare professionals in diabetes care.

CLINICAL IMPACT - PRACTICE OF EVIDENCE-BASED MEDICINE 1 AUDIT

To determine the clinical impact of the alphabet strategy in the care of our patients with type 2 diabetes, pre and post checklist implementation audits were conducted on over 400 consecutive patients attending our diabetes outpatient clinic^[13].

The average age of our cohort was 58 years, with mean duration of diabetes being 6 years. 54% were male. Ethnically, 87% were White Caucasian, 11% of South Asian (Indo-Asian) origin, and the remainder being of African-Caribbean. The average follow-up period between the two audits was 5 years.

Use of alphabet strategy resulted in significant improvements in average blood pressure, mean total and HDL cholesterol, performance of eye and foot examinations, and uptake of guardian drugs. Ninety-seven percent of subjects on lipid lowering agents in the post implementation audit were on statins. Significant deterioration was seen in glycaemic control over the mean 5 year follow-up, which can be partly explained by the effect of progressive ageing on glycaemic control^[8]. When adjusted for duration of diabetes, an improvement in HbA1c was seen. No significant change was seen in the number of smokers (Table 1).

CONTINUED BENEFITS - PRACTICE OF EVIDENCE-BASED MEDICINE 2

A repeat audit of subjects with T2DM attending the clinic was performed two years later to determine if the

Table 1 Effect of the Alphabet Strategy on change in achievement of target care processes

Alphabet strategy	Pre implementation	Post implementation	P value
A Smoking (%)	18.2 (77)	15.7 (66)	NS
B Blood pressure (mmHg)	146/82	140/76	< 0.0001
C Total cholesterol (mmol/L)	5.7	4.9	< 0.0001
HDL cholesterol (mmol/L)	1.1	1.39	< 0.001
D HbA1c (%)	7.9	8.3	< 0.0001
E Eye examination (%)	85.0	95.5	< 0.001
F Foot examination (%)	69.8	83.5	< 0.001
G Aspirin (%)	29.0	83.5	< 0.001
ACEI/ARB (%)	32.0	73.0	< 0.001
Lipid lowering (%)	16.8	55.0	< 0.001

NS: Not significant.

Table 2 Comparison of achievement of Alphabet Strategy components between practice of evidence-based medicine audits

Alphabet strategy	POEM 1 n = 420	POEM 2 n = 1071	P value
A Smoking status (%)	15.5	14.7	0.83
B Blood pressure (mmHg)	141/77	136/76	0.007
C Total cholesterol (mmol/L)	4.9	4.5	< 0.001
LDL cholesterol (mmol/L)	2.5	2.4	< 0.001
Creatinine (mmol/L)	109	105	0.036
D HbA1c (%)	8.3	7.9	0.09
E Eye examination (%)	95.5	97.1	0.72
F Foot examination (%)	83.5	97.3	< 0.001
G Aspirin (%)	83.5	88.0	0.20
ACEI/ARB (%)	73.0	74.4	0.75
Lipid lowering (%)	55.0	73.4	< 0.001

POEM: Practice of evidence-based medicine.

use of the AS continued to provide meaningful clinical benefits. Data on over 1000 subjects was collected^[14].

Performance of each essential care process according to the alphabet strategy was over 92%. Comparison of target care process achievement with the original Practice Of Evidence-based Medicine (POEM) audit is shown in Table 2. Improvements in all AS measured components were seen (Table 1).

ACHIEVING CLINICAL TRIAL STANDARDS IN ROUTINE PRACTICE

The clinical outcomes delivered by the alphabet strategy are comparable to those achieved in published landmark studies^[15]. Blood pressure, glycaemic and cholesterol targets in the Steno-2 study, The United Kingdom Prospective Diabetes Study (UKPDS), and the POEM audits are shown in Table 3. Table 4 shows

Table 3 Clinical trial standards of United Kingdom Prospective Diabetes Study and Steno-2 compared to those in practice of evidence-based medicine studies

Variable	Steno-2 (intensive arm)	UKPDS	Alphabet strategy	
			POEM	POEM2004
Systolic BP (mmHg)	≤ 130	≤ 144	≤ 140	≤ 130
Diastolic BP (mmHg)	≤ 80	≤ 82	≤ 80	≤ 80
HbA1c (%)	≤ 6.5	≤ 7	≤ 7	≤ 7
Cholesterol (mmol/L)	≤ 4.5	NA	≤ 5	≤ 4

POEM: Practice of evidence-based medicine; UKPDS: United Kingdom Prospective Diabetes Study.

Table 4 Percentage of practice of evidence-based medicine cohort attaining trial standards compared to original study treatment arms

	Intensive arm	POEM 1 post implementation	POEM 2	P value (intensive vs POEM 2)
Steno-2				
SBP ≤ 130 mmHg	45%	34%	36%	0.07
DBP ≤ 80 mmHg	70%	67%	67%	0.51
TC ≤ 4.5 mmol/L	72%	36%	54%	< 0.001
HbA1c ≤ 6.5%	15%	13%	15%	1.000
Aspirin	73%	83%	88%	< 0.001
Statin	71%	52%	69%	0.66
ACEI	66%	66%	58%	0.09
ARB	39%	10%	20%	< 0.001
Either ACEI/ARB	58%	73%	74%	0.001
UKPDS				
SBP ≤ 144 mmHg	50%	58%	73%	< 0.001
DBP ≤ 82 mmHg	50%	73%	74%	< 0.001
HbA1c ≤ 7%	50%	22%	29%	< 0.001

POEM: Practice of evidence-based medicine; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; UKPDS: United Kingdom Prospective Diabetes Study.

the proportion of people achieving these targets in the original studies compared to those in the POEM audits.

The percentage of POEM subjects reaching trial targets was comparable to those in the UKPDS and Steno-2 for the majority of categories. Significantly less people reached the total cholesterol target compared to Steno-2, but in a re-audit in 2013, 75% of our subjects achieved this goal. There were fewer people on ARBs, but individuals using either ACEI or ARB were higher than for Steno-2.

Almost three quarters of the POEM 2 population reached the UKPDS blood pressure target, but achieving an HbA1c of ≤ 7% was more difficult. Indeed in the recent National Diabetes Audit, centres with over 50% of their submitted cohorts achieving the slightly higher target HbA1c of ≤ 7.5% numbered only 10 out of 77 centres^[16]. However, it must be understood that our hospital provides secondary care for people with diabetes. Therefore our patient population is predominantly patients that cannot be managed in

Table 5 Change in care process performance following implementation of the alphabet strategy in a low-resource diabetes clinic

	Elements	Pre implementation	Post implementation	P value
A	Body mass index	99%	99%	NS
	Smoking status	99%	99%	NS
	Smoking cessation	100%	100%	NS
B	Blood pressure	99%	99%	NS
C	Total cholesterol	60%	99%	< 0.001
	Lipid profile	10%	64%	< 0.001
	Creatinine	5%	49%	< 0.001
	Proteinuria	48%	93%	< 0.001
D	Fasting and postprandial glucose	41%	97%	< 0.001
E	Eye examination	98%	100%	NS
F	Feet examination	95%	100%	NS
G	Aspirin therapy	6%	71%	< 0.001
	ACEI/ARB therapy	7%	57%	< 0.001
	Statin therapy	5%	38%	< 0.001
	All three	2%	20%	< 0.001

NS: Not significant.

primary care alone.

NATIONAL DIABETES AUDIT 2011/12 - SECONDARY CARE UNITS

In this national audit, the clinical effectiveness of the alphabet strategy was reflected in the beneficial showing of George Eliot Hospital in comparison with other hospitals. Performance of each of the seven out of eight NICE recommended processes occurred in 100% of our submitted cohort, a feat that no other trust achieved. Overall, 85.7% of the submitted cohort received all eight recommended care processes, placing it third out of seventy seven. For target care process achievement, George Eliot scored above average in all categories other than for HbA1c $\leq 6.5\%$ ^[16].

IMPLEMENTATION IN A NON-HIGH INCOME COUNTRY

A beneficial clinical change in care process performance was also demonstrated when the alphabet strategy was applied to a resource poor setting in India. An outpatient diabetes clinic run by a single diabetologist with the aid of a dietician and a nurse was selected for the study. The checklist was adapted for use to the limited local resources. Pre and post-implementation audits were conducted on 100 randomly chosen patients with type 2 diabetes. Principle improvements occurred in the assessments of cholesterol, creatinine and proteinuria, glycaemia, and the use of statins (Table 5).

QUESTIONNAIRE STUDY

As part of the Global Alphabet Strategy Implementation

Audit project, a questionnaire study was performed to gauge the opinions of healthcare professionals and patients on the potential of the alphabet strategy as a management checklist, as a patient-held diabetes care plan, and an education tool. Completed forms were available from 44 single centres located in 27 countries.

Most of the respondents considered the alphabet strategy an evidence-based and practical tool (98% and 91% respectively replying positively). Eighty-five percent of respondents thought that its use would potentially improve outcomes in their clinical practice. Over 70% said they would be likely to adopt the checklist in their clinical practice, although just over half thought it could be applied in their economic background. The strategy was regarded as a useful instrument for patient education. Indeed, over two-thirds of patient responders suggested that patients themselves should use it. However, there were some concerns about the checklist's indirect costs: HbA1c, creatinine, and lipid profile assessments together with the cost of statin implementation are prohibitive in low resource countries, with the expense borne entirely by the patient in private healthcare systems.

CHECKLISTS - ENGAGEMENT IS KEY

Interest has been gathering in the use of checklists and care bundles as a means of improving healthcare quality and lowering patient risk. However, there are many issues associated with their application and adoption, particularly social and cultural difficulties^[17]. After the adoption of the WHO Surgical Safety Checklist, several reports described a range of barriers including confusion regarding its proper use, lack of resource availability in low income countries, and individual personal beliefs and attitudes^[18]. The solutions provided by checklists should not be considered magic bullets. Mere provision of the WHO checklist to hospitals did not culminate in immediate clinical benefits, but rather months of groundwork to organisational systems and personnel were required to aid effectual implementation^[19].

The successful outcomes associated with the use of the alphabet strategy suggests it presents a technical solution to the complicated task of achieving effective diabetes care. Implementation of the alphabet strategy initially in our hospital trust was relatively uncomplicated. There was then one consultant diabetologist supported by an able diabetes team keen for patient-centred and evidence-based care. There now exists four consultant diabetologists, all with varying degrees of engagement with the alphabet strategy. Indeed a recent audit assessing care process performance showed considerable variation by consultant (Table 6).

Consultant A, the author of the alphabet strategy, achieved performance of all nine care processes in 80.8% of all patients directly seen. All other doctors fared significantly worse in performance of all care

Table 6 Care process performance by consultant, registrars, and junior doctors

Care process	Cons A/% n = 125	Cons B/% n = 132	Cons C/% n = 36	Cons D/% n = 101	Junior drs/% n = 86	Registrars/% n = 70
Smoking status	100	97.7	94.4	99	98.8	98.6
BMI	92.8	93.2	91.7	95	98.8	98.6
Blood pressure	100	100	100	100	100	100
Total cholesterol	100	100	100	100	98.8	100
Creatinine	100	100	100	100	100	100
Urine albumin creatinine ratio	87.2	78.8	75	82.2	74.4	78.6
HbA1c	100	100	100	100	100	100
Eye examination	97.6	98.5	97.2	99	97.7	97.1
Foot Examination	100	77.3	83.3	68.3	96.5	84.3
All care processes performed	80.8	59.1	55.6	51.5	73.3	67.1
P value all care processes vs Cons A (χ^2)	-	< 0.0001	< 0.0001	< 0.0001	0.057	< 0.0001

BMI: Body mass index.

processes, except interestingly for the junior doctors.

ALPHABET STRATEGY MATERIALS

Our healthcare education talks are centred around the alphabet strategy approach. A one-day alphabet strategy workshop has previously been delivered nationally in the United Kingdom and internationally under the auspices of the United Nations Development Programme in Bahrain (twice). Course evaluations have been consistently positive^[20].

A series of posters and leaflets discussing each of the elements of the alphabet strategy have proved popular and effective in group and individual education. The education posters were also rated favourably in our questionnaire study by healthcare professional and patients. As a result, they have been translated into French, Somali, Telugu, and Gujarati. Other patient resources include a "patient passport" - a diabetes care plan in the alphabet strategy format that allows people to track their clinical and biochemical data and identify management targets. Culturally adapted materials ensure that the key messages of the alphabet strategy are relayed to members of the South-Asian (Indo-Asian) community residing in our locality. For Muslim patients, Ramadan advice leaflets prepare individuals on self-management issues during their month of fasting.

Finally, clinical letters based on the strategy communicating treatment plans to primary care have been developed to facilitate shared management.

CONCLUSION

When used appropriately, the alphabet strategy can consistently deliver excellent clinical outcomes comparable to trial standards in landmark studies. We achieved these benefits despite a considerable outpatient workload and low levels of human resources: the average patient was seen two to three times per year in our unit, approximating a total of 45 min with a healthcare professional. The positive

improvements with the checklist have been achieved because healthcare providers and patients all subscribe to one methodical approach to diabetes care.

We believe diabetes care, like all forms of healthcare provision, should be effective, professional, responsible, and accountable. Our philosophy for the alphabet strategy is that it should follow the "POETIC" vision: (1) Patient-focused, Public health centred to improve outcomes, and Professionally guided and inspired; (2) Outcome-based, delivering relevant clinical improvements based on real and assessable outcomes; (3) Evidence-based, rooted in clinical evidence, up to date, and influenced by local audit; (4) Team-focused, allowing multidisciplinary cooperation and intervention to improve patient care; (5) Integrated across primary and secondary care, and other related health services; and (6) Cost efficient, using limited resources appropriately.

The alphabet strategy concept is freely available and not under copyright. All materials (lecture slides, patient education posters, patient held care plans, Ramadhan advice leaflets, clinic letter template) are available in the public domain, free to download and use, and easily adaptable to local resources and requirements. All patients with diabetes should be offered the foremost healthcare that resources allow, with none being refused effective or affordable care or therapy. The alphabet strategy can deliver real clinical benefits in diabetes care and has the scope to be adopted extensively across different economies.

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Pregnancy and neonatal outcomes in Indigenous Australians with diabetes in pregnancy

Victor Duong, Bronwyn Davis, Henrik Falhammar

Victor Duong, Royal Darwin Hospital, Tiwi NT 0810, Australia
 Bronwyn Davis, Centre for Nursing and Midwifery Research,
 College of Healthcare Sciences, Division of Tropical Health and
 Medicine, James Cook University, Townsville City QLD 4811,
 Australia

Henrik Falhammar, Department of Endocrinology, Metabolism
 and Diabetes, Karolinska University Hospital, 171 76 Solna,
 Sweden

Henrik Falhammar, Department of Molecular Medicine and
 Surgery, Karolinska Institutet, 171 76 Solna, Sweden

Henrik Falhammar, Menzies School of Health Research, Royal
 Darwin Hospital, Tiwi NT 0810, Australia

Author contributions: Duong V, Davis B and Falhammar H
 equally contributed to this paper.

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Correspondence to: Victor Duong, BBiomedSc, MBBS,
 Royal Darwin Hospital, 105 Rocklands Drive, Tiwi NT 0810,
 Australia. victor.duong@live.com.au
 Telephone: +61-8-89228888

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Abstract

AIM: To perform a systematic review of reported
 neonatal and pregnancy outcomes of Indigenous
 Australians with diabetes in pregnancy (DIP).

METHODS: Electronic searches of PubMed and Web of
 Science were carried out. Articles were selected if they
 contained original data on DIP outcomes in Indigenous
 Australians. There were no specific exclusion criteria.

RESULTS: A total of eight articles, predominantly from
 Queensland and Western Australia were identified
 once inclusion criteria were applied. Birth data from
 midwifery registries or paper charts encompassing
 years 1985-2008 were used. A total of 465591 pregnant
 women with and without DIP were included in the eight
 studies, with 1363 being Indigenous women with DIP.
 Indigenous Australians experienced increased rates
 of many known adverse outcomes of DIP including:
 macrosomia, caesarean section, congenital deformities,
 low birth weight, hypoglycaemia, and neonatal trauma.
 There were regional differences among Indigenous
 Australians, particularly regional/remote vs metropolitan
 populations where the regional/remote data showed
 worse outcomes. Two of the articles did not note a
 difference between Aboriginals and Caucasians in
 the rates of measured adverse outcome. Studies
 varied significantly in size, measured outcomes, and
 subsequent analysis.

CONCLUSION: The health disparities between In-
 digenous Australians and non-Indigenous Australians
 are further evidenced by poorer outcomes in DIP.
 This has broader implications for Indigenous health in
 general.

Key words: Diabetes; Gestational; Hyperglycaemia;
 Pregnancy; Indigenous; Aboriginal; Torres Strait Islander;
 Outcomes

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Core tip: A review of all published data in Australia concerning diabetes in pregnancy outcomes in Indigenous Australians was performed. Of the eight articles identified, Indigenous Australians were shown to have higher rates of adverse outcomes compared to the non-Indigenous population. Living in a remote region appeared to increase the risk of an adverse outcome. This article highlights further health disparities between Indigenous and non-Indigenous, but also exposes gaps in regional data.

Duong V, Davis B, Falhammar H. Pregnancy and neonatal outcomes in Indigenous Australians with diabetes in pregnancy. *World J Diabetes* 2015; 6(6): 880-888 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i6/880.htm> DOI: <http://dx.doi.org/10.4239/wjcd.v6.i6.880>

INTRODUCTION

It is well documented that diabetes during pregnancy poses multiple risks for the developing foetus as well as adverse outcomes for both mother and newborn^[1-4]. Common adverse pregnancy outcomes include foetal macrosomia, caesarean delivery, shoulder dystocia, congenital malformations, preterm delivery, neonatal hypoglycaemia, respiratory distress and hyperbilirubinaemia^[5-7].

Type 2 diabetes (T2DM) has been reported to be up to four times more prevalent in Indigenous Australians compared to non-Indigenous Australians^[8,9]. Furthermore, T2DM occurs at younger ages and complications occur earlier in addition to being more severe than in non-Indigenous Australians^[10,11]. There are parallels that can be drawn with Indigenous populations in overseas countries, with studies conducted in North America showing that the First Nations people also suffer an increased prevalence of diabetes and are subject to increased adverse outcomes as a result of diabetes during pregnancy, compared to non-Indigenous citizens^[12-14]. The high rates of T2DM in the Indigenous populations is thought to be related to obesity, high fat and carbohydrate dense dietary changes combined with a more sedentary lifestyle compared to the traditional hunter-gatherer lifestyle^[15]. As obesity is a risk factor for developing T2DM it has been hypothesised to be a cause of increased rates of gestational diabetes and pre-existing diabetes in women of childbearing age^[16].

The Indigenous population is spread out across Australia and a large proportion reside in communities located in rural or remote areas^[17] (Figure 1). Australia has two distinct Indigenous populations: Australian Aborigines and Torres Strait Islanders, the combined population estimated to be 669881 at the

2011 census, of which 90% were of sole Australian Aboriginal origin^[18]. Torres Strait Islanders are ethnically distinct from Australian Aborigines as they are primarily of Melanesian descent. They originate from the Torres Strait region, a region consisting of hundreds of islands between the tip of Cape York on the Australian mainland and Papua New Guinea^[19]. The Torres Strait diaspora are scattered all over Australia in present times. Indigenous women living in remote areas of Australia have been shown to be at greater risk for developing T2DM^[20]. This is related to obesity, physical inactivity, to poor food quality, availability and cost, in addition to poor economic disposition and genetic predisposition. Access to, and quality of services provided to rural and remote communities are frequently lacking due to the transient nature of health professionals working in these areas. Moreover, Indigenous women often present late for antenatal care, increasing the risk of an adverse pregnancy outcome.

The aim of this study was to conduct a systematic review of the current literature surrounding pregnancy and neonatal outcomes in DIP experienced by Indigenous Australian women.

MATERIALS AND METHODS

For this systematic review, the PRISMA guidelines for literature search and reporting were adhered to where they were applicable^[21]. PubMed searches were performed using the search terms "Aboriginal", "Torres Strait Islander", "Indigenous", "diabetes", "pregnancy", "gestational" and "Australia" (Figure 2). Relevant combinations of these terms returned around 40 unique articles. A MeSH search was then carried out using the combination of subject headings: "Pregnancy in Diabetics" or "Diabetes, Gestational" and "Australia". This returned a higher yield, with 185 articles identified. The Web of Science database was also searched using each of the initial PubMed search terms, however this did not reveal any further articles of interest. The last search was performed in November 2014. Papers which mentioned diabetes, pregnancy, outcomes or made reference to Indigenous Australians in their titles and abstracts were downloaded for further evaluation. Around 20 articles were identified, which involved Indigenous Australians and DIP. The references of these articles were screened to identify any further publications that may have been missed in the search, however this did not reveal any additional studies. Of the papers that were downloaded, prevalence of DIP was the topic of focus in roughly half of the articles, leaving a total of eight suitable papers focusing specifically on outcomes in Indigenous women with DIP (Figure 2). Inclusion criteria were articles that reported on pregnancy and neonatal outcomes in DIP experienced by Indigenous Australian women. There were no specific exclusion criteria. A quality analysis of each study was also

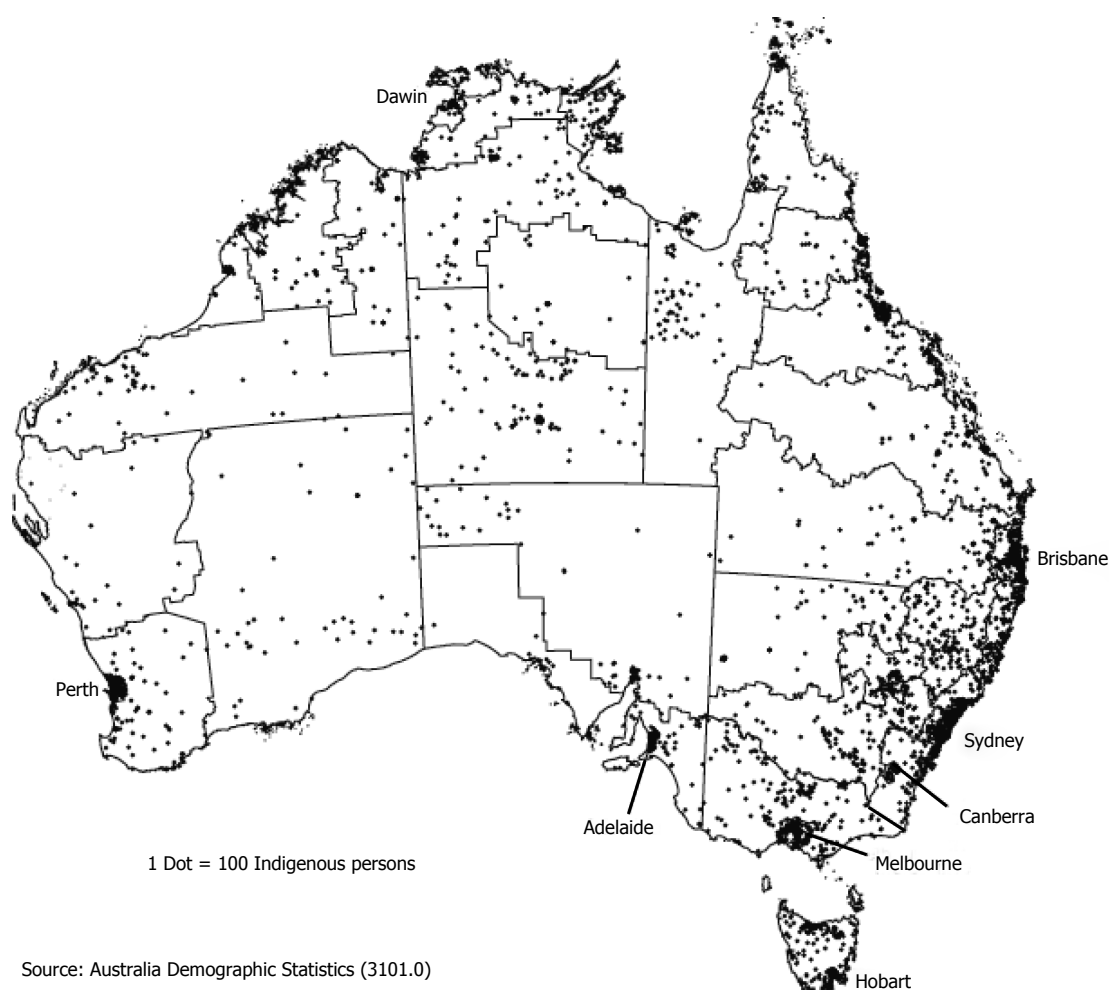


Figure 1 Graphic demonstrating the distribution of Indigenous Australians throughout Australia. Obtained from the Australian Human Rights Commission under Creative Commons^[17].

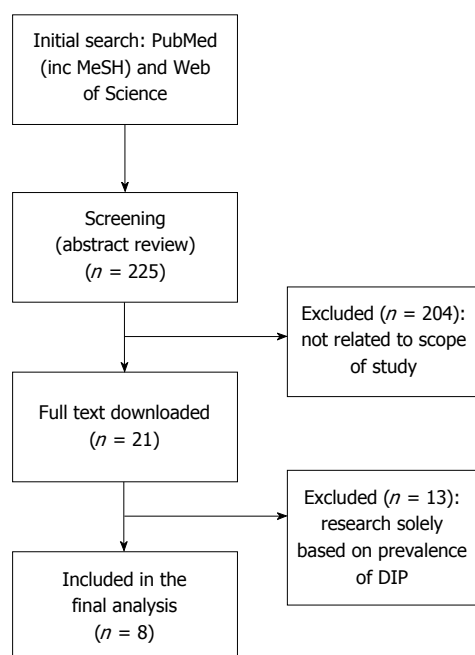


Figure 2 Article selection process for our systematic review. DIP: Diabetes in pregnancy.

performed, outlining merits and limitations of each.

RESULTS

The eight articles gathered from the PubMed database were published between 1985 and 2013. Most were state-wide studies, with three from Queensland (Qld), four from Western Australia (WA) and one from South Australia (SA). All were audits of paper charts or birth registers; in essence, retrospective cohort studies (Table 1). A total of 465591 pregnant women with and without DIP were included in the eight studies, with 1363 being Indigenous women with DIP. Outcomes were usually quoted in terms of rates of incidence, or sometimes where comparison to non-Indigenous was available, risk ratios. The quality of included studies varied (Table 2).

Our search revealed that Indigenous women experienced higher rates of most recordable complications of diabetes than non-Indigenous women, regardless of type 1, 2, or gestational diabetes (GDM). There were two exceptions. Sharpe *et al*^[22] found in their large cohort study in SA that although Aboriginal women had

Table 1 List of included studies, all describing pregnancy and neonatal outcomes in Indigenous Australians with diabetes in pregnancy

Ref.	Year	Title	Study region	Years studied	Total study size	Total women with DIP	Number of Indigenous women with DIP	Outcome measured	Findings
Stanley <i>et al</i> ^[25]	1985	Congenital malformations in infants of mothers with diabetes and epilepsy in Western Australia, 1980-1982	WA	1980-1982	62265	225	52	Congenital anomalies	Relative risk of malformations in Aboriginal DIP - 5.6 compared to 1.9 in non-Aboriginal DIP. Attributable risk however, is low
Bower <i>et al</i> ^[24]	1992	Birth defects in the infants of Aboriginal and non-Aboriginal mothers with diabetes in Western Australia	WA	1980-1984	111019	427	98	Congenital anomalies	Prevalence ratio for birth defects in Aboriginal children is 4.85 for insulin dependent DM and 3.64 for non-insulin dependent DM, compared to 2.08 and 3.64 respectively for non-Aboriginal children
Blair ^[23]	1996	Why do Aboriginal newborns weigh less? Determinants of birthweight for gestation	WA	1980's	1301	672	159	Birth weight	Aboriginal newborns weigh 180 g less than non-Aboriginal (DIP and non-DIP)
Sharpe <i>et al</i> ^[22]	2005	Maternal Diabetes and Congenital Anomalies in South Australia 1986-2000: A Population-Based Cohort Study	SA	1986-2000	282260	7681	432	Congenital anomalies	Congenital anomalies significantly higher in mothers with DIP, relative risk 2.01. No difference with ethnicity
Davis <i>et al</i> ^[28]	2009	Maternal and neonatal outcomes following diabetes in pregnancy in Far North Queensland, Australia	North Queensland	2004	50683 ¹	136	59	C-section, hypoglycaemia, resp distress, abnormal birth weight, term delivery	Compared with non-Indigenous women, Indigenous women had smaller babies, less term deliveries, more severe neonatal hypoglycaemia. Worse outcomes than national and state data
Falhammar <i>et al</i> ^[19]	2010	Maternal and neonatal outcomes in the Torres Strait Islands with a sixfold increase in type 2 diabetes in pregnancy over six years	North Queensland	1999, 2005/2006	454	37	32	C-section, large baby, neonatal trauma, hypoglycaemia	DIP infants heavier (700 g), taller (1.9 cm), more neonatal trauma and hypoglycaemia
Porter <i>et al</i> ^[26]	2011	What is the impact of diabetes for Australian Aboriginal women when pregnant?	WA	2000-2007	81617	5987	531	Birth weight, C-section, stillbirth	Indigenous infants' high birth weight, stillbirth rate = 22/1000 for GDM and 53/1000 for pre-existing DM, compared with 3/100 and 11/1000 for Caucasians
Davis <i>et al</i> ^[29]	2013	A threefold increase in gestational diabetes over two years: Review of screening practices and pregnancy outcomes in Indigenous women of Cape York, Australia	North Queensland	2006, 2008	261	31	31	C-section, birth weight, hypoglycaemia	Higher rates of C-section (66 vs 25%), higher birth weight and increased rate of hypoglycaemia (> 40%) in DIP vs non-DIP Indigenous mothers and babies

¹136 local mothers were compared to diabetic mothers in a national benchmark study ($n = 496$) and to all pregnant data in Queensland 2004 ($n = 50051$).
DIP: Diabetes in pregnancy; C-section: Caesarean section.

Table 2 Quality analysis of included studies, detailing strengths and limitations of each

Ref.	Title	Strengths	Limitations
Stanley <i>et al</i> ^[25]	Congenital malformations in infants of mothers with diabetes and epilepsy in Western Australia, 1980-1982	Large sample size	Retrospective cohort study Only one outcome measure (congenital malformations) Low number of Indigenous women with DIP Time period may not be relevant to modern era
Bower <i>et al</i> ^[24]	Birth defects in the infants of Aboriginal and non-Aboriginal mothers with diabetes in Western Australia	Large sample size	Retrospective cohort study Only one outcome measure (birth defects) Low number of Indigenous women with DIP
Blair ^[23]	Why do Aboriginal newborns weigh less? Determinants of birth weight for gestation	Large sample size	Retrospective cohort study DIP outcomes not main focus of paper
Sharpe <i>et al</i> ^[22]	Maternal Diabetes and Congenital Anomalies in South Australia 1986-2000: A Population-Based Cohort Study	Large sample size	Retrospective cohort study Only one outcome measure (congenital anomalies) Comparison between diabetic and non-diabetic with ethnic background as secondary comparator
Davis <i>et al</i> ^[28]	Maternal and neonatal outcomes following diabetes in pregnancy in Far North Queensland, Australia	Study question aligned with our study question, <i>i.e.</i> , outcomes of DIP in Australian Aboriginal women Large sample size	Retrospective cohort study Low number of Indigenous women with DIP
Falhammar <i>et al</i> ^[19]	Maternal and neonatal outcomes in the Torres Strait Islands with a sixfold increase in type 2 diabetes in pregnancy over six years	Assessment and comparison between two time periods	Retrospective cohort study No comparison with non-Indigenous women Low number of Indigenous women with DIP
Porter <i>et al</i> ^[26]	What is the impact of diabetes for Australian Aboriginal women when pregnant?	Study question mirrors ours Large sample size spanning 7 yr	Retrospective cohort study
Davis <i>et al</i> ^[29]	A threefold increase in gestational diabetes over two years: Review of screening practices and pregnancy outcomes in Indigenous women of Cape York, Australia	Comprehensive analysis of outcomes in Indigenous women with DIP	Retrospective cohort study No comparison with non-Indigenous women Low number of Indigenous women with DIP

DIP: Diabetes in pregnancy.

higher rates of pre-existing diabetes and gestational diabetes than Caucasian women, their rate of birth deformities was only slightly higher compared to the Caucasian group. Blair^[23] found that the increased presence of diabetes, urogenital infections, alcoholism and leprosy contributed to most of the lower birth weight in Indigenous children, but did not observe a higher impact of this compared to non-Indigenous children with similarly affected mothers. There was, however, a discrepancy of 180 g between pure-descent Aboriginal infants and Caucasian infants that was not accounted for by these conditions. Incomplete data on the pathologies and non-medical factors in Aboriginal women were cited as the cause of the discrepancy.

In contrast to the study by Sharpe *et al*^[22], Bower *et al*^[24] discovered in their analysis of midwifery data in WA from 1980-1984 that compared to non-diabetic Aboriginal mothers, birth defects in infants of Aboriginal women with pre-existing DM or gestational diabetes were over three times more common. Similarly, the risk in the Caucasian pre-existing diabetic population was 2.0-3.5 times higher but not increased at all in the gestational diabetes group compared to their non-diabetic counterparts. Thus, if Aboriginal diabetic mothers were compared directly to Caucasian diabetic mothers, there was a 10% increased prevalence of birth defects, which was statistically significant for gestational diabetes ($P = 0.02$) but not for pre-existing diabetes. In a cohort from WA with data from

1980-1982, Stanley *et al*^[25] in their analysis of diabetic and epileptic mothers showed a relative risk of 5.1 (95%CI: 2.6-13.0) for diabetic Aboriginal mothers having children with birth defects, in contrast with 1.7 (95%CI: 0.8-3.5) for Caucasian diabetic vs Caucasian non-diabetic women. There may be some overlap of data in these two similar studies, as the same state-wide database was used in overlapping time periods.

Porter *et al*^[26] in their analysis of the midwifery database in WA from 2000-2007, determined that for the time period studied, Aboriginal infants not only had greater birth weights compared to Caucasian infants when both had mothers with GDM, but when the mother had pre-existing diabetes the Aboriginal infants were smaller. Rates of elective caesarean section in Aboriginal women were 10% lower than for Caucasian women with diabetes, and were even found to be slightly lower than healthy Caucasian women. The other significant adverse outcome was that stillbirths were reported to be extremely high in both Aboriginal women with GDM or pre-existing diabetes (22/1000 and 53/1000 births respectively), while Caucasian women with GDM or pre-existing diabetes only had slightly higher rates (3/1000 and 11/1000 births respectively) compared to their non-diabetic counterparts (2/1000 births). The stillbirth rate in Aboriginal women with DIP was similar or even worse than some of the highest stillbirth rates worldwide in southern Africa and Asia (25 to 35/1000 births)^[27].

Falhammar *et al.*^[19] reported birth data from the Torres Strait Islands, obtaining information from two discrete time periods six years apart. The conclusions drawn were that Torres Strait Islander mothers with DIP experienced higher rates of expected complications compared to Torres Strait Islander mothers without DIP. Hypertension and previous spontaneous abortions were more prevalent, as were caesarean sections, with a fivefold elevation compared to the non-diabetic group in the latter year studied. Infants born to diabetic mothers were also heavier, longer, experienced more neonatal trauma, hypoglycaemia and IV dextrose use.

Two other studies originating from North Queensland examined maternal/neonatal outcomes in Indigenous women. Both included the Torres Strait Islander group in the analysis while recognising the ethnically distinct origin. Davis *et al.*^[28] in 2009 compared local DIP outcomes in Far North Queensland to state-wide and national outcomes. They found that Indigenous women with DIP had smaller babies, less term deliveries and more severe neonatal hypoglycaemia than the non-Indigenous cohort. Importantly, when comparing local and national Indigenous data, locals showed worse outcomes with more premature deliveries and lower APGAR scores. There were no significant differences between the two local populations of Aboriginals and Torres Strait Islanders.

Davis *et al.*^[29] in 2013 examined solely DIP vs non-DIP outcomes in the Indigenous Aboriginal population of Cape York in North Queensland. Analysis was done for two discrete years, 2006 and 2008. DIP women were found to have higher rates of caesarean section, higher birth weight and hypoglycaemia. Outcomes such as mean APGAR score and respiratory distress showed improvement in the latter studied year, after a period of intensive education on GDM screening and management protocols between the two years.

DISCUSSION

While it is widely acknowledged that Indigenous Australians suffer poorer health across a variety of disciplines, this is the first systematic review focusing specifically on outcomes related to hyperglycaemia in pregnancy. The studies identified from our literature search contained much variability in research focus and outcome measurement. Six out of eight studies were in accordance that Indigenous Australian women and their babies are subject to worse outcomes in DIP than their non-Indigenous counterparts, or at least their Indigenous counterparts without DIP. Maternal complications such as delivering prematurely or *via* caesarean section occurred at a higher rate. Neonatal complications including hypoglycaemia, macrosomia or low birth weight, and trauma were also increased to significant levels. The studies were equivocal as to whether there was a higher rate of birth defects compared to non-Indigenous babies. These complications for the infant correspond to poorer

health in adulthood, most importantly an increased risk of developing impaired glucose tolerance, obesity or the metabolic syndrome^[30-34]. For the mother, developing GDM in pregnancy places her at a higher risk of developing T2DM later in life and with subsequent pregnancies^[35]. Furthermore, experiencing a caesarean section may involve undue emotional distress, which is also financially and logistically disruptive as many Indigenous women live in remote communities and are relocated to larger regional centres for delivery. It also exposes them to the range of complications possible from undergoing a surgical procedure and places them at higher risk for future pregnancies. However, one study did show a lower frequency of elective caesarean sections in Indigenous mothers with DIP compared to Caucasian mothers with but also without DIP suggesting that inequality in the health care delivery exists, as discussed below. This may explain some of the differences found in outcomes.

In addition to poorer outcomes compared to non-Indigenous women, there is evidence to suggest that perinatal outcomes for Indigenous women living in rural or remote regions are poorer still, particularly in comparison to those women living in metropolitan areas^[19,28,36]. Two of the three studies performed in northern Queensland had shown that compared to state-wide statistics, people living in the study region experienced worse outcomes. This demonstrates that remoteness is likely to be a compounding factor towards a negative outcome in DIP, for reasons including limited food supply, substandard housing and living conditions, poor access to medical services and financial factors. Cultural barriers for women's non-engagement with mainstream health services in remote areas is multifaceted and incorporates cultural beliefs, conflicting cultural responsibilities, fear, guilt, shame, perceptions of culturally insensitive Western practices in pregnancy care and cultural indifference in health care providers^[37].

This review strikes similarities with Indigenous populations abroad, with a study conducted in Ontario, Canada showing that First Nations women received less antenatal and postpartum care, and that those with DIP were subject to higher birth weights and higher rates of jaundice, neonatal hypoglycaemia and shoulder dystocia^[12]. A separate investigation conducted in Alberta, Canada also confirmed worse perinatal outcomes due to DIP in First Nations women, however found that the prevalence of adverse outcomes varied between different provinces^[13]. Aljohani *et al.*^[14] found that in Manitoba, having gestational diabetes and First Nation status compounded the risk of shoulder dystocia. These similarities to other nations with Indigenous populations allow us to draw on and potentially apply any conclusion obtained from studies performed overseas, and vice-versa.

Early in our search it became apparent that literature focusing on pregnancy and neonatal outcomes in Indigenous Australians with DIP was scarce. Half

Table 3 Indigenous population by state as of 2011

State	Indigenous population	Proportion of total population
New South Wales	208000	2.9%
Victoria	47000	0.9%
Queensland	189000	4.2%
Western Australia	88000	3.8%
South Australia	37000	2.3%
Tasmania	24000	4.7%
Australian Capital Territory	6000	1.7%
Northern Territory	69000	29.8%

Source: Adapted from Australian Bureau of Statistics, Estimates of Aboriginal and Torres Strait Islander Australians, June 2011^[18].

of the studies that were eventually included in our analysis focused on one particular complication of pregnancy and assessed predisposing factors, with DIP and Indigenous status being a variable that may lead to the outcome in question. This limits the perspective on the full spectrum of outcomes of DIP, but also introduces an additional form of bias. Due to the variability of the research focuses and outcomes, detailed statistical analysis and direct comparisons, for instance performing a meta-analysis was not possible.

A point worth noting is that while the Northern Territory (NT) has the highest proportion of Indigenous Australians per capita, comprising 29.8% of the NT population at the 2011 census^[18], there were no studies originating from the NT. In both Queensland and Western Australia Indigenous people make up a much lower 4.8% and 3.2% respectively of the total population, however in some remote regions they make up 50%-90% of the population. These states were the biggest contributors to the data on diabetes in Aboriginal women and their pregnancy outcomes (Table 3). To alleviate the disparity in data output across states, an upcoming NT-based study will analyse many different aspects of DIP in Indigenous Australians^[38].

Due to the limited data available, not all of the known adverse outcomes of pregnancy in a diabetic mother could be fully assessed. Pre-eclampsia is known to occur at a higher rate in mothers with pre-existing diabetes^[5], however has not yet been analysed. Stillbirth, perinatal mortality and transient cardiomyopathy are significant recognised complications^[5,39,40], that require further study. Other complications with lacking data include biochemical derangements with uncertain long-term effects such as low iron stores, hyperbilirubinaemia and hypocalcaemia^[41-43]. With more research on the topic, we will be able to paint a clearer picture of specific outcomes that Indigenous women and their offspring may be more prone to developing. This may in turn be used as endpoints to gauge efficacy of management protocols and allocation of resources.

Diabetes in pregnancy presents itself as one of the many health disparities between Indigenous and non-Indigenous Australians. The rising prevalence of the disease and demonstrated poor outcomes com-

pared to non-Indigenous Australians ensures that this problem will remain topical until it is dealt with effectively. In addition, mothers and their infants who are affected by diabetes suffer consequences, which may not manifest until later in life. The main challenges that face Australian healthcare professionals are twofold: incorporating strict antenatal care into the cultural, spiritual and religious framework of Indigenous Australians, and dealing with the difficulties of delivering evidence-based medical care in rural and remote communities. More research into trends, specific outcomes and data from different regions of Australia will aid in building a clear image of the task at hand, as current data is limited. This will in turn assist in identifying novel intervention strategies to employ, and outcome measures by which to judge the effectiveness of these methods.

COMMENTS

Background

It is well recognised that in general, health outcomes of Indigenous Australians are inferior to those of non-Indigenous Australians. Complications related to having diabetes in pregnancy (DIP) are no exception to this trend. However, no systematic review has previously been performed to summarise the current state of pregnancy and neonatal outcomes experienced by Indigenous Australians with DIP.

Research frontiers

Although Indigenous Australians are known to suffer poorer obstetric outcomes compared to non-Indigenous Australians, less is known of specific outcomes and their relative prevalence.

Innovations and breakthroughs

Previous studies have been small or only investigated few outcomes. This systematic review included in total a large number of pregnant women with and without DIP in eight studies, and many were Indigenous women with DIP. Indigenous Australian mothers with DIP and their offspring experienced increased rates of macrosomia, caesarean section, congenital deformities, stillbirth, low birth weight, hypoglycaemia, and neonatal trauma. There were regional differences among Indigenous Australians, particularly regional/remote vs metropolitan populations, where the regional/remote data showed worse outcomes. However, two of the articles did not note a difference between Indigenous and non-Indigenous in the rates of measured adverse outcome.

Applications

This article summarises the main adverse outcomes that are experienced by Indigenous Australians with DIP. These outcomes can in turn be used as endpoints in assessing the impact of new interventions or policies to improve Indigenous health.

Terminology

Diabetes in pregnancy encompasses all conditions that may result in hyperglycaemia in pregnant women. This includes gestational diabetes mellitus diagnosed during pregnancy, or previously diagnosed type 1 or 2 diabetes mellitus.

Peer-review

The paper appears interesting and very well written paper.

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Editorial Board Member of *World Journal of Diabetes*, Guo-Ping Shi, DSc, Associate Professor of Medicine, Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, NRB-7, 77 Avenue Louis Pasteur, Boston, MA 02115, United States

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World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
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Explaining the increased mortality in type 1 diabetes

Chiara Mameli, Sara Mazzantini, Moufida Ben Nasr, Paolo Fiorina, Andrea E Scaramuzza, Gian Vincenzo Zuccotti

Chiara Mameli, Sara Mazzantini, Gian Vincenzo Zuccotti,
 Department of Pediatrics, "Ospedale dei Bambini V. Buzzi",
 University of Milan, 20154 Milan, Italy

Moufida Ben Nasr, Paolo Fiorina, Division of Nephrology,
 Boston Children's Hospital, Harvard Medical School, Boston,
 MA 02115, United States

Moufida Ben Nasr, Paolo Fiorina, Transplant Medicine,
 Ospedale San Raffaele, 20132 Milano, Italy

Andrea E Scaramuzza, Department of Pediatrics, Azienda
 Ospedaliera Luigi Sacco, University of Milan, 20154 Milan, Italy

Author contributions: Mameli C, Mazzantini S, Ben Nasr M, Fiorina P, Scaramuzza AE and Zuccotti GV conceived and designed this paper and drafted the report; all authors participated in critical review of the report; all authors had seen and approved the final version.

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Correspondence to: Andrea E Scaramuzza, MD, Department of Pediatrics, Azienda Ospedaliera Luigi Sacco, University of Milan, Via G.B. Grassi 74, 20154 Milan, Italy. scaramuzza.andrea@hsacco.it
 Telephone: +39-2-39042791
 Fax: +39-2-39042254

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Abstract

Despite large improvements in the management of glucose levels and in the treatment of cardiovascular risk factors, the mortality rate in individuals with type 1 diabetes (T1D) is still high. Recently, Lind *et al* found that T1D individuals with glycated hemoglobin levels of 6.9% or lower had a risk of death from any cause or from cardiovascular causes that is twice as high as the risk for matched controls. T1D is a chronic disease with an early onset (*e.g.*, pediatric age) and thus in order to establish a clear correlation between death rate and the glycometabolic control, the whole history of glycemic control should be considered; particularly in the early years of diabetes. The switch from a normo- to hyperglycemic milieu in an individual with T1D in the pediatric age, represents a stressful event that may impact outcomes and death rate many years later. In this paper we will discuss the aforementioned issues, and offer our view on these findings, paying a particular attention to the several alterations occurring in the earliest phases of T1D and to the many factors that may be associated with the chronic history of T1D. This may help us to better understand the recently published death rate data and to develop future innovative and effective preventive strategies.

Key words: Type 1 diabetes; Hyperglycemia; Death rates; Adolescence; Autonomic neuropathy; Children; Endothelial dysfunction; Exercise; Metabolic memory

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Core tip: Despite large improvements in the management of glucose levels and in the treatment of cardiovascular risk factors, the mortality rate in individuals with type 1 diabetes (T1D) is still high. A better understanding of the several different alterations occurring in the earliest phases of T1D and of the many factors that may be associated with a chronic history of T1D may help us to develop future innovative and effective preventive strategies.

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INTRODUCTION

Whether mortality in type 1 diabetes mellitus (T1D) is improved by intensive glycemic therapy has not been clarified yet. A number of studies have recently been published claiming that mortality rate is still higher than in age-matched controls without diabetes, despite improvements in management of glucose levels and treatment of cardiovascular risk factors^[1-3]. Lind *et al.*^[1] reported data on current life expectancy for adults with T1D in a population-based sample using Swedish national registries of adults with and without diabetes. The Authors found that individuals with T1D and glycated hemoglobin (HbA1c) level of 6.9% or lower had a risk of death from any cause or from cardiovascular causes that was twice as high as the risk for matched controls. The multivariable-adjusted hazard ratios for death from any cause according to the HbA1c level for individuals with T1D as compared with controls are reported in Table 1.

Livingstone *et al.*^[2] report data on current life expectancy for adults with T1D in a population-based sample using Scottish national registries of adults with and without diabetes. At the age of 20 years, women and men with T1D could expect to live 12.9 years (95%CI: 11.7-14.1) and 11.1 years (95%CI: 10.1-12.1), respectively, less than aged-matched adults without it. Finally, Orchard *et al.*^[3] report survival data on the selective cohort of North Americans with T1D who participated in the Diabetes Control and Complications Trial (DCCT)^[4] and its observational follow-up study, Epidemiology of Diabetes Interventions and Complications (EDIC)^[5]. They found that 27 years after entry into the trial, 6.5 years of initial intensive diabetes therapy was associated with a modestly lower all-cause mortality rate when compared with conventional therapy. Few editorials accompanied^[6] or commented^[7] these data, without suggesting any conclusive hypothesis about the reason why this happens. T1D is known to be associated with an increased risk of premature mortality among the affected individuals, as documented by a

recent systematic review on this topic by Morgan *et al.*^[8]. Authors identified thirteen relevant publications with mortality data, describing 23 independent studies. Standardized mortality ratios varied markedly ($P < 0.0001$). The increased mortality in childhood/adolescent-diagnosed with T1D was apparent across countries worldwide. Excesses were less marked in more recent studies and in countries with lower infant mortality and higher health expenditure. Given that good metabolic control has been shown to be effective reducing microvascular and macrovascular complication rates, one should expect that also the mortality rate might be reduced, but this is not the case^[4,5]. Therefore, we would like to propose our appraisal to these important findings.

THE IMPORTANCE OF CHILDHOOD YEARS OF T1D

All studies reporting mortality rates in T1D refer to adult individuals, most of the time with a diabetes which occurred in childhood. For instance all individuals studied in the Lind paper were at least 18-year-old at the moment of enrollment, with a mean age at baseline of 35.8 years and mean diabetes duration of 20.4 years^[1]. This implies that the average age for the onset of diabetes was 15.4 years, during their adolescence. The study does not provide any information at all with respect to HbA1c levels between diabetes onset and the time of data collection (on average 40 to 50 years after T1D diagnosis). From previous studies, (e.g., DCCT and EDIC), we know how “metabolic memory” provides an important footprinting to future long-term complications^[4,5,9]. We can thus argue that “metabolic memory” may partially be accounted for the higher death rate observed in individuals with T1D, whose onset was during childhood or adolescence. This aforementioned aspect reinforces the important of obtaining an optimal glycometabolic control in the first years of T1D. This is an important issue, since T1D incidence rate increases from birth, and peaks between the ages 10-14 years^[10], with an even increased incidence especially marked in the youngest children (0-4 years)^[11], making T1D the second most frequent chronic disease of childhood, after asthma. Further emphasis should be pointed to vascular complications, which start at the onset of the disease^[12], although the consequences become clinically evident later in adulthood^[13,14]. Once again, we may speculate that the reported excess mortality in adult individuals showing HbA1c < 6.9% in the study by Lind *et al.*^[1], may be the residual effect of previous cardiovascular insults started in infancy, childhood or adolescence. It is still unclear and partially unexplained why cardiovascular complications start so early in the disease history of T1D; indeed we can only speculate that a chronic state of mild hyperglycemia might be the culprit of cardiovascular morbidity, and thereby of excess death, as unaccounted in the Swedish observational trial^[1].

Table 1 Adjusted hazard ratios for death from any cause and death from cardiovascular disease among individuals with type 1 diabetes *vs* control according to the glycated hemoglobin

Mean HbA1c	Hazard ratios	
	Death from any cause	Death from cardiovascular disease
≤ 6.9%	2.36 (95%CI: 1.97-2.83)	2.92 (95%CI: 2.07-4.13)
7.0%-7.8%	2.38 (95%CI: 2.02-2.80)	3.39 (95%CI: 2.49-4.61)
7.9%-8.7%	3.11 (95%CI: 2.66-3.62)	4.44 (95%CI: 3.32-5.96)
8.8%-9.6%	3.65 (95%CI: 3.11-4.30)	5.35 (95%CI: 3.94-7.26)
≥ 9.7%	8.51 (95%CI: 7.24-10.01)	10.46 (95%CI: 7.62-14.37)

Adapted from Lind *et al*^[1]. HbA1c: Glycated hemoglobin.

Indeed, according to the A1c-Derived Average Glucose study group, a HbA1c value of 6.9% indicate an average glucose level as high as 151 mg/dL (8.4 mmol/L)^[15].

Additionally, the HbA1c measurement has some limitations itself. It has been shown to be unreliable in several different clinical scenarios, such as anemia or hemolysis, in presence of implanted mechanical heart valves, hypothyroidism, or during the use of medications such as erythropoietin^[16]. Moreover, there is a recognized biological variability in the glycation process of the hemoglobin molecule in response to hyperglycemia. This is the result of a different glycation rate in "high glyating" *vs* "low glyating" subjects, where the same mean blood glucose was associated with an HbA1c level of 9.6% *vs* 7.6%, respectively^[17]. To summarize, besides the issues related to the use of HbA1c, multiple factors contributed to the augmented risk of death in T1D individuals despite a good metabolic control. Indeed, several challenges are offered by the constantly evolving age-appropriate care needed by diabetic individuals transitioning from infancy to adulthood^[18].

ENDOTHELIAL DYSFUNCTION AND EARLY ATHEROSCLEROSIS

Several different systems show altered homeostasis early along the course of diabetes^[19-21]. Among them, the endothelium is definitely one of the most important and earlier targeted organs. Evidence suggests that impairment in nitric oxide-mediated smooth muscle vasodilation is an early pathophysiologic process and underlies the onset endothelial dysfunction, a key event for the development of atherosclerosis^[22]. Among factors that may worsen endothelial function in individuals with T1D, we should mention: a long disease duration^[23], a severely altered glycemic control^[24], high low density lipoprotein cholesterol levels^[25], high levels of advanced glycated end products^[26], and altered mitochondrial dynamics^[27]. Our group recently observed a high prevalence of endothelial dysfunction (76.7%) in adolescents with T1D for a mean duration of 9 years, particularly in those individuals with impaired glycometabolic control, subclinical signs of autonomic neuropathy and sedentary lifestyle. We did not observe any correlations between endothelial dysfunction and diabetes duration or individuals' age. A

HbA1c below 7.5% (58 mmol/mol) and regular physical activity of at least 4 h per week, were indeed associated with better endothelial function. Atherosclerosis, the late event of endothelial dysfunction, is frequently linked to the likelihood of death from cardiovascular origin, especially in individuals with T1D. Compared to non-diabetic subjects, individuals with T1D show an increased risk up to 10-fold to develop atherosclerotic plaques, starting since childhood and adolescence^[28]. Furthermore, intima media thickness measurement of the carotid artery is considered another valid surrogate marker for cardiovascular risk allowing assessment of atherosclerotic changes at a very early stage^[29]. Finally, Paroni *et al*^[30] showed that hyperhomocysteinemia in individuals with T1D may further increase the risk of endothelial dysfunction.

CARDIOVASCULAR AUTONOMIC NEUROPATHY

Cardiac autonomic neuropathy is an often overlooked and common complication of diabetes mellitus and by itself it is associated with increased cardiovascular morbidity and mortality^[31], together with cardiac abnormalities typical of individuals with diabetes^[32,33]. Data demonstrate the dual (vagal and sympathetic) control of heart rate and the dominant role of respiration in the genesis of heart rate and blood pressure fluctuations, suggesting that reduced vagal control of the sinoatrial node and impaired vascular regulation are the two main pathophysiological alterations^[34]. Few years ago, our group investigated the autonomic performance of 93 children and adolescents with uncomplicated well-controlled T1D compared to age-matched controls. We found a significant increase in arterial blood pressure, a blunted baroreceptor reflex, and an increase of the low-frequency component of systolic arterial pressure variability. These findings entail the simultaneous impairment of the capability of the vagal system to influence the heart function, together with an increased sympathetic vasomotor regulation^[21]. A follow-up study conducted 1-year later showed further impairment of the neuro vegetative performance, thereby suggesting early progression of the autonomic disturbance^[21]. Interestingly, a small weekly increase in exercise in these same individuals can greatly help to improve cardiac autonomic neuropathy.

INFLAMMATION AND OXIDATIVE STRESS

In the last fifteen years several groups worked in the direction of uncovering the association between the increased cardiovascular risk in individuals with T1D and inflammation. Schaumberg *et al*^[35] measured levels of inflammatory biomarkers at baseline and after a 3-year follow-up in a random sample of 385 participants of the DCCT cohort. Results were controversial and emphasized the extremely complex interaction between

inflammation, T1D and insulin therapy. Some of the inflammation indexes were high in both intensive and conventional insulin treatment groups; others were higher in the intensive insulin therapy group, others in the conventional one. What seemed to be linked to increased inflammation status in individuals using intensive insulin therapy was the weight gain they showed^[35], underlining the need for a more effective weight control in individuals with T1D. Indeed, a recent study by Valerio *et al.*^[36] found that T1D adolescents, particularly females, showed a considerable occurrence of abdominal adiposity and metabolic syndrome. That is why pediatric diabetologists need to make every effort to achieve normal weight and better health outcomes in their young T1D patients. Davì *et al.*^[37] found that newly T1D diagnosed individuals showed significantly augmented lipid peroxidation and platelet activation, paralleled by a higher degree of systemic inflammation. This data strongly support the idea of a significantly noxious effect of even the earliest form of damage triggered by the disease. The biochemical picture depicted is suggestive of a true acute inflammatory response accompanying the disease in its very earliest, hence pediatric, phase^[37]. The SEARCH Case-Control Study showed that young individuals with T1D, when compared to healthy controls, were characterized by excess inflammation despite good glycemic control^[38]. Interestingly, Folli *et al.*^[39] demonstrated that persistent cellular changes of anti-oxidative machinery and of aerobic/anaerobic glycolysis are present in individuals with T1D (with or without end-stage renal disease), and these abnormalities may play a key role in the pathogenesis of hyperglycemia-related vascular complications. Restoration of euglycemia and removal of uremia with kidney pancreas transplant can correct these abnormalities. Some of these identified pathways may become potential therapeutic targets for a new generation of drugs^[39].

HYPOGLYCEMIC EVENTS

Another possible explanation for the increased death rate in individuals with T1D despite good glycemic control may be hypoglycemia. The T1D Exchange Registry seems to confirm this hypothesis^[40]. Elderly individuals and children younger than 5 years seem to be the two populations at greater risk^[41,42]. A value of HbA1c in the low range ("good" metabolic control) may not only be associated with well controlled glucose control but with recurrent episodes of hyper/hypoglycemic oscillations. We may speculate that, in the study by Lind *et al.*^[41], one of the factors potentially explaining the persistence of a sizeable mortality hazard ratio in individuals with low HbA1c could be a high rate of hypoglycemic events in those individuals.

HOW TO IMPROVE OUTCOMES

T1D is a complex disease whose management may be extremely awkward and demanding^[43]. Diet and

exercise, in combination with a correct insulin therapy, play a pivotal role in obtaining and maintaining the best glycemic control possible. In the evaluation of a subgroup of individuals from the treatment group of the DCCT cohort, Delahanty *et al.*^[44] established the relation between diet and glycemic control beyond the sole intensive insulin therapy. A higher content of total and saturated fat, associated with a lower carbohydrate intake are linked to worse glycemic control, thereby further increasing the cardiovascular risk^[44]. Adequate fibers intake, usually lower than suggested, is also recommended. Indeed, fibers offer a beneficial dietary profile: (1) by reducing or at least delaying the overall glucose absorption; (2) by blunting post-prandial glycemic peaks, and finally (3) by impacting low-density lipoproteins by enhancing biliary acid secretion. For the aforementioned reasons, a proper nutritional education is a crucial part of diabetes management and needs to be promoted in all pediatric individuals with T1D and their families^[45]. Interestingly, recent study reported that children with T1D show less healthy food habits than same age healthy subjects^[46].

Routine physical exercise is known to have beneficial effects on the cardiovascular system in the general population, and even more in individuals with T1D^[47]. For this reason, we should strongly encourage individuals with T1D to participate in regular physical activity since childhood. One hour of moderate, aerobic exercise every day is currently recommended. Lucini *et al.*^[48] recently found the favorable effects of moderate increase (10%) in spontaneous exercise load in adolescents with T1D. Similarly, in children with T1D (mean age 11 years old), 60 min per day of exercise improves endothelial dysfunction, a well-known risk factor for cardiovascular diseases^[49]. Moreover, in the recent years, technology has helped to reduce the impact of T1D especially in children^[50]. Continuous glucose monitoring has emerged as one of the most significant innovation in the management of children with T1D. The combination of continuous glucose monitoring and insulin pumps, provides better glycemic control with less hypoglycemic episodes^[51,52]. The ultimate technological advance of such automated insulin administration systems, currently under development, is the completely automated glycemic management, the closed-loop system also known as "external artificial pancreas"^[53].

Finally, we would like to highlight recent stem cell-based trials, for which expectations in the scientific community and among individuals with T1D are high^[54]. One of the most promising is cord blood stem cells that have been demonstrated to become a powerful tool not only for regenerative medicine but for autoimmune (e.g., T1D) and inflammatory diseases as well^[55,56]. Recently, a novel hematopoietic stem cell-based strategy has been tested in individuals with new-onset T1D, suggesting that remission of the disease is possible by combining hematopoietic stem cell transplantation and immunosuppression; however safer hematopoietic stem cell-based therapeutic options are required^[57].

MORTALITY IN INDIVIDUALS WITH TYPE 2 DIABETES

As the prevalence of type 2 diabetes (T2D) continues to increase worldwide, diabetes-related morbidity and mortality increase as well. There is scarce evidence on the effect of HbA1c reduction on mortality rate in T2D individuals. Recently a study by Skriver *et al.*^[58] in a large cohort ($n = 11205$) of Danish individuals with T2D, showed that HbA1c variability was associated with mortality irrespective of the magnitude of absolute change in HbA1c. An increased mortality was observed even in those individuals with a HbA1c $\leq 8\%$ if presenting a higher HbA1c variability^[58]. However, in T2D individuals many factors other than glycometabolic control may contribute to increase the mortality rate (e.g., hypertension, obesity, dyslipidemia, elevated uric acid and insulin resistance). Therefore, an early diagnosis and a prompt management of T2D comorbidities is required^[59-62].

CONCLUSION

In conclusion, the recent findings describing an increased mortality in individuals with T1D as compared to age-matched population, even in the presence of on-target HbA1c, are important. Whenever the outcomes of a chronic disease like T1D are being studied, it is important to acquire data from the onset. Indeed, any events in the early phase may affect its future course and especially its final outcome (*i.e.*, death rates). A better understanding of the several alterations occurring in the earliest phases of T1D and of the factors that may be associated with the chronic history of T1D may help us to develop future innovative and effective preventive strategies.

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Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome

Richard C Strange, Kate E Shipman, Sudarshan Ramachandran

Richard C Strange, Institute for Science and Technology in Medicine, Keele University Medical School, England ST4 6QG, United Kingdom

Kate E Shipman, Sudarshan Ramachandran, Department of Clinical Biochemistry, Good Hope Hospital, Heart of England NHS Foundation Trust, Sutton Coldfield B75 7RR, United Kingdom

Sudarshan Ramachandran, Department of Clinical Biochemistry, University Hospital of North Staffordshire, England ST4 6QG, United Kingdom

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Correspondence to: Dr. Sudarshan Ramachandran, Department of Clinical Biochemistry, Good Hope Hospital, Heart of England NHS Foundation Trust, Rectory Road, Sutton Coldfield B75 7RR, United Kingdom. sud.ramachandran@heartofengland.nhs.uk
Telephone: +44-121-4247246
Fax: +44-121-3111800

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Abstract

Despite the well-recognised role of vitamin D in a wide range of physiological processes, hypovitaminosis is common worldwide (prevalence 30%-50%) presumably arising from inadequate exposure to ultraviolet radiation and insufficient consumption. While generally not at the very low levels associated with rickets, hypovitaminosis D has been implicated in various very different, patho-physiological processes. These include putative effects on the pathogenesis of neoplastic change, inflammatory and demyelinating conditions, cardiovascular disease (CVD) and diabetes. This review focuses on the association between hypovitaminosis D and the metabolic syndrome as well as its component characteristics which are central obesity, glucose homeostasis, insulin resistance, hypertension and atherogenic dyslipidaemia. We also consider the effects of hypovitaminosis D on outcomes associated with the metabolic syndrome such as CVD, diabetes and non-alcoholic fatty liver disease. We structure this review into 3 distinct sections; the metabolic syndrome, vitamin D biochemistry and the putative association between hypovitaminosis D, the metabolic syndrome and cardiovascular risk.

Key words: Vitamin D; Hypovitaminosis D; Metabolic syndrome; Type 2 diabetes mellitus; Insulin resistance; Cardiovascular disease; Atherogenic dyslipidaemia; Hypertension; Non-alcoholic fatty liver disease

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Core tip: The metabolic syndrome is common, affecting about 40% of Americans. It is defined by combinations of risk factors for cardiovascular disease (CVD) including insulin resistance and abdominal obesity. Research implicates hypovitaminosis D in the causation and phenotype of the syndrome and we present relevant data. While hypovitaminosis appears a risk factor for components of the syndrome and its outcome, the mechanism is unclear. The risks associated with varying

levels of hypovitaminosis and the benefits of vitamin replacement are unknown. However, unravelling the association between hypovitaminosis and the syndrome is warranted as even a modest decrease in CVD risk would confer substantial benefits.

Strange RC, Shipman KE, Ramachandran S. Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome. *World J Diabetes* 2015; 6(7): 896-911 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i7/896.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i7.896>

INTRODUCTION

Much research over the last 30 years has shown that the pleiotrophic actions of 1, 25 dihydroxy-vitamin D [$1,25(\text{OH})_2\text{D}$] are central to cell, organ and organism homeostasis. Thus, along with its historic functions as a mediator of calcium and bone metabolism, $1,25(\text{OH})_2\text{D}$ has effects on a wide range of physiological processes. It is perhaps surprising, given its perceived importance to public health, to find that hypovitaminosis D is common worldwide (prevalence 30%-50%). This deficiency presumably arises from failure to firstly, ensure adequate exposure to ultraviolet radiation (UVR) because of skin cancer fears and secondly, consume food with sufficient levels of the vitamin. Vitamin D status is identified by low serum levels of biologically inactive 25-hydroxylated vitamin D [$25(\text{OH})\text{D}$]. While generally not at the very low levels associated with rickets, hypovitaminosis D has been implicated in various very different, pathophysiological processes. These include a putative effect on the development of neoplastic, inflammatory, demyelinating, cardiovascular and diabetic conditions. While the impact of hypovitaminosis D on health remains unclear, accumulating data indicates it confers increased disease risk and in some cases worse outcome.

In the context of this review, the finding that hypovitaminosis D is associated with impaired glucose homeostasis is of particular interest. A meta-analysis of 28 studies demonstrated that higher serum $25(\text{OH})\text{D}$ levels were associated with a 55% reduction in diabetes, a 51% decreased risk of the metabolic syndrome and a 33% lower risk of cardiovascular disease (CVD)^[1]. Further, treatment with vitamin D supplements over 2 mo improved fasting glucose levels and insulin resistance homeostasis model assessment for insulin resistance (HOMA-IR) in 100 patients with type 2 diabetes^[2]. It is suggested that the mechanism for this latter finding involves improved sensitivity of target tissues such as the liver, muscle and bone to insulin as well as enhanced beta cell function. Given that many risk factors for CVD are clustered in the highly prevalent metabolic syndrome, which is characterised by insulin resistance and abdominal obesity, it is reasonable to speculate a significant role for the vitamin in the development of the syndrome and its sequelae of diabetes and CVD.

In this review we focus on the association between hypovitaminosis D and the metabolic syndrome and how this may contribute to increased CVD risk. We present 3 sections describing firstly, the metabolic syndrome, secondly, vitamin D biochemistry and thirdly, the putative association between hypovitaminosis D, the syndrome and CVD risk.

METABOLIC SYNDROME; HOW IT WAS IDENTIFIED

The relationship between sensitivity to insulin, obesity and glucose homeostasis was first observed by the Swedish physician Eskil Kylin^[3]. He described a syndrome comprising hyperglycaemia, hypertension and hyperuricaemia and suggested insulin resistance as a possible causative factor^[3]. Subsequently Himsworth *et al.*^[4] laid the foundations for the classification of type 1 and 2 diabetes by showing that while some patients were insulin sensitive (younger, normal weight and blood pressure) others are insulin insensitive (older, more obese, hypertensive and atherosclerotic). Vague, in studies on gender-related obesity patterns described android obesity (now termed central obesity and linked with diabetes and atherosclerosis) and suggested a hormonal aetiology with over-activity of the pituitary-adrenal axis playing a key role^[5].

Such observations were brought together by Reaven^[6] in his Banting Lecture to the American Diabetes Association in 1988. He termed the combination of hypertension, dyslipidaemia and glucose intolerance as syndrome X and proposed that this mix of phenotypes provided a pathophysiological basis for atherosclerosis. Obesity, was also seen as a further essential component and following a number of iterations (dyslipidaemic hypertension, deadly quartet, insulin resistance, hazardous waist), the combination of phenotypes is now termed the metabolic syndrome^[7] with the International Classification of Disease code of 277^[7,8].

Classification of the metabolic syndrome

Various groups including the World Health Organisation^[9], European Group for the Study of Insulin Resistance^[7], American Association of Clinical Endocrinologists^[10], National Cholesterol Education Program - Adult Treatment Panel III^[11] and, more recently, the International Diabetes Federation (IDF)^[12,13] have provided definitions of the metabolic syndrome (Table 1). While all are based on the characteristics presented by Reaven^[6], there are various inclusion thresholds. A form of consensus was arrived at in 2009^[14] with the IDF, National Heart, Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis and the International Association for the Study of Obesity agreeing on threshold levels that were similar to those originally proposed by the IDF. Guidelines for classifying metabolic syndrome in children over 10 years of age were also issued^[15] and population and gender specific

Table 1 Thresholds defining the metabolic syndrome issued by individual organisations

	WHO 1998 (Alberti 1998)	EGIR (Balkau 1999)	NCEP/ATP III 2001 (NCEP 2002)	AACE (2003) (Einhorn 2003)	IDF consensus 2005 (Zimmet 2005)	IDF consensus (10 to < 16 yr) (Zimmet 2007)
Definition	IGT, IFG, T2DM or lowered insulin sensitivity Plus 2 of the following	Plasma insulin > 75 th percentile Plus 2 of the following	3 of the following	IGT or IFG plus any of the following based on clinical judgement	See below	
Europoid waist circumference (cm)	W:H > 0.90 M W:H > 0.85 F or BMI > 30 kg/m ²	≥ 94 M ≥ 80 F	≥ 102 M ≥ 88 F	BMI ≥ 25 kg/m ²	≥ 94 M ≥ 80 F or BMI > 30 kg/m ² Plus 2 of the following	> 90 th percentile Plus 2 of the following
Triglyceride [mg/dL (mmol/L)]	> 150 (1.7)	> 150 (1.7)	≥ 150 (1.7)	> 150 (1.7)	> 150 (1.7)	≥ 150 (1.7)
HDL [mg/dL (mmol/L)]	< 35 (0.91) M < 39 (1.01) F	< 39 (0.91)	< 40 (1.03) M < 50 (1.29) F	< 40 (1.03) M < 50 (1.29) F	< 40 (1.03) M < 50 (1.29) F	< 40 (1.03)
BP (mmHg)	≥ 140/90	≥ 140/90 or on treatment	≥ 130/85	≥ 130/85	SBP ≥ 130 or DBP ≥ 85 or on treatment	SBP ≥ 130 and/or DBP ≥ 85
Glucose [mg/dL (mmol/ L)]	IGT, IFG or T2DM	IGT or IFG (but not diabetes)	≥ 100 (5.6) (Grundy) or diabetes	IGT or IFG (but not diabetes)	≥ 100 (5.6)	≥ 100 (5.6) or known T2DM
Others	Microalbuminuria ACR > 30 mg/g			Other features of IR ¹		

¹Includes polycystic ovary syndrome, family history or ethnic group susceptible to type 2 diabetes, sedentary lifestyle and advancing age. ACR: Albumin creatinine ratio; BMI: Body mass index; DBP: Diastolic blood pressure; F: Female; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; IR: Insulin resistance; SBP: Systolic blood pressure; M: Male; T2DM: Type 2 diabetes mellitus; W:H: Waist to hip ratio; WHO: World Health Organization; HDL: High density lipoprotein; IDF: International Diabetes Federation; EGIR: European Group for the Study of Insulin Resistance; NCEP: National Cholesterol Education Program; AACE: American Association of Clinical Endocrinologists; BP: Blood pressure; IR: Insulin resistance.

waist circumference thresholds were published to define central obesity^[13]. The prevalence for the metabolic syndrome varies between countries. Based on the IDF classification a 40% prevalence in the United States has been reported^[16].

Is there a clinical value in identifying the metabolic syndrome: It is not surprising, given the presence of known risk factors, to find that the metabolic syndrome confers an approximately two-fold increased relative risk of CVD^[17]. However, it is important to determine whether this impact is the effect of the metabolic syndrome (added risk due to a clustering of risk factors) or just the sum of its defining phenotypes. Studies using different CVD endpoints indicate the latter is the case. For example, Eddy *et al.*^[18] used data from NHANES III (third national health and nutrition survey) to simulate a population matching that of the United States, estimated its metabolic syndrome prevalence (using the various definitions) and associated this with CVD. While the number of individuals identified by the various metabolic syndrome classifications differed, they reported that fasting glucose levels > 110 mg/dL (6.1 mmol/L) were a better predictor of CVD than the presence of the metabolic syndrome classified by any of the definitions^[18]. Further, using change in atheroma volume as an endpoint, Bayturan *et al.*^[19] reviewed 3459 patients enrolled in 7 trials that used intravascular ultrasonography to measure plaque progression. While the metabolic syndrome was significantly associated [odds ratio (OR)

= 1.29, 95%CI: 1.09-1.53] with increased atheroma volume, the relationship was not significant (OR = 1.04, 95%CI: 0.79-1.37) when adjusted for its individual components; serum triglycerides ≥ 150 mg/dL (1.7 mmol/L), body mass index (BMI) ≥ 30 kg/m², high density lipoprotein cholesterol (HDL-C) < 40 mg/dL (1.0 mmol/L) in men or < 50 mg/dL (1.3 mmol/L) in women, blood pressure ≥ 135/85 mmHg or treatment of hypertension^[19]. In this multifactorial model, only serum triglyceride concentrations (≥ 150 mg/dL) remained significantly associated with plaque progression^[19].

These findings (and others) question the clinical value of identifying the metabolic syndrome in patients. Indeed, the identification is dependent on the thresholds of each of the contributing factors. Thus, for example, if age-related thresholds were used there would be a marked change in the numbers of affected individuals. While in theory its identification does not appear to add anything to prognosis in an individual patient, we and others^[20] argue that it has clinical value. As the metabolic syndrome is based on related and modifiable CVD risk factors, its identification encourages a holistic approach rather than a focus on the individual aspects (glycaemia, dyslipidaemia, weight reduction and blood pressure management) of the patients' condition. It therefore, has value in encouraging the clinician to address CVD risk using a multifactorial approach. It is also arguably useful in a research setting when considering the role of possible risk factors.

We also believe it is important to consider the

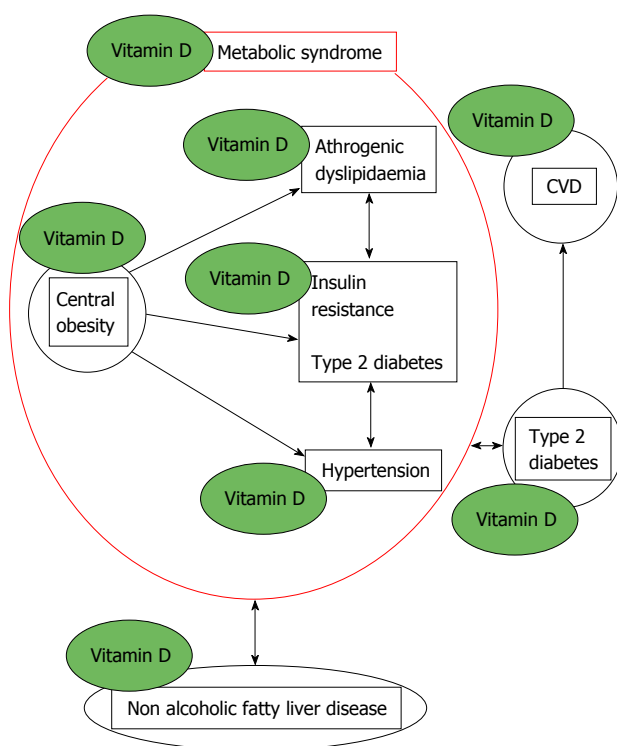


Figure 1 Simplified illustrations of the component risk factors of the metabolic syndrome, the complex relationships between them and the outcomes leading to increased morbidity and mortality. We also identify the areas that may be affected by hypovitaminosis D which are covered in this review. CVD: Cardiovascular disease.

metabolic syndrome as a heterogeneous entity. Indeed, in patients with the syndrome, we have shown that following treatment with statins and fibrates, outcomes can vary considerably indicating the presence of subgroups both known (gender, baseline lipids, and concurrent therapy) and unknown^[21-24].

Metabolic syndrome - putative pathway to CVD:

While it is accepted that central obesity and insulin resistance are core drivers of the metabolic syndrome, the timescale and inter-relationships between these and other factors that lead to an individual being classified with the syndrome and the consequent increased risk of CVD remain unclear^[25-27]. Clearly, while obesity and insulin resistance are common in adults worldwide they are rare in childhood indicating that environmental factors interacting with a genetic predisposition drive the development of the syndrome from birth through childhood to its identification in adulthood. Once an individual develops the metabolic syndrome, the combination of risk factors leads to an increased risk of CVD (Figure 1).

Obesity is a recognised risk factor associated with mortality, this probably due to the link between obesity and risk of developing diabetes, hypertension, atherogenic dyslipidaemia and CVD^[28]. However, the National Health and Nutrition Examination Survey (NHANES) indicated that individuals with a BMI between 30 and 35 kg/m² demonstrated only a modest increase

in mortality compared to those with BMI 18.5-25 kg/m²^[27,29]. These findings suggest the presence of a subgroup of obese individuals who are not at high risk of metabolic disturbances or increased mortality. Their presence may be a reason for the relatively modest increase in overall mortality in obese subjects. It has been speculated that the link between obesity and CVD may be *via* insulin resistance^[27]. Individuals with high insulin sensitivity and not fulfilling the ATP III metabolic syndrome criteria are considered to be a "metabolically healthy obese" group^[29].

The concept that not all obesity is bad in the context of developing CVD is interesting. Abdominal obesity, visceral as opposed to subcutaneous fat, appears to be critical in the development of insulin resistance^[30]. Abdominal adipose tissue was initially considered an inert storage depot for triglycerides (glycerol and fatty acids). The current view however, is that it is also an active endocrine organ. Intra-abdominal obesity, a classifying characteristic of the metabolic syndrome promotes insulin resistance (the reverse of insulin sensitivity), perhaps by secreting metabolically active substances (adipokines) and making available an increased quantity of free fatty acids^[30,31].

Insulin resistance, the other key factor in the metabolic syndrome, is defined as a condition where greater than normal levels of the peptide are needed to clear a glucose load (and effect its other metabolic actions). Thus, for a given blood glucose level the amount of insulin secreted is high. Impairment of sensitivity appears to be a contributing factor to all of the features of the metabolic syndrome in addition to having a direct causative role in the pathogenesis of type 2 diabetes. It can be considered a pre-diabetic state in non-diabetic patients, conferring a 5 fold increased risk of developing diabetes^[32]. Insulin resistance has also been demonstrated to be associated with hypertension, atherogenic dyslipidaemia and higher amounts of the atherogenic small dense low density lipoprotein cholesterol (LDL-C), features associated with the metabolic syndrome^[20,33].

Thus, in addition to weight reduction measures, reducing insulin resistance, a feature that may be an intermediate factor linking obesity with morbidity and mortality, must be addressed in patients with the metabolic syndrome. Apart from abdominal obesity there are other factors that may modify insulin resistance. Physical fitness (as measured by aerobic capacity) has been seen to increase insulin sensitivity^[34].

VITAMIN D BIOCHEMISTRY

Vitamin D, in addition to its role in calcium and bone metabolism, has pleiotrophic effects in many cell types in many life forms. These include a potential role in the actions of insulin and development of obesity (Figure 1). Thus, not surprisingly hypovitaminosis D has been linked with hypertension, atherogenic dyslipidaemia and increased CVD risk (Figure 1). An association has

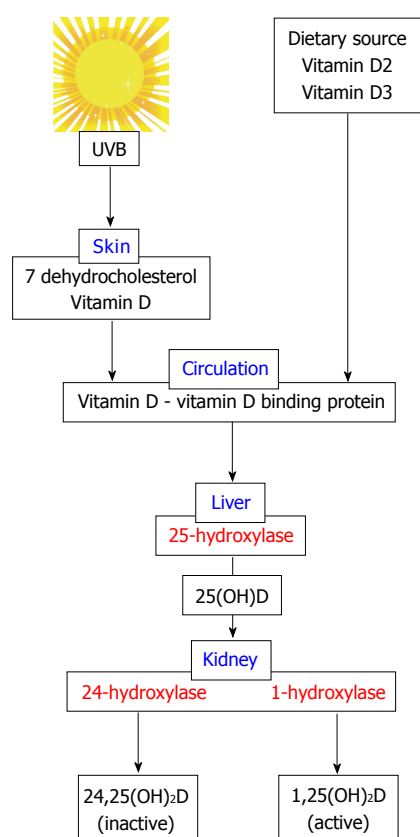


Figure 2 Simplified synthetic pathway leading to the formation of the active metabolite 1,25(OH)₂D. UVB: Ultraviolet B.

also been noted with non-alcoholic fatty liver disease independent of the features classifying the metabolic syndrome. Hypovitaminosis D can be addressed by both lifestyle measures and supplementation; hence, it is important to understand the relationship between vitamin D and the metabolic syndrome at both mechanistic and epidemiological levels.

Vitamin D synthesis

Bioactive vitamin D, 1,25(OH)₂D is synthesised in a pathway involving different organs and intermediates (Figure 2). Some inactive chemicals are produced that may have a regulatory role but will not be considered further. The first step in the pathway is the photochemical production of cholecalciferol in the skin from 7-dehydrocholesterol. Thus, production of bioactive 1,25(OH)₂D can only be initiated in skin *via* a photochemical process. Accordingly, animals have to eat foods containing the vitamin or be exposed to sunlight to allow its photosynthesis in skin.

Cholecalciferol is produced in the stratum basale and stratum spinosum layers of skin following reaction of 7-dehydrocholesterol with ultraviolet B (UVB) (270–300 nm). It is noteworthy that the concentration of 7-dehydrocholesterol falls with increasing age resulting in reduced capacity to synthesise vitamin D₃. This effect is marked; for example, the skin of a 70-year-old subject has approximately 25% of the 7-dehydrocholesterol compared with that of a young adult^[35]. Cholecalciferol

(and ergocalciferol) is carried in blood to the liver and hydroxylated at position 25 to form 25(OH)D. The final step in the pathway is hydroxylation of circulating 25(OH)D at the 1 position to form biologically active 1,25(OH)₂D. This occurs in the kidney, and other tissues, and is followed by its release into blood bound to vitamin D binding protein and transported to target organs.

How vitamin D works

Systemic or locally produced 1,25(OH)₂D binds to the vitamin D receptor (VDR), a nuclear receptor that dimerises with the retinoid X receptor and, in turn, becomes a regulator of transcription^[36]. Dimerisation allows interaction with the vitamin D response element on target genes initiating transcription^[37]. The VDR is a member of the steroid receptor superfamily and is responsible for regulating transcription in many responsive genes. Indeed, more than 200 genes, including those that regulate cell differentiation and proliferation as well as multiple metabolic systems, are targets for vitamin D.

Skin pigmentation, UVR and vitamin D

Vitamin D photosynthesis is long-established among animals implying a key role in metabolism. Phytoplankton in the sea have synthesised vitamin D for more than 500 million years and land vertebrates for more than 350 million years. Further, the sophisticated biochemical systems used by humans to balance the harmful and beneficial effects of sunlight demonstrate the evolutionary pressures on these processes. Protection from UVR has been provided by the development of a sunscreen; eumelanin. Eumelanin absorbs UVR, reducing its penetration and, thereby, formation of potentially harmful free radicals (reactive oxygen species) in the skin. The migration of humans from Africa to environments of often low and highly seasonal UVR placed pressure on the original constitutive, dark-skinned phenotype^[38]. Thus vitamin D₃ synthetic ability, following movement into higher latitudes, was enabled by polymorphic change in genes that determine skin pigmentation, such as melanocortin 1 receptor, with the resulting development of partially depigmented phenotypes capable of tanning. Thus, the present range of skin pigmentation results from a requirement to promote cutaneous UVR-induced vitamin D₃ synthesis (depigmented phenotype) and simultaneously prevent UVR-induced damage (pigmented phenotype)^[38].

Studying the relationship between UVR exposure, vitamin D status, skin type and disease risk is complicated by historical and recent population movements resulting in many people living under solar regimes very different to those in which their ancestors developed mechanisms to balance sunlight's harmful and beneficial effects. The health penalties of these movements are still under assessment though the potentially serious consequences of chronically low exposure are now being recognised. This of course, does not mitigate the need to ensure that the risks associated with inappropriate and excessive UVR exposure in terms of skin and other

cancers continues to be emphasised.

Environmental factors affecting exposure to UVR

The amount of UVR reaching earth varies with the angle at which radiation passes through the atmosphere (solar zenith angle), its path length through air, the presence of clouds and pollution in the lower atmosphere^[39,40]. Consequently, place and time of day and season are important. Outside of tropical latitudes, ensuring a year-round, adequate level of vitamin D synthesis is problematic because large solar zenith angles and long path lengths result in increased absorption and scattering of UVR. During the year the availability of vitamin D₃-inducing UVB wavelengths varies with latitude and outside the tropics there is little or no UVB in sunlight except at high altitudes for much of the year. For example, the equator sees only about a 20% variation while 50° N (circle of latitude that crosses the English Channel, Belgium, Czech Republic, Russia, Mongolia and Canada) sees around 250% variation. Indeed, between November-February, people living at latitude 50° N and higher receive no effective vitamin D₃-inducing UVB and can effect no vitamin synthesis^[39,40]. This latitude effect is compounded by dark skin pigmentation; the higher the eumelanin content the lower the vitamin D₃ production. Thus, for many individuals there is insufficient UVB over the year to allow adequate vitamin D synthesis and therefore a need to consume vitamin D₃-rich foods such as oily fish. An additional problem in ensuring adequate vitamin D status, particularly away from the equator, is presented by modern urban lifestyles. Exposure to UVR is limited by clothing, shade-seeking behaviour, often because of skin cancer fears, and occupations that result in 80%-90% of work time being spent indoors.

Assessing vitamin D status and defining hypovitaminosis

Exposure to sunlight or dietary intake of vitamin D increases the serum concentration of 25(OH)D making this a ready indicator of body vitamin D status. Establishing a link between chronic hypovitaminosis and disease risk clearly requires definition of a normal serum concentration of 25(OH)D. The serum 25(OH)D concentrations that identify hypovitaminosis D are not fully defined though the following ranges have been suggested; deficiency: ≤ 12 ng/mL (30 nmol/L), insufficiency: 12-20 ng/mL and satisfactory status: ≥ 20 ng/mL (50 nmol/L). However, given the well-recognised seasonal variation in vitamin synthesis, particularly in northerly latitudes, any reference range needs to be considered in the context of season. Recently Tandeter described an Individual Mean Annual vitamin D level termed the "IMAD level" and a recovery formula "RF" that may be used to calculate a mean that encompasses values from four seasons^[41].

Relationship between season and vitamin D status

Understanding the temporal relationship between

seasons, solar radiation and vitamin D photosynthesis is important if epidemiological approaches are used to establish associations between these variables, disease risk and outcome. Furthermore, the impact of relative acute or chronic hypovitaminosis on the relationship between seasons and disease pathogenesis is unclear. For example, if chronic hypovitaminosis D was pathological, a visible consequence might take some time to be clinically evident and therefore not easily associated with the seasons^[42].

Surprisingly given the potential impact of vitamin D on public health, there is little data on the relationship between seasons, serum vitamin concentrations and lag time between firstly, solar radiation and building up of adequate levels of the vitamin and secondly, which chronic patterns of hypovitaminosis have most impact on the pathogenesis of particular diseases^[42]. Thus, while the causal link between skin exposure to solar UVR and serum vitamin D cyclicity is recognised, neither the mathematical relationship between the peaks and troughs of serum 25(OH)D concentrations during the year nor how (or if) particular patterns affect disease risk have been well defined. Kasahara *et al.*^[42] also provide a model describing the seasonality of serum 25(OH)D concentrations in the United States that could be extrapolated to other studies^[41]. They argued that in the temperate northern hemisphere, serum 25(OH)D concentrations vary during the year because production is determined by the area of skin exposed to UVR and the intensity of the radiation. Thus, serum vitamin concentrations demonstrate maximum levels in late summer and lowest in late winter. This presumably reflects significant photosynthesis and gradual accumulation of vitamin D during the early spring months and a gradual use of reserves in months immediately after photosynthesis ceases when there is little sunlight. Thus, serum vitamin D concentrations demonstrate a seasonal lag pattern that is influenced by how much atmosphere sunlight must pass through before reaching the human body.

ASSOCIATION BETWEEN HYPOVITAMINOSIS D, THE METABOLIC SYNDROME AND CVD RISK

Importance of vitamin D: Population studies using mortality/morbidity as outcome

Many studies suggest that low serum vitamin D concentrations, even when above those associated with rickets, are deleterious. A variety of criteria have been used as clinical endpoints. For example, Schöttker *et al.*^[43] studied the association between serum 25(OH)D concentrations and mortality in a meta-analysis of data from eight prospective cohort studies involving 26018 men and women aged 50-79 years from Europe and the United States. The outcome measures were all-cause, cardiovascular, and cancer mortality. As expected, 25(OH)D concentrations were higher in summer and in

men. During follow-up a total of 6695 study participants died; 2624 of these subjects died of CVDs and 2227 of cancer. Despite levels of 25(OH)D strongly varying with country, gender and season, the association between 25(OH)D concentration and all-cause and cause-specific mortality was consistent^[43]. The lowest 25(OH)D quintile was associated with increased all-cause mortality, cardiovascular mortality and cancer mortality (in those with a history of cancer)^[43]. The inverse association across quintiles was consistent across countries, genders, season and age groups despite 25(OH)D cut-off values varying according to these characteristics^[43].

Associations between UVR exposure and disease risk and outcome have been reported for a wide range of pathologies, although in most cases conflicting data have also presented. Corresponding studies using serum 25(OH)D also show conflicting data. For example, we have presented data indicating that UVR may influence disease risk by a vitamin D mediated mechanism in the pathogenesis of prostate cancer^[44,45] and multiple sclerosis^[46] though we emphasise that these associations remain unproven and any mechanistic basis is uncertain^[47].

Metabolic syndrome and seasons

Clearly, any suggestion that risk of the metabolic syndrome is partly determined by vitamin D status would be helped by evidence that the incidence of the syndrome, and/or its component phenotypes, is linked with availability of the vitamin and/or the seasons. Some evidence supporting this view is available. Kamezaki *et al.*^[48] reported such links in 1202 Japanese males (44 ± 10 years) who were assessed in summer and winter in 2008 for the metabolic syndrome defined using the criteria proposed by the NCEP, the IDF and the Japanese Society of Internal Medicine (JSIM). The prevalence rates of NCEP, IDF, and JSIM defined metabolic syndrome in winter were 3.8%, 15.1% and 12.4% and in summer, 3.2%, 10.7% and 8.4% respectively^[48]. Blood pressure changes were most significantly correlated with this seasonal variation in metabolic syndrome prevalence^[48].

However, inconsistent results regarding the putative association of key components of the metabolic syndrome with season have been reported including more insulin resistance and higher triglyceride concentrations during the summer in some, winter in others and some showing no significant seasonal variation. Taiwanese subjects described by Chen *et al.*^[49] were studied in winter (January and February) and summer (July and August) in 2002. They found higher levels of fasting insulin, HOMA-insulin resistance and triglycerides, but lower levels of HDL-C in summer compared with winter. The prevalence of metabolic syndrome in summer was higher than in winter; difference of 7.7% in both genders ($P = 0.0092$ in men, $P = 0.0037$ in women). After controlling for BMI and other risk profiles, summer was independently and positively associated with fasting insulin and insulin resistance regardless of metabolic syndrome^[49].

A further interesting association between the meta-

bolic syndrome and season is the report by Rintamäki *et al.*^[50] showing a significant association between seasonal changes in mood and behaviour and the metabolic syndrome. Individuals with the syndrome had greater seasonal changes in mood and behaviour.

Metabolic syndrome and vitamin D status: Observational studies

Considerable research has focussed on associations between vitamin D levels and the prevalence of the metabolic syndrome and its component features. Many studies demonstrate an inverse relationship between serum 25(OH)D and diabetes, metabolic syndrome, insulin resistance and beta cell function^[51,52]. The NHANES data confirmed the inverse relationship between 25(OH)D levels and diabetes and insulin resistance in the non-Hispanic white and Mexican American, but not in the non-Hispanic black populations^[53,54].

A meta-analysis of 28 studies (between 1990 and 2009) including 99745 participants (age range: 40.5-74.5 years) by Parker *et al.*^[1] investigated the effects of vitamin D on the risk of CVD, diabetes and the metabolic syndrome^[1]. Higher levels of vitamin D were seen to be associated with reduction of all the outcomes studied among middle aged and elderly individuals. The 28 studies reported 33 ORs when considering the association between 25(OH)D and cardiometabolic outcomes; 29 of these ORs suggested an inverse relationship with 3 indicating an opposite effect with 1 analysis remaining non-significant^[1]. The pooled OR was 0.57 (95%CI: 0.48-0.57). Prevalence of the metabolic syndrome was the outcome in 8 of the studies; all these showing a significant association between high 25(OH)D levels and reduced metabolic syndrome prevalence (OR = 0.49, 95%CI: 0.38-0.64).

Ju *et al.*^[55] studied the relationship between serum 25(OH)D levels and metabolic syndrome in the general adult population using a dose-response meta-analysis based on studies reporting risk ratios for metabolic syndrome in categories of serum 25(OH)D concentrations. The pooled OR for the metabolic syndrome per 25 nmol/L (10 ng/mL) increment in the 25(OH)D concentration was 0.87 (95%CI: 0.83-0.92), based on 16 cross-sectional studies and 1.00 (95%CI: 0.98-1.02) for 2 cohort and nested case-control studies^[55]. The dose-response meta-analysis showed a generally linear, inverse relationship between 25(OH)D levels and the metabolic syndrome in the cross-sectional studies [probability (P) value for linear trend < 0.001]. They concluded that vitamin D status was associated with metabolic syndrome risk in cross-sectional but not longitudinal studies^[55].

Song *et al.*^[56] reported a cross-sectional study comprising 778 Korean adults. Metabolic syndrome was defined according to the American Heart Association/ National Heart, Lung, and Blood Institute criteria and the Korean Society for the Study of Obesity. The overall prevalence of the metabolic syndrome was 18.9%^[56]. After multiple adjustments, compared with the highest

quartile serum 25(OH)D level group (19.9-55.9 ng/mL), the OR for metabolic syndrome in the lowest level group (4.2-9.7 ng/mL) was 2.44 (95%CI: 1.32-4.48). The intermediate quartiles (9.8-14.1 ng/mL) and (14.3-19.8 ng/mL) had ORs of 2.20 (95%CI: 1.24-3.90) and 1.81 (95%CI: 1.02-3.20) respectively when compared to the highest quartile. Among the components of metabolic syndrome, the adjusted ORs for elevated blood pressure and high triglycerides in the lowest 25(OH)D level were 1.81 (95%CI: 1.15-2.85) and 2.74 (95%CI: 1.64-4.57) respectively^[56].

Thus, it is clear from these observational surveys that a relationship may exist between 25(OH)D levels and glucose homeostasis, metabolic syndrome and type 2 diabetes. These population studies do not hint as causation as 25(OH)D status and other established risk factors were not measured at or prior to diagnosis. Thus, prospective studies are required that take into account other confounding factors such as serial weight measurements, physical activity and family history.

Metabolic syndrome and vitamin D status: Prospective studies

A number of prospective studies have also presented data that support the proposal that low serum 25(OH)D concentrations are associated with increased risk of the development of the metabolic syndrome. For example, Gagnon *et al.*^[57] studied 4164 adults (mean age 50 years; 58% women; 92% Europeans). Over the following 5 years, 528 incident cases (12.7%) of the metabolic syndrome were identified^[57]. Compared with the reference category [highest quintile 25(OH)D ≥ 34 ng/mL], the metabolic syndrome risk was significantly higher in people with 25(OH)D in the first (< 18 ng/mL) and second (18-23 ng/mL) quintiles [OR = 1.41 (95%CI: 1.02-1.95) and 1.74 (95%CI: 1.28-2.37) respectively]^[57]. Serum 25(OH)D was inversely associated with waist circumference ($P < 0.001$), triglycerides ($P < 0.01$), fasting glucose ($P < 0.01$), and HOMA-IR ($P < 0.001$) but not with 2-h plasma glucose ($P = 0.29$), HDL-C ($P = 0.70$), or blood pressure ($P = 0.46$)^[57].

More recently Kayaniyil *et al.*^[58] examined the prospective association of 25(OH)D with the metabolic syndrome in a multi-ethnic cohort of non-diabetic adults with pre-existing risk factors in Ontario, Canada. Of 654 participants enrolled at baseline, 489 attended a 3 year follow-up visit. Multivariate logistic regression analyses indicated a decreased risk of the metabolic syndrome at follow-up per standard deviation increase in baseline 25(OH)D after adjustment for sociodemographics, season, baseline and change in supplement use, physical activity and insulin resistance (OR = 0.63, 95%CI: 0.44-0.90)^[58].

Associations between the defining components of the metabolic syndrome and vitamin D status:

Observational, prospective and interventional studies

The observational and prospective studies previously

described demonstrate associations between 25(OH)D concentrations and the metabolic syndrome, but were not designed to explore mechanistic aspects. We now review the effect that 25(OH)D levels may have on the defining characteristics of the metabolic syndrome; abdominal adiposity, insulin resistance (and beta cell function), hypertension and atherogenic dyslipidaemia.

Karatas *et al.*^[59] investigated the association between 25(OH)D levels and all components of the metabolic syndrome in 287 Turkish subjects. Of these, 214 participants were either obese (BMI ≥ 30 kg/m²) or overweight (BMI: 25-29.9 kg/m²). Metabolic syndrome was classified using IDF criteria. Multiple logistic regression analyses were carried out with metabolic syndrome, abdominal obesity, low HDL-C, hypertriglyceridaemia and hypertension as the dependent variable and with 25(OH)D as a continuous independent variable in one set of analyses and 25(OH)D levels stratified as deficiency (< 20 ng/mL), insufficiency (20-29.9 ng/mL) and sufficient (reference level) groups as a factorised independent variable in further analyses. The analyses were corrected for age, gender and season. Hypovitaminosis was significantly more common in the overweight/obese individuals with and without the metabolic syndrome^[59]. There was a significant inverse relationship between triglyceride levels and serum 25(OH)D concentration. No significant associations between 25(OH)D and HDL-C, hypertension and insulin resistance were observed.

Obesity has been associated with hypovitaminosis D, perhaps *via* multiple mechanisms^[60,61]. The nature of this association was investigated by a bi-directional genetic study that suggested higher BMI resulted in lower 25(OH)D levels but with the reverse effect being small^[62]. They concluded that weight reducing interventions would be expected to reduce the prevalence of hypovitaminosis D^[62]. In contrast Salehpour *et al.*^[63] carried out a 12 wk study following cholecalciferol supplementation and showed a significant decrease in body fat mass in both healthy and obese women compared to the placebo arm^[63]. These conflicting findings make it essential that both interventions (weight reduction and vitamin D replacement) are studied in detail with suitably designed trials. Other studies investigating mechanisms, unlike Vimalleswaran *et al.*^[62], have indicated a bi-directional association between obesity and hypovitaminosis D. It has been seen from animal studies that vitamin D may play a part in adipogenesis and energy metabolism. The VDR is expressed in adipose tissue pre-maturation^[64] and in early adipogenesis^[65]. The presence of a role in adipogenesis is also suggested by adipocyte atrophy seen in VDR knockout mice^[66].

The relationship between volume of adipose tissue and vitamin D status, at least as reflected in serum 25(OH)D concentrations, is unclear. Vitamin D is sequestered in adipose tissue and it has been speculated that obesity, by increasing the volume of distribution of available adiposity, will lead to lower serum vitamin D levels^[67,68]. This view is contradicted by Pramyothin *et al.*^[69] who measured vitamin D levels in the subcutaneous

abdominal fat of 17 patients undergoing gastric bypass. Vitamin measurements were made at surgery and over a 12 mo follow-up period^[69]. It was found that vitamin D levels in adipose tissue varied considerably and no significant change in serum 25(OH)D was noted during follow-up despite intake of supplements (> 2500 U/d).

There has been speculation that behaviour traits associated with obesity, such as reduced outdoor exercise levels, could be associated with decreased exposure and reduced vitamin D synthesis. Results from studies investigating this possible association have varied^[70,71]. Thus, although a clear association is evident between adiposity and vitamin D levels the nature of this association has yet to be determined. It is important to establish this relationship as central adiposity is a key driver in the development of the metabolic syndrome.

Dysfunction of insulin secretion by pancreatic beta cells and insulin resistance are considered to be causative drivers in the aetiology of type 2 diabetes^[26]. Insulin secretion may be affected by lipotoxicity, due to increased free fatty acids, and glucotoxicity, due to elevated serum glucose and lipid accumulation within the beta cells^[72]. We have seen that insulin resistance is a core component of the metabolic syndrome. Contrasting findings are evident in observational studies investigating the relationship between 25(OH)D levels and insulin sensitivity. Chiu *et al.*^[52], in Californian students of mixed ethnicity, and Kamycheva *et al.*^[73], in a study of patients with hyperparathyroidism, [patients grouped by the median 25(OH)D concentration] noted a positive correlation between insulin sensitivity and 25(OH)D levels. However, there have been other studies which have not shown the above association, these having been carried out in patient groups characterised by obesity^[74], non-diabetic status^[75] and the metabolic syndrome^[76]. A prospective study of 524 non-diabetic individuals by Forouhi *et al.*^[77] showed an inverse association between 25(OH)D levels and the risk of insulin resistance and elevated blood sugars^[77]. However, the Mini-Finland Health Survey did not demonstrate a significant correlation between 25(OH)D quartiles and the onset of diabetes when the analysis was corrected for BMI and activity^[78].

Vitamin D supplementation has been seen to alter insulin sensitivity in non-diabetic patients, but not in patients diagnosed with type 2 diabetes^[79,80]. Pittas *et al.*^[81] demonstrated that, when compared to placebo, vitamin D had a positive effect on insulin resistance and glycaemic control (non-primary outcome) in a randomised controlled study of patients with impaired fasting glucose^[81]. A complex mechanism is suggested by the SURAYA trial of obese south Asian women as insulin resistance was seen to improve only when supplementation elevated the 25(OH)D concentration above 80 nmol/L this perhaps indicates either a dose response or threshold effect^[82].

Given the association between 25(OH)D levels and obesity it is expected that there would be a similar

relationship with the lipid concentrations; however, study results have varied. A large study in Norway, both longitudinal ($n = 2159$) and cross sectional ($n = 10105$) demonstrated that higher levels of cholesterol, HDL-C and LDL-C and lower levels of triglyceride were associated with reduced 25(OH)D concentrations^[83]. A survey of 108711 patients who had multiple 25(OH)D and lipid profiles measured revealed a similar relationship between 25(OH)D and cholesterol and LDL-C levels^[84]. Further, optimal levels of 25(OH)D were associated with higher HDL-C^[84]. More confusion has arisen as vitamin D supplementation following their cross sectional survey in patients with hypovitaminosis did not lead to consistent changes in the lipid profile^[84]. Jorde *et al.*^[85] reviewed the findings of 22 cross sectional and 10 placebo controlled double blind randomised controlled trials and concluded that, while the cross sectional studies demonstrated a uniform inverse relationship between 25(OH)D and triglyceride levels, the intervention studies with vitamin D supplementation have led to varied results. They concluded that these intervention studies were not adequately designed to specifically investigate the relationship between 25(OH)D and lipids and speculated that the relationship between 25(OH)D and lipids could be either direct or *via* changes in parathyroid hormone and/or calcium concentrations^[85].

Many studies using mouse and human hepatoma cell lines^[86,87] and VDR knockout mice^[88] have been carried out to understand the observed associations between 25(OH)D and lipid concentrations. Some have examined the effect of VDR on bile acid synthesis, and cholesterol levels, once again with inconsistent results^[89].

Hypertension, one of the defining components of the metabolic syndrome, has been reported to display a seasonal and geographical variability raising the possibility of sun exposure having a role^[90]. Even before this observation Resnick *et al.*^[91] in 1986 suggested that vitamin D metabolites were associated with hypertension potentially *via* the renin-angiotensin system^[91]. Both animal and cross-sectional human studies have suggested vitamin D to be an inhibitor of the renin-angiotensin system in VDR knockout^[92] and 1α -hydroxylase knockout^[93] mice with significantly raised renin activity and plasma angiotensin 2 concentrations. The effects were reversed in the 1α -hydroxylase knockout mice by administration of $1,25(\text{OH})_2\text{D}$ ^[93]. Vascular smooth muscle and endothelial cells express VDR and the 1α -hydroxylase enzyme indicating that vitamin D may influence endothelial function which could lead to arterial stiffness and hypertension, in addition to plaque formation^[94]. The change in endothelial function could be due to either a direct effect or *via* improved blood pressure.

Most of the surveys such as NHANES III^[95], the German National Health Interview and Examination Survey^[96] and the 1958 British Birth Cohort^[97] investigating the relationship between vitamin D and hypertension have pointed to an inverse association. However, there have been studies that have not shown this

association^[98,99]. Once again the mixed findings could have been due to confounding variables common in multifactorial pathology. Similarly prospective studies too have not been consistent with regards to outcome^[100,101]. Further, interventional trials have also resulted in varied results^[102,103]. A meta-analysis of 11 interventional trials showed a modest reduction in diastolic blood pressure (3.1 mmHg), but this was not accompanied by any significant change in systolic blood pressure^[104]. It was evident that most of the studies were not designed to investigate the association in question. Although observational studies have suggested endothelial dysfunction in individuals with hypovitaminosis D^[105,106] results following vitamin D supplements have been missed. While some intervention trials have shown a beneficial effect on endothelial function^[107,108] others have not^[109,110]. Thus, it is clear that although most studies indicate an association between vitamin D status and blood pressure the findings from observational, prospective and interventional studies have not been unanimous.

We have seen that much of the work presented above, with the exemption of Karatas *et al.*^[59], has focussed on individual associations between hypovitaminosis D, vitamin D supplementation and components of the metabolic syndrome. As evident from Figure 1 these factors are inter-related and it is essential that future studies take this into account.

Benefits in mortality, CVD and onset of type 2 diabetes observed following vitamin D supplements

As we have seen previously there is considerable evidence that hypovitaminosis D is associated with increased CVD risk although the mechanisms still remain largely unclear. It is essential to determine if this increase in risk can be reversed by supplements. Many questions remain that can only be answered by long term intervention studies. It is important to estimate benefit in the overall study group as well as subgroups based on age, gender, ethnicity, CVD risk, vitamin D levels and other baseline characteristics. Further, benefit associated with different replacement dosage must be evaluated. To this date no large intervention trial fulfilling the above criteria has reported findings.

Vacek *et al.*^[111] in 2012 carried out an observational retrospective study of 10889 patients seen in a secondary care cardiology setting. Hypovitaminosis (< 30 ng/mL) was diagnosed in 70.3% of this cohort. Vitamin D supplements were taken by 31.6% of the vitamin D deficient patients and 21.3% of patients with normal values and the association between treatment and all-cause mortality studied. Hypovitaminosis D was significantly associated with mortality in patients not on vitamin D replacement (OR = 3.72, 95%CI: 2.563-5.396)^[111]. In contrast hypovitaminosis was not significantly associated with mortality in patients on supplements (OR = 1.46, 95%CI: 0.760-2.799). This analysis was not carried out with CVD mortality and morbidity as an outcome measure. It must be noted

that this study was in a selected population and was retrospective and observational. Gotsman studied the impact of vitamin D supplements on mortality in 3069 patients with heart failure^[112]. Supplementation was associated with significantly reduced mortality (HR = 0.68, 95%CI: 0.54-0.85). However, no convincing data exists regarding the benefits in mortality that may be related to vitamin D supplements in a healthy population.

There are very few studies examining CVD risk reduction following vitamin D supplementation. A systematic review of 17 prospective and randomised trials using vitamin D and/or calcium supplements showed vitamin D supplements, at approximately 1000 IU/d, caused a 10% relative risk reduction that was not significant when compared to placebo^[113]. When the analysis was restricted to the 5 prospective studies of patients receiving vitamin D a reduction in CVD related mortality was observed. It must be noted that 4 of these studies consisted of patients receiving dialysis, a high risk group. Interestingly calcium supplementation did not appear to influence any of the outcome measures.

No large randomised control trial has been carried out with onset of metabolic syndrome/diabetes as the primary outcome. The RECORD study where patients were randomised to receive 800 IU/d of vitamin D recorded onset of type 2 diabetes as a secondary outcome (primary outcome was fracture rate)^[114]. A non-significant 33% relative risk reduction was seen. Similarly, onset of diabetes was the monitored outcome in the Womens Health Initiative Calcium/Vitamin D Trial with 33951 women randomised to either 400 IU/d of vitamin D or placebo for 7 years and no significant benefit was observed^[115]. Most other studies have included smaller patient numbers and have not demonstrated reduced incidence of type 2 diabetes or the metabolic syndrome. Further, no convincing evidence exists that supplementation reduces the progression from the metabolic syndrome to type 2 diabetes.

Mixed results have been observed when insulin sensitivity has been determined following treatment with vitamin D. Mitri *et al.*^[116] demonstrated a significant improvement in insulin secretion in 92 individuals at high risk of developing diabetes following randomisation to either 2000 IU/d of vitamin D supplements or placebo. Nagpal *et al.*^[117] determined the effect vitamin D supplementation (3 doses of 120000 IU) had on insulin sensitivity compared to placebo in 71 healthy male volunteers with central obesity. Insulin sensitivity was seen to improve in the treatment arm^[117]. However, there have been other trials demonstrating no improvement in insulin sensitivity. Luo *et al.*^[118] treated 21 Chinese patients with type 2 diabetes and hypovitaminosis D (≤ 50 nmol/L) with 2000 IU/d of vitamin D for a 3 mo period. No changes were observed in any of the metabolic syndrome parameters, HbA1c or in insulin requirements^[118]. George *et al.*^[119] published a systematic review of 15 trials assessing the effects of vitamin D supplementation compared to placebo on fasting glucose,

glycaemic control and insulin resistance. When all the studies were combined no significant improvement in outcomes was observed. When the analyses were restricted, to patients with diabetes or impaired glucose tolerance, significant but small improvements were observed in both fasting glucose and insulin sensitivity, but no changes seen in HbA1c^[119].

All the studies described above leave an impression that vitamin D supplementation could potentially be beneficial. However, current evidence does not allow us to identify patient groups that would benefit maximally.

Vitamin D and type 2 diabetes

We have focussed this review on hypovitaminosis D in the metabolic syndrome and its defining components as well as CVD. We have described that hypovitaminosis D appears to be related to the metabolic syndrome, potentially a pre-diabetic state and its component characteristics such as obesity and insulin resistance. Thus, we would expect there to be a relationship between vitamin D levels and type 2 diabetes. We have also described current evidence as to the effects of vitamin D supplementation on diabetes control. In addition to actions that may be mediated *via* obesity and insulin resistance which we have described above, hypovitaminosis D appears to have a direct effect on glycaemic control. It has been suggested that vitamin D could have a role in ensuring calcium influx into cells which may be essential to the actions of insulin in skeletal muscle and adipocytes^[120]. There have been hints that elevated parathyroid hormone levels may blunt the actions of insulin^[121]. Although outside the boundaries of this review, an association between type 1 diabetes and hypovitaminosis D also suggests at a direct action of vitamin D on insulin action that may also be relevant to type 2 diabetes^[122].

Vitamin D and non-alcoholic fatty liver disease

Individuals with the metabolic syndrome of long duration are considered to be at greater risk of developing hepatic steatosis^[123]. A two or three hit hypothesis has been proposed^[124]. The first hit is considered to be the damage caused by fatty infiltration associated with insulin resistance and obesity^[124]. The second and third hits are thought to be due to hepatic injury resulting from mechanisms linked to oxidative stress and impaired cellular regeneration^[124]. Hepatic fatty infiltration could progress through non-alcoholic steatohepatitis and liver fibrosis to liver cirrhosis. Management of this spectrum has focused on improving the metabolic syndrome phenotype with weight reduction and management of dyslipidaemia and hyperglycaemia^[125].

As hypovitaminosis D is related to the metabolic syndrome we would expect an association with non-alcoholic fatty liver disease. A review of 6800 patients on the NHANES III database showed that those with an elevated serum alanine transaminase activity were seen to have lower vitamin D concentrations compared to matched controls with normal enzyme levels, the analysis

being corrected for the metabolic syndrome^[126]. This association (independent of age, gender, triglycerides and insulin resistance) was also observed by Barchetta *et al.*^[127] in a study of 262 patients. Further, vitamin D levels were lower in patients with non-alcoholic fatty liver disease diagnosed by liver biopsy^[128]. Hypovitaminosis D has been associated with altered regulation of inflammatory and anti-oxidant pathways in addition to influencing the metabolic syndrome phenotype; all the hits postulated in the aetiology of steatosis^[129]. At present there is no conclusive evidence that vitamin D supplementation could lead to clinical improvement of hepatic steatosis. Interestingly treatment with agents such as ursodeoxycholic acid, which increases vitamin D concentrations, has shown some improvement in non-alcoholic steatohepatitis with alanine transaminase levels used as the outcome^[130]. However, ursodeoxycholic acid may possess direct anti-inflammatory anti-oxidant properties which may be significant confounding factors.

CONCLUSION

It is clear that hypovitaminosis D has extra-skeletal effects that impact on the development of various pathologies including those that make up a large majority of morbidity and mortality; cancer, CVD and diabetes. In this review we have focussed on the association between hypovitaminosis D and the metabolic syndrome. Recently there has been a significant increase in the number of individuals with the metabolic syndrome. Indeed, as much as 40% of the United States population suffers from the condition comprising some or all of a cluster of CVD risk factors. Although the metabolic syndrome does not confer additional risk compared to the component risk factors we believe it helpful to the clinician and researcher to classify patients because it encourages a holistic approach to CVD risk reduction and study of the inter-relationships between the different relevant factors respectively.

There is considerable confusion surrounding the association between vitamin D and the metabolic syndrome, its component factors, CVD and mortality. Although studies have not been unanimous in their findings we are left with the impression that hypovitaminosis D is probably associated with all the above outcomes. However, the nature of this relationship in subgroups (*e.g.*, gender, age groups, ethnicity, *etc.*) is not clear. The risk associated with varying levels of vitamin D has not been estimated. Mechanisms that lead to increased prevalence of the components of the metabolic syndrome and its associated risk have not been worked out. Even more confusing is whether there is any benefit in vitamin D replacement therapy as trials have been contradictory.

However, there appears to be sufficient evidence to make the unravelling of the association between hypovitaminosis D and the metabolic syndrome a priority. Today both conditions are of high prevalence. This suggests that even if a modest decrease in CVD risk is

observed following vitamin D replacement it will translate to substantial overall benefits. Due to the modest price of supplements and relative safety, the cost benefits could be in favour of vitamin D replacement.

What is required are well designed studies, both prospective and intervention. In addition to estimating overall benefit, they must be sufficiently powered to study subgroups as well as risk and benefits at varying serum vitamin D concentrations as well as replacement regimes. It is only following the availability of this data that clear recommendations can be made with regards vitamin D replacement in patients with the components of the metabolic syndrome.

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Mechanisms of hypoglycemia unawareness and implications in diabetic patients

Iciar Martín-Timón, Francisco Javier del Cañizo-Gómez

Iciar Martín-Timón, Francisco Javier del Cañizo-Gómez, Section of Endocrinology, Hospital Universitario Infanta Leonor, Facultad de Medicina, Universidad Complutense, 28031 Madrid, Spain

Author contributions: Martín-Timón I and del Cañizo-Gómez FJ contributed equally to this work.

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Correspondence to: Dr. Francisco Javier del Cañizo-Gómez, Professor, Chief, Section of Endocrinology, Hospital Universitario Infanta Leonor, Facultad de Medicina, Universidad Complutense, Avda Gran Vía del Este 80, 28031 Madrid, Spain. fjcanizog@salud.madrid.org
 Telephone: +34-91-1918000
 Fax: +34-91-1918878

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Abstract

Hypoglycemia unawareness (HU) is defined at the onset of neuroglycopenia before the appearance of autonomic warning symptoms. It is a major limitation to achieving tight diabetes and reduced quality of life. HU occurs in approximately 40% of people with type 1 diabetes

mellitus (T1DM) and with less frequency in T2DM. Though the aetiology of HU is multifactorial, possible mechanisms include chronic exposure to low blood glucose, antecedent hypoglycaemia, recurrent severe hypoglycaemia and the failure of counter-regulatory hormones. Clinically it manifests as the inability to recognise impending hypoglycaemia by symptoms, but the mechanisms and mediators remain largely unknown. Prevention and management of HU is complex, and can only be achieved by a multifactorial intervention of clinical care and structured patient education by the diabetes team. Less known regarding the impact of medications on the development or recognition of this condition in patients with diabetes. Several medications are thought to worsen or promote HU, whereas others may have an attenuating effect on the problem. This article reviews recent advances in how the brain senses and responds to hypoglycaemia, novel mechanisms by which people with insulin-treated diabetes develop HU and impaired counter-regulatory responses. The consequences that HU has on the person with diabetes and their family are also described. Finally, it examines the evidence for prevention and treatment of HU, and summarizes the effects of medications that may influence it.

Key words: Hypoglycemia unawareness; Impaired awareness of hypoglycemia; Hypoglycemia associated autonomic failure; Diabetes mellitus; Counter-regulation

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Core tip: This review describes novel mechanisms by which people with insulin-treated diabetes develop hypoglycemia unawareness (HU), the consequences that HU has on the person with diabetes and their family, the evidence for prevention and treatment of HU, and the effects of medications that may influence it.

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INTRODUCTION

Hypoglycemia is usually defined as a plasma glucose level < 70 mg/dL (3.9 mmol/L)^[1]. Since the brain is permanently dependent on glucose, strong counter-regulatory mechanisms exist to quickly increase glucose levels to protect the human body from the negative consequences of hypoglycemia. Counter-regulatory response to hypoglycemia (Figure 1) includes inhibition of the endogenous insulin secretion and stimulation of glucagon, catecholamines (norepinephrine, epinephrine), cortisol and growth hormone secretion, which all together stimulate hepatic glucose production and cut down glucose utilization in peripheral tissues, increasing in this way plasma glucose levels. As glycaemia comes down, the activation of the autonomic nervous system leads to neurogenic symptoms (palpitations, sweating, hunger, anxiety, tremors, etc.), which allows the perception of hypoglycaemia (hypoglycaemia awareness) (Figure 2).

Hypoglycemia unawareness (HU) is defined as the onset of neuroglycopenia before the appearance of autonomic warning symptoms^[2] or as the failure to sense a significant fall in blood glucose below normal levels^[3]. In patients with type 1 (T1DM) or type 2 diabetes mellitus (T2DM), recurrent hypoglycemia has been shown to reduce the glucose level that precipitates the counter-regulatory response necessary to restore euglycemia during a subsequent episode of hypoglycemia^[4,5].

HU was observed in 40% T1DM patients^[6] and less frequently in T2DM patients with low C-peptide levels. The presence of HU increases the risk of severe hypoglycaemia (six-fold for T1DM^[7] and 17-fold for T2DM^[8]). HU is more common in individuals with longer duration of diabetes, history of recent and/or recurrent hypoglycaemic events, patients with intensive glycaemic therapy and in advanced age^[9].

Presently, the major risk factors for the development of HU are duration of the disease and improved metabolic control. The severity of HU was associated with longer diabetes duration and with a history of frequent low glycaemic levels^[6], whereas aging and the blood glucose decreasing rate using professional continuous glucose monitoring systems (CGMS), which falls from near blood glucose level, were risk of severe HU^[10]. Data from Pittsburgh Epidemiology of Diabetes Complications^[11] showed that diabetes duration, HbA1c and intensive insulin therapy predicted HU in men, whereas severity and frequency of hypoglycemia, QTc interval and hypertension predicted HU in women. Thus, women are more likely to have HU, which unlike in men, is also marginally related to hypertension, QTc interval

and hypoglycemia. On the other hand, in patients with T1DM, HU was 3.4-fold more common among patients homozygous for Gly16 than among patients with other variants of the Arg16Gly polymorphism, so that T1DM patients who carry two alleles of the Gly16 variant of ADRB2 are at increased risk of developing HU^[12]. Finally, in both T1 and T2DM patients with impaired HU, hypoglycemia-induced electroencephalogram changes, such as increased theta band amplitude, were not affected by antecedent hypoglycemia^[13].

This article reviews recent advances in how the brain senses and responds to hypoglycemia, novel mechanisms by which people with insulin-treated diabetes develop HU and impaired counter-regulatory responses. The consequences that HU had on the person with diabetes and their family is also described. Finally, it examines the evidence for prevention and management of HU, and summarizes the effects of medications that may influence it.

MECHANISMS OF HU

Aberrant glucose counter-regulation (as a result of a failure in the reduction of insulin production and an increase in glucagon release), and HU (as the result of an attenuated increase in sympathoadrenal activity) are the components of hypoglycemia-associated autonomic failure (HAAF) in diabetic patients. HAAF is most often caused by recent/recurrent iatrogenic hypoglycemia, and indeed HAAF is maintained by recurrent hypoglycemia^[14,15] (Figure 3).

Diverse causes of HAAF and HU in diabetes^[16]

Catecholamines: Previous hypoglycemia leads to a blunted catecholamine response to a following episode of hypoglycemia. This has been demonstrated in several studies; for example Ramanathan *et al.*^[17] showed that intravenous infusion of adrenergic blockers on one day of a hypoglycemia prevent the counter-regulatory failure in the response on the next day of hypoglycemia. This study implicates that HAAF needs a previous hypoglycemia (with its sympathoadrenal responses). If we use this hypothesis to think in a possible pharmacologic treatment, we can conclude that blocking the action of catecholamines we can limit the development of HAAF and protect against subsequent hypoglycemia; but unfortunately, blocking the action of catecholamines in periphery we would tend to an increase in the severity of hypoglycemia. We would need to develop a selective adrenergic receptor modulators that favourably change central nervous system response without modify the beneficial peripheral effects of the sympathoadrenal response.

Sleep: Sleep is a peripheral mediator of HAAF linked with catecholamine response. Patients with T1DM, while they are sleeping, they have a significantly decreased epinephrine response to hypoglycemia^[18], and also a

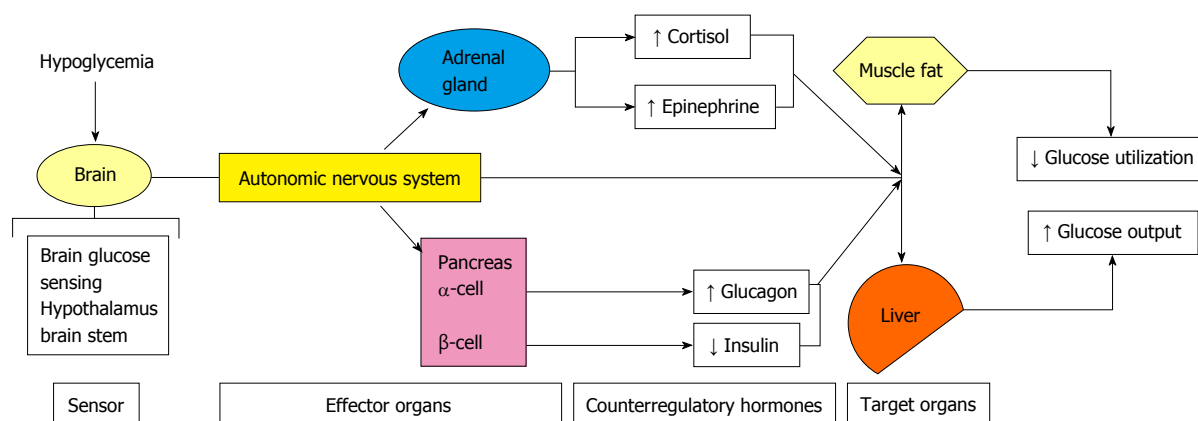


Figure 1 Counter-regulatory response to hypoglycemia.

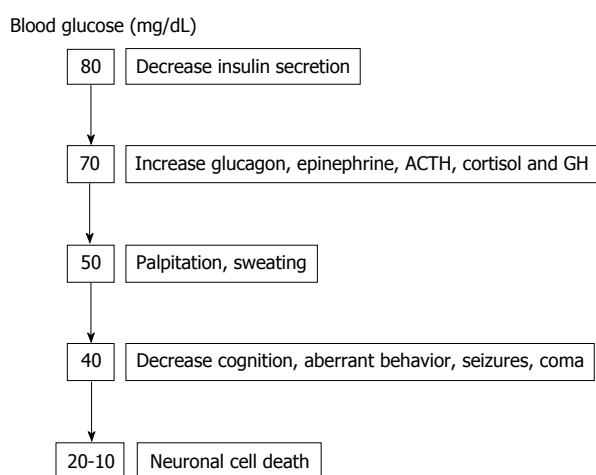


Figure 2 Symptoms and signs associated with progressive hypoglycemia. ACTH: Adrenocorticotrophic hormone; GH: Growth hormone.

reduced awakening from sleep during hypoglycemia^[19]. So, because of the HU and the impaired adrenomedullary response, we can explain some of the overnight deaths of healthy young people with T1DM.

Cortisol: Hypoglycemia is associated with an elevation in systemic corticosteroids, and this has been proposed to feedback to the hypothalamus contributing to HAAF^[20-22]. However it remains controversial if the endogenous hypercortisolemia is of sufficient magnitude to blunt the counter-regulatory response to hypoglycemia^[23,24]. It has been shown that corticotrophin releasing hormone agonist impair the counter-regulatory response to a subsequent hypoglycemia, suggesting a possible role in HAAF^[25].

Opioids: Preclinical and clinical studies with opioids demonstrated a rise in endogenous opioids during hypoglycemia, for example naloxone (an opioid receptor blocker), increased the sympathoadrenal response to hypoglycemia, and when is infused during previous hypoglycemia, it prevent HAAF^[26,27]. Hence there is a potential therapeutic function for opioid receptor blockade to protect against HAAF.

Exercise: The inability to reduced circulating insulin during exercise, lead T1DM patients, at an increased risk for hypoglycemia during or after exercise. In addition to, during exercise the opioid beta endorphin is released to activate the sympathoadrenal response. In a recent study, healthy individuals who exercised and elevated endorphin levels, they had reduced catecholamine response during hypoglycemia in the next day^[28], suggesting that endogenous opioids, again, play a role in HAAF, and that blocking their action may protect against exercise-autonomic failure.

Recurrent hypoglycemia and HU

Clinically HAAF can be viewed as both, maladaptive or adaptive response^[29]. At one end, patients with T1DM and HU make tests of cognitive function during hypoglycemia better than patients with HU. Additionally, the time necessary for complete cognitive recovery after restoration of normoglycemia is faster in patients who have HU^[30]. HAAF in humans may be similar than in rats; rats with recurrent moderate hypoglycemia had less brain cell death^[31] and less mortality during or following marked hypoglycemia than those without recurrent hypoglycemia. On the other hand, HAAF is without doubt a maladaptive response if we consider that defective glucose counter-regulation and HU rise the risk of severe hypoglycemia with its morbidity and potential mortality^[32].

Although it is well established that recurrent hypoglycemia leads to HU, the mechanism responsible for this are unknown. Several current mechanistic hypotheses are discussed below.

The brain glucose transport or glucose metabolism hypothesis

Several studies have identified specific brain regions that exhibit decrease glucose uptake. In diabetic patients with and without HU, the effects of acute moderate hypoglycemia and the condition of HU on regional brain uptake of the labeled glucose analog [(18)F]fluorodeoxyglucose (FDG) using positron emission tomography were examined^[33,34]. In the group with hypoglycemia awareness, there was an increase

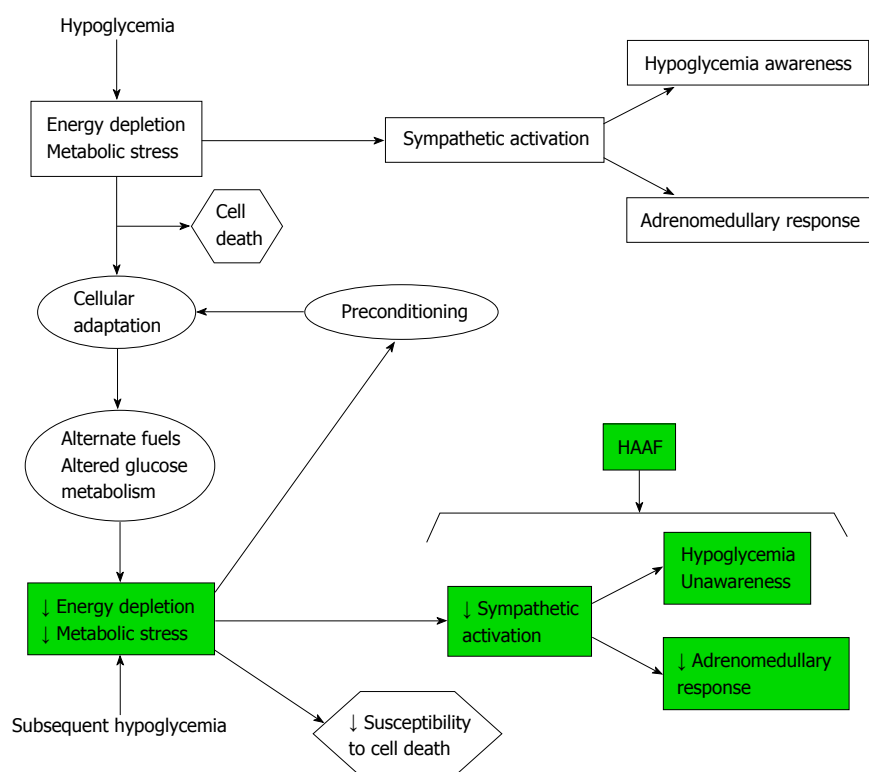


Figure 3 Recurrent hypoglycemia leads to cellular adaptation and hypoglycemia-associated autonomic failure. HAAF: Hypoglycemia-associated autonomic failure.

in the normalized FDG uptake in a subthalamic brain region^[33], in left amygdale and in bilateral ventral striatum^[34] in response to hypoglycemia; whereas in the group with HU the uptake in these brain regions fell significantly^[33,34]. Reduced responses in these brain regions in HU, suggest habituation of higher behavioral responses to hypoglycemia as a basis for unawareness, and demonstrated a change in its metabolic function associated with the failure to trigger a counter-regulatory response. On the other hand, in subjects with T1DM and HU a positive correlation was observed between thalamic response and epinephrine response to hypoglycemia, suggesting that this brain region may be involved in the coordination of the counter-regulatory response to hypoglycemia^[35]. During recurrent hypoglycemia, cerebral blood flow reduced significantly in the thalamus and hypothalamus of T1DM subjects, compared to healthy controls^[36], suggesting that there is reduced neuronal activation in these brain regions that participate in glucose sensing and/or coordination of counter-regulation response in subjects with T1DM that likely contributes to the development of HU.

It has been hypothesized that recurrent hypoglycemia leads to HU through an alteration in the glucose transport or metabolism. Altered glucose transport or metabolism as a cause of HU is less substantiated in humans. Subjects with T1DM and HU had significantly higher brain glucose concentrations compared to that in controls under the same conditions^[37]. These data suggest that changes in brain glucose transport or metabolism may occur as a result of recurrent hypoglycemia.

The brain glycogen supercompensation hypothesis:

It has been hypothesized that increased brain glycogen contributes to the development of HU and impaired sympathoadrenal responses by providing energy for the brain during periods of systemic hypoglycemia. Experimental studies and in humans have shown that after one or more episodes of hypoglycemia, increased glycogen content in the brain^[38,39]. Subsequent studies indicated lower glycogen content in brain of humans with T1DM, implying that glycogen supercompensation does not contribute to the development of HU^[40]. The most important question to resolve is whether changes to brain glucose levels, physiologically or pharmacologically induced, may provide people who suffer from recurrent hypoglycemia a therapeutic benefit to preserve both the sympathoadrenal response and HU.

The brain fuel hypothesis:

When there is a decrease in the supply of glucose from the periphery, the brain may be able to keep your metabolic processes by increasing uptake of alternative carbon fuels such as lactate or ketones. Plasma lactate concentrations are approximately tenfold higher than those of acetate, making it a primary candidate as an alternative brain fuel during hypoglycemia. On the other hand, increased of blood-brain barrier monocarboxylic acid (MCA) transport and metabolism among T1DM individuals with HU may be a mechanism to supply the brain with non-glucose fuels during episodes of acute hypoglycemia and may contribute to the maintenance of brain energetic during hypoglycemia and to the syndrome of HU, independent

of diabetes^[41]. Finally, in T1DM patients with HU, upregulation of the MCA transporter promotes increased brain lactate uptake^[42].

The brain neuronal communication hypothesis:

Neuronal communication relies on the release of classical neurotransmitters, such as Gamma-Aminobutyric Acid (GABA), a potent inhibitory neurotransmitter. GABA levels in ventromedial hypothalamus (VMH) interstitial fluid are decreased during acute hypoglycemia^[43]. Recurrent hypoglycemia leads to a significant increase in VMH GABA concentrations^[44], that fail to decrease normally during subsequent hypoglycemia, and which correlates with the reduced glucagon and epinephrine responses^[45]. These data suggest that recurrent hypoglycemia results in increased VMH GABA inhibitory tone, and that altered GABA tone may be an important common mediator in the development of HAAF, especially in diabetic patients.

CONSEQUENCES OF HU

Consequences of HU on morbidity, mortality, and cardiovascular outcomes

People who have HU have a much greater risk of severe hypoglycemia, up to six fold, with its attendant morbidity^[46,47]. HU may result in many serious forms of morbidity including seizure, coma, fractures and joint dislocation and cardiac arrhythmias, and is occasionally fatal.

Severe episodes of hypoglycemia or HU requiring the assistance of another have been shown to be associated with an increased risk of mortality in both the Action to Control Cardiovascular Risk in Diabetes (ACCORD)^[48] and the Action in Diabetes and Vascular Disease^[49] studies. On the other hand, *post hoc* analysis of the ACCORD study cohort, to examine the relationship between frequent and unrecognized hypoglycemia and mortality, 10096 ACCORD study participants were included. In this study, recognized and unrecognized hypoglycemia was more common in the intensive group than in the standard group; and in the intensive group, a small but statistically significant inverse relationship was identified between the number of hypoglycemic episodes and the risk of death among participants^[50]. This latter finding does not mean that we should change our clinical practice and include frequent episodes of hypoglycemia in the targets of T2DM patients and cardiovascular risk factors. Instead, we must strive to achieve optimal glycemic control in our patients, without episodes of hypoglycemia.

Consequences of HU on adults with T1DM

Several prospective studies as the Diabetes Control and Complications Trial^[51] and the Stockholm Diabetes Intervention Study^[52] suggests that cognitive function does not deteriorate in patients with T1DM who suffer recurrent hypoglycemia, at least less than 10 years of these studies.

Gold *et al.*^[53] to compare the degree of cognitive

dysfunction experienced by T1DM patients who had normal awareness of the onset of hypoglycemia with patients who had history of impaired awareness of hypoglycemia, found that T1DM patients with HU exhibited more profound cognitive dysfunction during acute hypoglycemia which persisted for longer following blood glucose recovery. Intellectual activity is likely to be affected and cause sub-optimal performance during this recovery period. Recent investigations with advanced imaging techniques have demonstrated that adults with T1DM appear to call upon a greater volume of the brain to perform a working memory task during hypoglycemia^[54]. These findings suggest that adults with T1DM must recruit more regions to preserve cognitive function during hypoglycemia than adults without the disease.

Evidence of clinical audit in T1DM patients with intensive insulin therapy with HU showed that these patients had less adhesion to changes in insulin regimens to compare them with patients with hypoglycemia awareness, despite the observed increase in clinical contacts^[55]. Neuroimaging studies have shown that patients with HU showed a reduced activation in appetitive motivational networks associated with integrated behavioral responses to hypoglycemia^[34]. This may suggest that in some patients with HU behavioral strategies are more important than educational strategies; however treatment of HU will require a combination of both strategies, behavioral and educational, along with the use of technology, such as therapy with continuous insulin pump and online glucose monitoring^[56].

Consequences of HU in children and adolescents with T1DM

A significant proportion of children and adolescents with T1DM have HU. Screening for HU is an important component of routine diabetes care and can identify patients at increased risk of severe hypoglycemic events^[57]. The youngest patients are most vulnerable to the adverse consequences of hypoglycemia. Ongoing maturation of the central nervous system puts these children at greater risk for cognitive deficits as a consequence of HU^[58]. HU is a significant problem for children and adolescents with T1DM and the major risk factor for development of hypoglycemia^[57]. Those children with T1DM diagnosed before age of 6, who suffer repeated and severe episodes of hypoglycemia may have more increased range of cognitive dysfunction, brain abnormalities^[59], structural brain changes^[60], lower mental abilities latter on in life, and behavior problems than those who do not have HU until latter^[61,62].

Consequences of HU on subjects with T2DM

HU is less common in T2DM patients. Two retrospective surveys of subjects with insulin-treated T2DM showed that only 8% and 9.8% respectively had HU estimated by a validated scoring system^[8,46]. However, in the patients with HU the incidence of severe hypoglycemia was nine-fold and 17-fold higher respectively than

those with normal hypoglycemia awareness^[8,46]. In several studies, using continuous monitoring system, asymptomatic hypoglycemia was detected in 47%^[63] and 56%^[64] of subjects with T2DM, treated with different treatment regimes. These findings suggest that HU may be more prevalent in T2DM than is appreciated.

Severe hypoglycemia, due to HU, was associated in T2DM patients with cardiovascular and neurological complications^[1,48]. In patients with T2DM and coronary artery disease, severe hypoglycemia was associated with ischemic electrocardiogram changes and chest pain, and may account for sudden mortality^[65,66]. In a retrospective study in T2DM subjects, the patients who experienced outpatient severe hypoglycemia were also shown to have a 79% higher odds ratio of experiencing acute cardiovascular events than patients without severe hypoglycemia^[67]; and a case-control study in patients with T2DM showed a 65% increase in the odds of myocardial infarction with severe hypoglycemia within the previous two weeks; the risk of myocardial infarction persisted elevated for up to six months following a hypoglycemic event^[68].

Behavioral changes, cognitive impairment, seizures, coma and a mortality rate estimated at between 4.9% and 9% are well-known neurological complications of severe and prolonged hypoglycemia secondary to HU^[69-71]. Severe hypoglycemia secondary to HU can cause neuronal cell death and may damage regions of the brain that oversee memory, especially in older people with T2DM^[72].

Finally, a frequently problem in T2DM is nocturnal hypoglycemia. Undetected nocturnal hypoglycemia often contributes to HU. Nocturnal hypoglycemia has been associated with cardiac arrhythmias resulting in sudden death^[73].

Consequences of HU on the elderly

Patients in the older age-groups are especially vulnerable to HU. Aging modifies the cognitive, symptomatic, and counter-regulatory hormonal responses to hypoglycemia^[74]. Older adults with diabetes are at much higher risk for the geriatric syndrome, which includes falls, incontinence, frailty, cognitive impairment and depressive symptoms^[75]. In the elderly subjects, episodes of severe hypoglycemia are more likely to be followed by changes in the blood brain circulation which may further increase the risk of neurological damage in this population^[76,77]. In older patients with T2DM, Whitmer *et al.*^[72] found a significant association between the number of severe hypoglycemic episodes and dementia; with ≥ 3 episodes almost doubling the risk more episodes of severe hypoglycemia secondary to HU had increasing likelihoods of being subsequently diagnosed with dementia. Another authors also found an association between severe hypoglycemia and cognitive impairment in these patients^[78]. These reports suggest that severe hypoglycemia and HU in older people with

diabetes may be associated with cognitive decline^[79].

Consequences of HU during pregnancy

Pregnancy is associated with a high risk of severe hypoglycemia in diabetic subjects. History of HU has been documented as risk factors of severe hypoglycemia during pregnancy^[80-82]. Reduced sympathoadrenal responses during hypoglycemia may contribute to defective glucose counter-regulation and HU^[83,84]. In pregnant woman severe hypoglycemia episodes and HU occur three to five times more frequently in first trimester than third trimester when compared with the incidence in the year preceding the pregnancy^[80,81,85] and may lead to severe morbidity and even death^[86].

Consequences of HU on quality of life and social impact

Hypoglycemia and HU are associated with significant reductions in quality of life measures in both T1DM and T2DM patients^[87-89]. The wellbeing of patients may be affected both from the effects of hypoglycemia and from fear of recurrence^[89,90]. A positive association was found between severity and/or frequency of hypoglycemic events and greater fear of hypoglycemic episodes^[71]. As a result fear of hypoglycemia makes the patients to promote compensatory behaviors in a way to have less episodes of hypoglycemia such as decreased insulin doses resulting in negative glycemic control, and an increased risk of serious health consequences^[91]. Patients with recurrent hypoglycemia and HU were more likely to have a lower quality of life in several parameters including depression and anxiety^[89,92,93], increased pain and limitations in mobility and usual activities^[89], and decline in the quantity and quality of sleep^[94]. On the other hand, young adults with T1DM reported the presence of interpersonal conflict, and difficulty talking about issues related to hypoglycemia with significant others^[95], that may carry over to their work life, where hypoglycemia has been linked to reduced productivity^[88].

Despite that many countries require documentation that severe hypoglycemia and HU is not occurring before persons with diabetes are permitted to have a license to operate a motor vehicle; HU has not consistently been associated with an increased risk of car collisions^[96-98].

Consequences of HU on family members

In the subjects with diabetes, HU can have a profound impact on the lives of their family members, and are often reliant on immediate relatives or partners to detect and treat hypoglycemia episodes. A recent study based in-depth interviews with 24 adult family members of persons with T1DM and HU, showed that family members restricted their own lives in order to help the person with HU to detect and treat hypoglycemia^[99]. In this study, some family members of people with HU, report that they are afraid of their partners, during episodes of hypoglycemia because of their aggressive behavior and their personality changes, making it difficult managing their treatment. The study showed that family

members of patients with HU restricted their own lives in order to help the person with HU to detect and treat hypoglycemia, and felt anxious about the safety of the person with HU; which sometimes leads family members to neglect their own health, leading to resentment over time^[100]. On the other hand, personality changes during hypoglycemia events of the person with diabetes, such as aggression, also caused, in some family members, physical fear of your partner or relative, and made treatment difficult. Family members emphasized that there is an unmet need for information and emotional support for caregivers, and the researchers suggest that proactive support for the families of patients with diabetes and HU should be considered and provided by healthcare professionals^[99].

Psychological consequences of HU

The psychological consequences of HU include subsequent fear to hypoglycemia, and secondary poor treatment compliance, increased anxiety and decreased levels of satisfaction and happiness. Fear of hypoglycemia will be a barrier to achieving good glycemic control. The hypoglycemia fear survey (HFS) used to measure behaviors (HFS-B) and worries (HFS-W) related to hypoglycemia in adults with T1DM, such as maintaining higher blood glucose levels than recommended, and limiting exercise or physical activity, or concerns may have about hypoglycemic episodes, such as nocturnal episodes; have been shown to be significantly higher in women than in men and among patients who have experienced severe hypoglycemia in the past compared with those that have not^[100]. If patients experience repeated severe hypoglycemic events, both the patient's and the physician's subsequent treatment policy are affected. In one study that reviewed hospital records and examined daily insulin doses and HbA1c levels before and after an episode of severe hypoglycemia in patients with insulin-dependent diabetes, it was found that, in 69% of these cases, either the physician or patient or both decreased the daily insulin dose. Furthermore, physicians decreased the insulin dose in a third of patients in whom the cause of hypoglycemia was preventable and due to a cause other than erroneous administration of excess insulin^[101].

Economic consequences of HU

The economic consequences of severe hypoglycemia events and HU in patients with diabetes are higher than that of a mild episode and have been examined in a number of studies in Europe and United States^[102-105]. Reported costs of a severe hypoglycemic event varied from approximately \$80 to \$5000, depending on the requirement for resources including hospitalization, emergency services, healthcare professionals and diagnostic test.

A United Kingdom study estimated the total cost of emergency treatments of 244 episodes of severe hypoglycemia in 160 patients with T1DM and T2DM over the course of one year. The total cost was approximately

£92078 (£400 per episode)^[102]. On the other hand, in a Swedish study the total cost (direct and indirect) of severe hypoglycemia in T2DM patients was between \$12.90 and \$14.10 for one month period^[90].

An analysis of several United States studies, the estimated annual total cost attributable to severe hypoglycemia was between \$1400 and \$1500^[106]. In this analysis the estimated work days lost per hypoglycemic event was between 0.22 and 6.60 d^[103]. A recent study estimated that in patients with diabetes who experienced severe hypoglycemia, the lost of productivity ranged from \$15.26 to \$93.47 per severe hypoglycemic event, representing 8.3-15.9 h of lost work time per month^[106]. Among the patients who experimented a severe hypoglycemic event at work, 18.3% missed work for a mean duration of 9.9 h, whereas the patients who had severe hypoglycemic event outside working hours, 22.7% arrived late for work or missed a full day^[104]. If the hypoglycemia has occurred during the night, the number of working hours lost increased to 14.7 h^[104].

PREVENTION AND MANAGEMENT OF HU

Prevention of HU

Prevention of HU is an important part of modern day intensive diabetes therapy. To prevent HU, the goal is the complete avoidance of hypoglycemia, which is very difficult to achieve^[105]. Blood glucose monitoring, individualized targets and educational programs are important in the bid to prevent and manage HU.

Blood glucose monitoring: CGMS, that can detect hypoglycemia, represents an important technological advance on the methods used for self-monitoring of blood glucose, and they are welcome to both patients and clinicians^[106]. The ability of CGMS systems is to advise patients when glucose levels fall too low or rise too high, and has the potential to reduce the duration of hypoglycemia and hyperglycemia events^[107,108]. Also, CGMS can be used for objective detection of patients with HU^[109]. In adult patients with long-standing T1DM, a fasting level of C peptide of ≤ 0.6 ng/mL, and a HbA1c $\leq 9\%$, hypoglycemic episodes with a duration more than 90 minutes detected by CGMS, identified patients who had HU with an 88% specificity and 75% sensitivity^[109]. On the other hand, the epinephrine response to hypoglycemia in adolescents patients with T1DM with HU was greater after the use of real-time CGMS with low glucose alarms than with standard medical therapy alone^[110]. This suggests that real-time CGMS is a useful clinical tool to improve HU in adolescents with T1DM^[110]. Choudhary *et al.*^[111] assessed the effect of CGMS on the frequency of severe hypoglycemia episodes, using the Gold scoring method^[46] in 35 people with T1DM who have HU, *via* retrospective audit. A significant decline was observed in the mean rate of severe hypoglycemia (8.1 to 0.6 events per year) and also in HbA1c level

(8.1% to 7.6%), between its initiation and the end of the 1-year follow-up period; while the mean Gold score did not change significantly^[111]. These results support previous reports that CGMS can lower the incidence of severe hypoglycemia in patients with T1DM and HU, with no impact on the severity of HU over a 1-year period. A randomized cross-over study to assess the effects of CGMS use on glycemic levels and quality of life in patients with T1DM and HU, using the change in the Gold scoring as one of the secondary endpoints, is currently in progress and the results will not be available until 2015^[112].

The impact of closed-loop CGMS, which link CGM technology with insulin pumps, whereby insulin infusion is programmed to stop automatically when glucose levels drop below a pre-determined glycemic threshold, on reducing the incidence of hypoglycemia events appears to be limited and so their usefulness in improving HU is debatable^[16].

Individualized targets: In diabetic patients with HU blood glucose targets should be relaxed but not abandoned. Appropriate targeting of plasma glucose may help patients and practitioners achieve HbA1c goals, reduce excessive self-testing and minimize the occurrence of severe hypoglycemic events^[113]. Glycemic goals should be individualized with some degree of safety particularly for patients with long duration of diabetes, patients who have a high risk of HU and severe hypoglycemia development, and/or subjects with multiple co-morbidities^[114,115]. Basically, an HbA1c goal of less than 7% remains recommended, but is there a safe range for HbA1c? In patients with T1DM undertaking insulin therapy, the rates of severe hypoglycemia were increased among those with HbA1c < 6% and therefore it was suggested that using current therapy, an HbA1c of between 6%-7% represents the best compromise between the risk of severe hypoglycemia and that of developing microvascular complications^[116].

Educational programs: The central objective of a hypoglycemia-reversal program is to prevent any period of hypoglycemia for at least four weeks. In diabetic patients with HU an appropriate educational program includes an emphasis on regular snacks at right times, warnings to take special care at periods of greater risk such as before lunch, moderation in alcohol intake and about the danger of delayed hypoglycemia after heavy alcohol intake or prolonged exercise. Diabetes self-management education can have physical and psychosocial benefits, and results in behavior changes with positive influence in outcome. A self-awareness intervention of 8 sessions, each lasting 3 h, was designed to determine whether there are psychosocial and physical benefits of self-awareness intervention in 29 adults with T1DM and HU. Post-intervention the participants detected more cues of euglycemia and hypoglycemia and experienced significant increases in integration and metabolic control^[117].

In a randomized, prospective multi-centre trial, the effect of a specific training program for patients with hypoglycemia problem was compared with a control group receiving a standardized education program aiming of at avoidance of hypoglycemia by optimization of insulin therapy^[118]. Compared to control group, the specific training program demonstrates additional benefits in terms of improving HU, reducing mild hypoglycemia, and detecting and treating low blood glucose^[118]. In the Dose Adjustment for Normal Eating-Hypoglycemia Awareness Restoration study, a 6-wk pilot intervention using motivational interviews and cognitive behavioral techniques around hypoglycemia, in 23 people with HU; support the importance of educational programs to improve HU. One year after the intervention HU had improved, mean rates of severe hypoglycemia fell from 3 to 0 per person per year, and worry and behavior around hypoglycemia improved^[119]. In a sub-study of HypoCOMPASS trial aimed to assess the restoration of impaired hypoglycemia awareness and defective hypoglycemia counter-regulation by an educational strategy targeted at hypoglycemia avoidance, in 18 adults patients with T1DM; following the 6-mo intervention the mean glucose concentration at which participants first experienced symptoms of hypoglycemia significantly increased from baseline (from 2.6 to 3.1 mg/dL), and counter-regulatory responses to hypoglycemia were also enhanced^[120].

Jointly, the results of these three studies suggest that interventions that include education around hypoglycemia avoidance may help to decrease HU.

Treatment of HU

The treatment options for the management of HU are listed in Table 1.

Optimizing insulin treatment: It is important that in patients with a history of recurrent hypoglycemia and HU, the time of episodes be identified and the treatment regimen be adjusted accordingly^[121]. Compared with regular insulin, rapid-acting insulin analogs have a more rapid onset of action, higher peak action, and shorter duration of action, which more closely approximates endogenous mealtime insulin response, allowing more flexibility in the time of meals and exercise, and, consequently, a lower risk of severe hypoglycemic events^[122]. Similarly, long-acting insulin analogs exhibit a more consistent, longer, and flatter action profile than NPH insulin, and demonstrate a lower risk of hypoglycemia, particularly nocturnal^[123,124]. In diabetic patients with HU substitution of regular insulin with rapid-acting insulin analogs (aspart, lispro or glulisine) reduces frequency of daytime hypoglycemia; and substitution of long-acting insulin analogues (detemir or glargine) for intermediate-acting insulin (NPH or premix) reduces the frequency of nocturnal and day time hypoglycemia^[121,125]. Compared with insulin glargine, the newest basal analog insulin degludec offers a more constant time-action profile, a long duration of action, and a lower risk of

Table 1 Treatment options for the management of hypoglycemia unawareness and mechanisms of action

Treatments options	Mechanism of action
Optimizing insulin treatment	Avoidance of hypoglycemia
Pharmacological therapy	
β 2-adrenergic agents	Enhancement of adrenaline effect
Methylxanthine derivatives (caffeine, theophylline)	Central nervous system stimulation
Serotonin reuptake inhibitors (fluoxetine, sertraline, paroxetine)	Unknown. It has been hypothesized that the effect could be mediated by an atypical presentation of serotonin syndrome that will lead to autonomic dysfunction
KATP channel modulators	Modulation of hypoglycemia sensing
Other treatments	
Islet cell transplantation	Improving metabolic control
Fructose	Modulation of hypoglycemia sensing

hypoglycemia^[126,127]. While clinical experience with insulin degludec is limited, a meta-analysis evaluating 5 clinical trials of 3372 subjects with T2DM demonstrated a 17% lower rate of overall hypoglycemia and a 32% lower rate of nocturnal hypoglycemia with insulin degludec, compared with insulin glargine^[128]. These characteristics may facilitate the achievement of glycemic control with insulin degludec with fewer hypoglycemic events in patients with HU.

An alternative approach is to use continuous subcutaneous insulin infusion (CSII). A study was designed by Giménez *et al*^[129] to evaluate the effect of CSII on hypoglycemia awareness and on glucose profile in a cohort of T1DM subjects in which 95% had established HU and had experienced two or more episodes of severe hypoglycemia in the preceding two years, for a 24-mo period. Severe hypoglycemic episodes fell from 1.25 per subject-year to 0.05 after 24 mo, an improvement in all the aspects of quality of life, and an improved symptomatic response to experimentally-induced hypoglycemia was observed^[130]. Previous studies^[130-132] have also shown a reduction in hypoglycemia with CSII, particularly when a short-acting insulin analogue is used^[2,133]. The decrease is partly due to better pharmacokinetic delivery of insulin and a 15%-20% reduction in insulin requirements compared with multiple doses of insulin^[134]. Substitution of CSII for NPH insulin in patients with T1DM, especially at bedtime, resulted in a lower frequency of hypoglycemic episodes, and improved counter-regulatory and symptomatic responses during subsequent acute hypoglycemia^[135]. On the other hand, administration of bolus doses of glucagon at times of impending hypoglycemia during CSII lowered the frequency of hypoglycemia^[136].

Pharmacological therapy: β -adrenergic antagonists or β -blockers alter the effects of epinephrine and could have potential effects on glucose homeostasis and the hypoglycemic counter-regulatory system. The more troubling concern regarding β -blockers is their potential effect on HU and blunting of the return to euglycemic levels after hypoglycemia has occurred, through the suppression of all adrenergically mediated symptoms of hypoglycemia. In patients with T1DM without HU, adrenergic symptoms did occur at lower glucose levels

when subjects were treated with β -blockers^[137]. Cardio-selective β -blockers cause less alteration in the perception of hypoglycemia and may have an effect on correction of hypoglycemia than do their noncardioselective counterparts^[138]. These agents should not be avoided in patients with diabetes but should be used with the same caution as when any new medication is added to a patient's therapeutic regime.

It has been suggested that people with HU may have reduced β -adrenergic sensitivity, and this can be reversed by strict avoidance of hypoglycemia^[139]. In T1DM patients, the use of β -adrenergic agonist terbutaline was associated with statistically significant higher glucose levels compared to control subjects during the first half and second half of the night, and with reduction of nocturnal hypoglycemic episodes (22 in the control group vs 1 in the group of terbutaline). β -adrenergic agonist had therefore been suggested as possible therapeutic options for HU, at the cost of inducing morning hyperglycemia. One of the concerns about using β -adrenergic agonist for the treatment of HU was associated with reduced β_2 sensitivity observed *in vitro*. A recent study from De Galan *et al*^[140] showed that sensitivity to β_2 -adrenergic receptor agonist stimulation is preserved in T1DM patients with HU. No long-term clinical trials to evaluate the usefulness of β -adrenergic agonist in the prevention of HU have been reported.

Several studies have evaluated the effects of the methylxanthines derivatives caffeine and theophylline on HU and the counter-regulatory response to hypoglycemia. Both have been shown to augment symptom intensity and improve counter-regulatory responses in patients with T1DM with and without HU^[2,141]. Using functional magnetic imaging, caffeine can restore regional brain activation normally lost during acute hypoglycemia^[142]. In another trial designed to assess the impact of caffeine on the frequency and perception of hypoglycemia over a 3-mo period; patients receiving caffeine (200 mg/twice-daily) had statistically significant more symptomatic hypoglycemia episodes and more intense warning symptoms than patients receiving placebo^[143]. These results suggest that modest amounts of caffeine enhance the sensitivity of hypoglycemia warning symptoms in patients with T1DM without increasing the incidence of severe hypoglycemia. de Galan *et al*^[144] planned one

study to evaluate the impact of theophylline on the response to hypoglycemia in 15 patients with T1DM who had a history of HU and 15 matched healthy control subjects. When compared with placebo, theophylline (2.8 mg/kg) improves the counter-regulatory response to a perception of hypoglycemia in the group with T1DM with HU^[144]. Although modest doses of caffeine and theophylline may be effective at reducing HU in patients with T1DM at a low cost and without significant toxicity, larger doses may carry risk, and large trials are needed to determine efficacy, toxicity and dose-response curves.

The development of HU was associated with the use of selective serotonin reuptake inhibitors (SSRIs) in three patients with T1DM treated with different SSRIs (fluoxetine, sertraline and paroxetine) for depression and who were previously able to recognize and treat hypoglycemia symptoms^[145]. HU occurred in all three patients within weeks of starting SSRI therapy. HU reversed after discontinuation of SSRI therapy^[145]. The mechanism by which SSRIs might be associated with HU is unknown, but it has been hypothesized that the effect could be mediated by an atypical presentation of serotonin syndrome that will lead to autonomic dysfunction^[146]. These observations suggest that in some patients, treatment with SSRIs may alter the perception of hypoglycemia, and should be used with caution in diabetic subjects with HU.

Infusion of the opioid-receptor antagonist naloxone increases the plasma epinephrine response to hypoglycemia and, when administered during hypoglycemia prevents attenuation of the plasma epinephrine response to subsequent hypoglycemia in humans^[26,27].

Administration of a selective Kir6.2/SUR-1 K_{ATP}-channel agonist increases the epinephrine response to hypoglycemia in rats^[147]. However, systemic administration of the nonselective K_{ATP}-channel agonist diazoxide suppresses the glucagon response and has no effect on the epinephrine response to hypoglycemia in nondiabetic humans^[148]. These results suggest that K_{ATP}-channel modulators are not effective in humans, possibly due to inability to cross blood-brain barrier.

Other treatments: Islet cell transplantation (ICTx) prevents severe hypoglycemia^[149], and restores some counter-regulatory hormone secretion^[150]. In a retrospective study conducted in 31 T1DM recipients of ICTx, HU was assessed using the Clark hypoglycemic score (minimum = 0; maximum = 7; no hypoglycemia = 0; HU \geq 4)^[151] twice. A reduction in the proportion of patients with HU was observed post-ICTx (pre vs post-ICTx: 87% vs 13%) and a significant increase in glucose threshold that resulted in symptoms (pre vs post-ICTx: 41.4 mg/dL vs 58.4 mg/dL)^[152]. These results were sustained even after the patient's stratification based in islet function, graft dysfunction and graft failure^[152]. These results suggest that improved metabolic control achieved with ICTx can restore hypoglycemia awareness in patients with T1DM, persisting even after islet graft failure.

Fructose infusion amplifies epinephrine and glucagon responses and increases glucose production during hypoglycemia in humans^[153]. Fructose is a promising treatment but has not been tested in clinical trials.

CONCLUSION

HU is a complex, difficult-to-study phenomenon that carries with it great risk to patients. HU is common in people with T1DM and is observed with less frequency in insulin-treated T2DM. Exposure to antecedent hypoglycemia, especially repeated episodes, is an important factor in the pathogenesis of HU. Although enormous advances have been made in our knowledge of the mechanisms of HU, further research is needed to elucidate the pathophysiology of counter-regulatory impairment and HU, and enable the development of more targeted strategies that support glucose counter-regulation and consequently reduce hypoglycemia. Numerous research studies have begun to uncover the mechanisms by which the central nervous system responds and adapts to hypoglycemia. Understanding these mechanisms will lead to better management and therapies that reduce the risk for hypoglycemia. Studies aiming to improve or even reverse HU have met with variable success and a number of research groups are considering new candidate pathways to develop a therapy. Therefore, until effective measures are developed to reverse HU, part of the role of the healthcare professional should be to educate people with diabetes on the risks associated with HU and should discuss hypoglycemia prevention strategies with their patients, so that they can have a better chance of achieving their glucose control goals while avoiding the morbidity and mortality associated with hypoglycemia.

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Relationship between diabetes and periodontal infection

Fernando Llambés, Santiago Arias-Herrera, Raúl Caffesse

Fernando Llambés, Department of Stomatology, Dentistry
University of Valencia, 46010 Valencia, Spain

Santiago Arias-Herrera, Section of Graduate Periodontology-
Faculty of Odontology, Complutense University, 28040 Madrid,
Spain

Raúl Caffesse, Complutense University, 28040 Madrid, Spain

Author contributions: Llambés F, Arias-Herrera S and Caffesse R contributed equally to this work; Llambés F designed the research; Llambés F, Arias-Herrera S and Caffesse R performed the research; Llambés F, Arias-Herrera S and Caffesse R contributed new reagents/analytic tools; Llambés F, Arias-Herrera S and Caffesse R analyzed the data; Llambés F, Arias-Herrera S and Caffesse R wrote the paper.

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Correspondence to: Fernando Llambés, DDS, PhD, Department of Stomatology, Dentistry University of Valencia, la Pechina 51, esc2, pta3, 46010 Valencia, Spain. fernando.llambes@gmail.com
Telephone: +34-96-3518437
Fax: +34-96-3106744

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Abstract

Periodontal disease is a high prevalent disease. In the United States 47.2% of adults ≥ 30 years old have been diagnosed with some type of periodontitis. Longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis, with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease. Periodontal treatment can be successful in diabetic patients. Short term effects of periodontal treatment are similar in diabetic patients and healthy population but, more recurrence of periodontal disease can be expected in no well controlled diabetic individuals. However, effects of periodontitis and its treatment on diabetes metabolic control are not clearly defined and results of the studies remain controversial.

Key words: Diabetes; Diabetes mellitus; Periodontitis; Periodontal disease; Periodontal treatment; Scaling and root planning; Non surgical periodontal treatment; Antibiotic; Glycosylated hemoglobin; C-reactive protein

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Core tip: Longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis, with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease. Periodontal treatment can be successful in diabetic patients, but more recurrence of periodontal disease can be expected in non well controlled diabetic individuals. However, effects of periodontitis and its treatment on diabetes metabolic control are not clearly defined and results of the studies remain controversial. Recommendations for future investigations are included in this review.

Llambés F, Arias-Herrera S, Caffesse R. Relationship between

diabetes and periodontal infection. *World J Diabetes* 2015; 6(7): 927-935 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i7/927.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i7.927>

PERIODONTAL DISEASE

What is periodontal disease?

Periodontal disease is the destruction of the tissues that support the tooth by accumulation and maturation of oral bacteria on teeth.

Periodontal diseases include two major entities, gingivitis and periodontitis. Gingivitis is characterized by reversible inflammation of periodontal tissues whereas periodontitis also presents destruction of tooth supporting structures, and may lead to tooth loss. Existing evidence indicates that gingival inflammation (gingivitis) is required for periodontitis, however some gingivitis never transform to periodontitis^[1,2]. This is because bacterial plaque accumulation is necessary for the onset of both entities but individual susceptibility is required to develop periodontitis^[2,3].

The currently used classification of periodontal diseases was introduced by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions^[4]. Since the current classification has been used only in the last years, a substantial part of the existing literature on the prevalence and extent of periodontal diseases in various populations is still based on earlier classification systems.

Due to its high prevalence in current populations, it has become a public health priority. Epidemiologic studies have determined that about 50% of the population suffer from gingivitis and approximately 14% show periodontitis^[5]. This percentage was higher in a recent study on United States population, which showed that 47.2% of adults ≥ 30 years old had periodontitis. Prevalence of periodontitis increased with age up to the point that 70.1% of adults ≥ 65 years old were affected by periodontal disease^[6]. Men exhibit worse periodontal status than women [(56.4% vs 38.4%), as well as those with limited education (66.9%) and income (65.4%)]. These factors, together with cigarette smoking are increased risk factors for periodontal progression^[7].

Etiology and pathogenesis of periodontal disease

Microorganisms in combination with individual host susceptibility and environmental factors are the main etiologic factors of periodontal diseases.

Plaque accumulation on teeth produces gingivitis, but the degree of inflammation and destruction of the alveolar bone that supports teeth depend on the host susceptibility^[8].

Oral bacteria can damage periodontal tissues through the action of matrix-degrading enzymes and molecules that affect host cells. The transition from gingivitis to

periodontitis involves the spreading of the inflammatory front to deeper areas in the connective tissue. However the reason why this happens is not well established. One etiopathogenic mechanism could involve the presence of bacteria or their products, such as lipopolysaccharides, in the periodontal connective tissue. They may induce an immune response with production of interleukins and tumor necrosis factor (TNF), which play an important role in the regulation of inflammatory processes. This inflammation stimulates the production of secondary mediators, which amplify the inflammatory response. Simultaneously, the presence of these cytokines reduces the ability to repair damaged tissue by cells such as fibroblasts, and finally, bacterial products and this inflammatory cascade stimulate osteoclastogenesis, leading to alveolar bone destruction^[9,10] (Figure 1).

Several studies have shown how gingival inflammation can be modulated by a number of conditions. Systemic diseases, steroid hormones variations, nutritional deficiency, the intake of drugs, diabetes, tobacco smoking and other conditions have comprehensive and profound effects on the host, resulting in an increased response to bacterial plaque accumulation^[10].

The high prevalence of *Helicobacter pylori* (*H. pylori*) among the microorganisms isolated from the oral environment induce to think that it may have an effect in the development of periodontal disease. Umeda *et al.*^[11] determined that periodontal patients showed a higher level of *H. pylori* than healthy subjects but, there was no significant correlation between the presence of *H. pylori* and the severity of periodontitis^[12]. The addition of periodontal treatment to eradication therapy may reduce *H. pylori* recurrence compared with eradication therapy alone in periodontal patients suffering from gastric diseases associated with *H. pylori*^[13].

Clinical manifestation of periodontal disease

Clinical signs of gingival inflammation (gingivitis) involve enlarged gingival contours due to edema or fibrosis, color transition to a red and/or bluish red hue, elevated sulcular temperature, bleeding upon probing and, increased gingival exudates (Figure 2).

Periodontitis clinical features include clinical attachment loss (CAL), alveolar bone loss (BL), periodontal pocketing and gingival inflammation. In addition, enlargement or recession of the gingiva; increase tooth mobility, drifting, and even tooth exfoliation may occur (Figure 3)^[14].

Diagnosis of periodontal disease

Clinical evaluation includes periodontal probing (Figure 4) to evaluate: (1) Probing depth: the distance a periodontal probe penetrates into a periodontal pocket measured from the gingival margin to its bottom; (2) Clinical attachment level: The distance from the cemento-enamel junction to the bottom of the periodontal pocket; (3) Bleeding on probing. Bleeding after probing to the base of the periodontal pocket has been

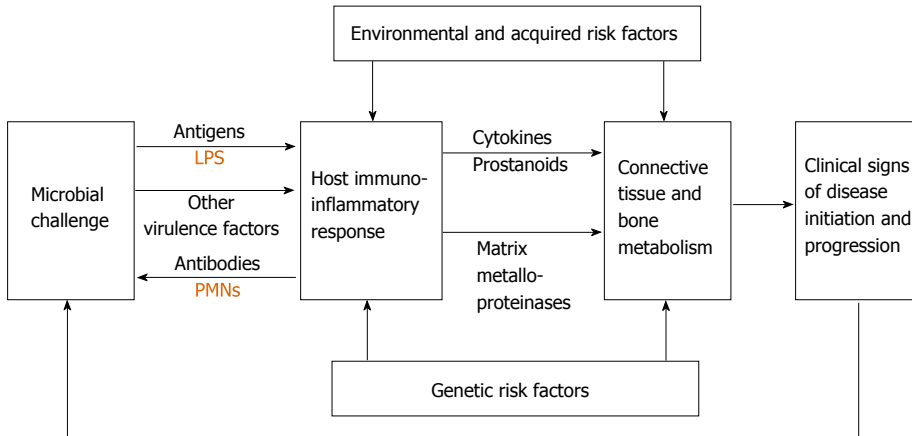


Figure 1 Etiology and pathogenesis of periodontal diseases. Adapted from: Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. *Periodontol* 2000 1997; 14: 9-11.



Figure 2 Clinical features of plaque-induced gingivitis associated with systemic diseases (diabetes mellitus-associated gingivitis).



Figure 3 Clinical features of chronic periodontitis in diabetic subject.

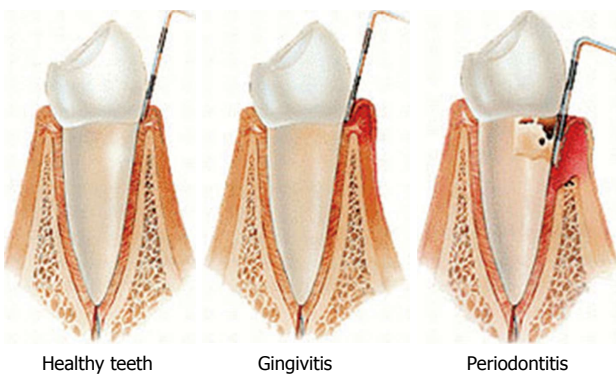


Figure 4 Clinical diagnosis of periodontitis.

a common way to identify presence of subgingival inflammation; and (4) Tooth mobility and furcations. The movement of a tooth in its socket resulting from an applied force can be classified into three categories. Furcation involvement is defined as BL affecting the base of the root trunk of a tooth where two or more roots meet.

Radiographic evaluation will show if alveolar bone that support tooth roots is lost. In a healthy situation alveolar bone will remain 1-2 mm below the crown of

the teeth. If bone is located further from the crown, it means that loss has occurred (Figure 5).

Classification of periodontal disease

In 1999, the American Academy of Periodontology organized an international symposium with the aim of reaching a consensus regarding the classification of periodontal diseases and disorders, resulting in eight categories: gingival diseases, chronic periodontitis, aggressive periodontitis, periodontitis as manifestation of systemic diseases, necrotizing periodontal diseases, periodontal abscesses, periodontitis associated with endodontic lesions and, developmental or acquired deformities and conditions^[4,15,16].

It is possible to include in this classification additional subcategories such as "diabetes mellitus-associated chronic periodontitis" and "diabetes mellitus-associated aggressive periodontitis" under the category of periodontitis as manifestation of systemic diseases.

INTERRELATIONSHIP BETWEEN PERIODONTITIS AND DIABETES

Investigations have demonstrated associations between periodontitis and various systemic diseases^[17,18] such as cardiovascular disorders^[19,20], respiratory diseases^[21,22], osteoporosis^[23,24], immunodeficiencies^[25] and also diabetes

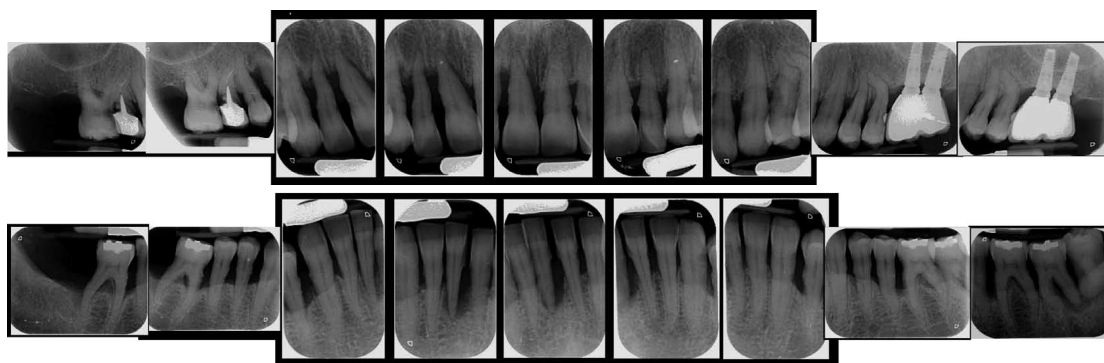


Figure 5 Radiographic diagnosis of periodontitis.

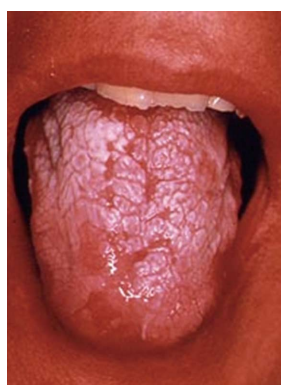


Figure 6 Clinical features of acute pseudomembranous candidiasis.

mellitus^[26].

As already mentioned, longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis, with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease^[27-30].

Effect of diabetes on periodontal disease and periodontal treatment

Diabetes has been associated to different oral diseases such as salivary and taste dysfunction, oral bacterial and fungal infections (*i.e.*, candidiasis), and oral mucosa lesions (*i.e.*, stomatitis, geographic tongue, traumatic ulcer, lichen planus,...)^[31,32]. Diminished salivary flow and burning mouth are other oral characteristics in diabetic patients with poor glycemic control. Also, different oral pathologies such as, lichen planus, leukoplakia and lichenoid reactions are associated to diabetic subjects due to immunosuppression and/or drugs used. In addition, delayed mucosal wound healing, mucosal neuro-sensory disorders, decay lesions and tooth loss have been reported in diabetic patients^[33]. Xerostomia is a frequent symptom found in diabetic patients on oral hypoglycemic agents, and it may facilitate the onset of some fungal opportunistic infection. Candidiasis has been reported in patients with poorly controlled diabetes (Figure 6).

Evidence suggests that diabetes leads to worsening of periodontal disease, and a significant association between

diabetes and periodontitis has been demonstrated. Periodontal disease has a higher incidence in diabetic patients, and it is more prevalent and severe if compared with a healthy population^[27,34]. Lalla *et al.*^[35] determined the prevalence of periodontitis in different age cohorts. It was 4.8 times higher among diabetic patients compared to non diabetics when the 15 to 24-year age cohort was considered, and 2.3 higher in the 25-34 year group. Also, CAL was higher in diabetic patients when the 15 to 55-year age cohort was considered. Lim *et al.*^[36] estimated that the glycemic control was the most important risk factor related to severity and extent of periodontitis. Other authors like Lalla *et al.*^[37] established that the rate of periodontal destruction is related to inappropriate glycemic control in diabetic patients so that accurate metabolic control could be important to prevent periodontal complications. Thus, glycemic control and the diabetes onset are critical factors in periodontal disease progression but it should be considered that substantial heterogeneity exists within diabetics^[38].

Glycosylated hemoglobin (HbA1c) allows the control of serum glucose levels in an interval of 120 d and is a useful decision-making tool. Diabetes micro- and macrovascular complications are related to increased levels of HbA1c. The risk of periodontitis is 3-fold times higher among diabetic patients^[39], being its prevalence and severity even greater in diabetic patients presenting elevated HbA1c levels^[40].

Different hypotheses have been proposed to explain the influence of diabetes mellitus on periodontitis but they are all currently under investigation and remain somewhat controversial. Two similar but distinct pathogenic pathways may justify the biologic plausibility, a possible common origin of the two diseases which results in a host susceptible to either diseases^[41], or a direct causal relationship in which, through the effects of advanced glycosylation end products (AGEs), diabetes triggers an increased inflammatory phenotype in cells^[5,27]. Studies have shown how chronic hyperglycemia produces AGEs that can bind to specific receptors (RAGE) on different cells such as fibroblast, endothelial cells and macrophages^[42]. Thereby, macrophages are transformed into hyperactive cells that produce pro-inflammatory cytokines such as interleukins 1 β and 6 (IL-1 β , IL-6)

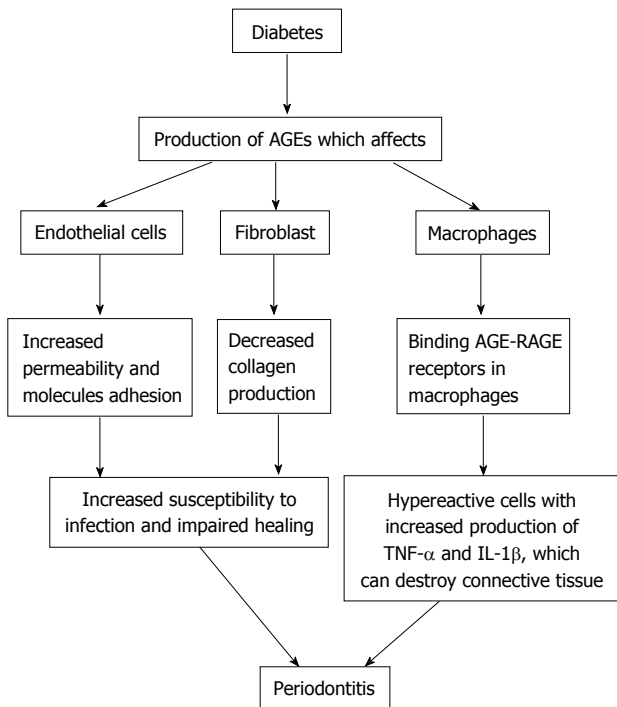


Figure 7 How diabetes mellitus could contribute to the development of periodontal disease (Llambés F, Caffesse R, Arias S). TNF: Tumor necrosis factor; IL-1 β : Interleukins 1 β ; AGE: Advanced glycosylation end product; RAGE: Receptors AGE.

and TNF- α . AGEs can also alter endothelial cells which will become hyperpermeable and hyperexpressive for adhesion molecules, while fibroblasts will show decreased collagen production^[43]. Therefore, AGEs produced by chronic hyperglycemia can produce hyper inflammatory responses, vascular modifications, altered healing and increased predisposition to infections (Figure 7). Lalla *et al.*^[44] supported the hypothesis that the activation of RAGE contributes to pathogenesis of periodontitis in diabetic patients. Increased accumulation of AGEs and their interaction with RAGE in diabetic gingiva leads to hyper production of proinflammatory cytokines, vascular dysfunction, and loss of effective tissue integrity and barrier function.

Despite these facts, periodontal treatment can be successful in diabetic patients. Short term effects of periodontal treatment are similar in diabetic patients and healthy population^[45-47] but, more recurrence of periodontal disease can be expected in non well controlled diabetic individuals^[26].

Effect of periodontal disease and its treatment on diabetes

The National Health and Nutrition Examination Survey 2009-2010 reported that prevalence of diabetes was 12.5% among periodontal patients, but only 6.3% in subjects without periodontitis^[48].

If diabetic individuals are at a higher risk for periodontitis, it is also important to determine what effects periodontitis and its treatment may have on diabetes. It would be reasonable to think that perio-

dontal inflammation, as any other infections, can have an adverse effect on diabetes glycemic control, compromising diabetes management in these individuals. Most evidence on this issue is derived from interventional and observational studies, indicating that periodontitis affects the glycemic control of diabetic patients. HbA1c values < 7% are related with proper glycemic levels whilst > 8% values represents poorly controlled glycemia.

Longitudinal studies have demonstrated that severe periodontitis is associated with poorly controlled glycemia, higher HbA1c levels and development of diabetic systemic complications^[1,30,49]. It also has been reported that periodontitis is associated with a slight elevation of HbA1c in non-diabetic subjects (periodontitis may potentially increase the incidence of diabetes), although a clear-cut association could not be established^[50].

Studies assumed that periodontal infection may impair glycemic control by increasing insulin tissue resistance^[26]. Hence, glycemic level could be improved by non-surgical periodontal treatment removing bacterial plaque accumulation and decreasing gingival inflammation. This assumption is based on studies that observed an improvement in diabetes glycemic control following periodontal therapy^[46,51]. It should be considered that other studies did not find such causal relationship, maybe due to inadequate time for periodontal tissues healing, or because periodontitis had not been properly resolved^[30,52]. Another reason may be the influence of factors such as diet, physical exercise or use of antidiabetics that can alter significantly HbA1c, and make more difficult to observe the metabolic effect of periodontal treatment^[45].

Effect of non-surgical periodontal therapy on diabetes glycemic control

Several studies have investigated the effect of non-surgical periodontal therapy on the glycemic control of diabetic patients. Both non-diabetic and diabetic patients show similar short-term outcomes after non-surgical periodontal therapy in terms of probing depth reductions, gain in CAL and changes in subgingival microbiota^[53]. If glycemic control is considered as treatment outcome after non-surgical periodontal therapy, results vary (Table 1).

Different studies on patients with type 1 diabetes mellitus have not found an additional beneficial effect of periodontal treatment in glycemic control. Llambés *et al.*^[45] obtained changes in mean HbA1c of about 0.07%, without statistical significant difference after non-surgical periodontal treatment in type 1 diabetic patients after 3-mo. Similarly, Seppälä *et al.*^[54] reported that in poorly-controlled type 1 diabetic patients, non-surgical periodontal therapy had no effect on HbA1c. The same results were observed in the study performed by Aldridge *et al.*^[55] who stated no changes in HbA1c levels after non-surgical periodontal therapy in 22 type 1 diabetics with severe periodontitis.

On the other hand, Faria-Almeida *et al.*^[46] reported

Table 1 How periodontal therapy affects diabetes glycemic control

	Ref.	Design	Sample	Follow-up	Outcome	Results
Type 1	Aldridge <i>et al</i> ^[55]	Randomized clinical trial	23 subjects	2 mo	HbA1c	No changes
	Smith <i>et al</i> ^[47]	Controlled clinical trial	18 subjects	2 mo	HbA1c	No changes
	Christgau <i>et al</i> ^[53]	Cohort study	7 subjects	4 mo	HbA1c	No changes
	Llambés <i>et al</i> ^[45]	Randomized clinical trial	30 subjects	3 mo	HbA1c	0.06% reduction (no changes)
Type 2	Stewart <i>et al</i> ^[75]	Controlled clinical trial	72 subjects	10 mo	HbA1c	6% reduction
	Kiran <i>et al</i> ^[51]	Randomized clinical trial	44 subjects	3 mo	HbA1c	0.8% reduction
	Faria-Almeida <i>et al</i> ^[46]	Cohort study	20 subjects	6 mo	HbA1c	5.7% reduction
	Dağ <i>et al</i> ^[56]	Controlled clinical trial	45 subjects	3 mo	HbA1c	No changes
	Auyeung <i>et al</i> ^[57]	Cohort study	75 subjects	12 mo	HbA1c	No changes
	Engelbreton <i>et al</i> ^[58]	Randomized clinical trial	257 subjects	6 mo	HbA1c	No changes
	Gay <i>et al</i> ^[59]	Randomized clinical trial	126 subjects	4 mo	HbA1c	No changes

that non-surgical periodontal therapy significantly reduce HbA1c levels about 5.7% in type 2 diabetics, while Dağ *et al*^[56] and Auyeung *et al*^[57] reported that this therapy alone significantly reduced HbA1c levels only in well-controlled diabetics. Smith *et al*^[47] reported that mechanical periodontal therapy alone did not produce a significant change in glycemic control in diabetic patients.

Recently, Engelbreton *et al*^[58] indicated that non-surgical periodontal therapy in type 2 diabetics with chronic periodontitis did not improve diabetes glycemic control. According to these findings the use of nonsurgical periodontal treatment in order to reduce levels of HbA1c would not be justified. Lately, Gay *et al*^[59] in a randomized clinical trial where 152 type 2 diabetic patients with periodontitis were treated, determined that no statistically significant differences were found in the changes of HbA1c levels.

Furthermore, current systematic reviews report glycemic control improvement, with a HbA1c reduction of approximately 0.4%, after non-surgical periodontal treatment^[60]. A mean reduction of -0.36% of glycosylated HbA1c in subjects with type 2 diabetes has been determined recently^[61]. However, the clinical significance of this effect is still unknown. It has been reported that each 1% reduction of HbA1c may be associated with 35% reduction in the risk of microvascular complications^[62]. To the best of our knowledge, no studies have evaluated changes in HbA1c levels in non-diabetic patients after non-surgical periodontal therapy.

Effect of non-surgical periodontal therapy in combination with antimicrobials on diabetes glycemic control

Two studies have examined the added benefit of chlorhexidine as adjunct to non-surgical periodontal therapy in diabetic patients. Christgau *et al*^[53] demonstrated that non-surgical periodontal therapy in combination with subgingival irrigation with 0.2% chlorhexidine did not improve HbA1c levels. The same results were achieved when 0.12% chlorhexidine was considered^[63].

Iwamoto *et al*^[64] demonstrated a 0.8% reduction in HbA1c in type 2 diabetics after non-surgical periodontal

therapy and subgingival use of minocycline gel.

Studies in which systemic antibiotics were used along with mechanical therapy showed a significant improvement in glycemic control in diabetic patients. This may be due to the additional benefits of systemic antibiotics, such as their antimicrobial and host modulation effects, as well as their inhibition of non-enzymatic glycosylation^[63,65-67].

Non-surgical periodontal therapy combined with 100 mg doxycycline is associated with a mean HbA1c reduction of 0.6% in type 2 diabetics patients^[65]. There is not enough evidence about the use of tetracyclines but it seems to play a role in limiting tissue destruction. Lately, a modest improvement in glycemic control was detected after nonsurgical therapy plus azithromycin^[68]. However, Llambés *et al*^[45] show that non-surgical periodontal treatment combined with systemic doxycycline has no effect on HbA1c of type 1 diabetic patients^[43].

Effect of surgical periodontal therapy on diabetes glycemic control

Scarce available evidence makes it impossible to determine the response after periodontal surgical treatment in diabetic patients. Diabetic subjects usually show improved periodontitis after surgical periodontal treatment. However, if poor diabetic control is present, more recurrence of periodontal pockets and unfavorable long term response is expected after surgical treatment^[53,69]. Effects of surgical periodontal treatment on HbA1c are currently unknown.

The exact mechanism linking periodontitis/periodontal inflammation and HbA1c levels is still not clearly known. In periodontitis, there is an increased production of pro-inflammatory mediators, such as TNF- α , IL-6, IL-1 β and interferon gamma (IF- α), and increased levels of acute-phase proteins, such as C-reactive protein (CRP). All these mediators have important effects on glucose and lipid metabolism. TNF- α , IL-6 and IL-1 β are insulin antagonist and lipid metabolism is hampered by TNF- α . Elevated levels of CRP lead to insulin resistance. IF- α induces apoptosis of pancreatic β cells^[70]. Non-enzymatic glycosylation of hemoglobin is not induced by inflammation, but rather results from hyperglycemia caused by insulin resistance^[44]. Thus, this could explain

why subjects with periodontitis have high HbA1c levels.

According to these reports, it can be presumed that control of periodontal inflammation after therapy may reduce the levels of local and circulatory mediators, such as IL-6 and TNF- α . Both may trigger acute phase proteins such as CRP, and impair intracellular insulin signaling. Consequently, if these mediators were reduced by periodontal treatment, this could theoretically, help in diabetes control. However, this mechanism remains to be confirmed. Some studies have shown that periodontal disease severity is correlated with blood CRP levels in diabetic patients^[71,72], however CRP levels are not reduced after periodontal treatment^[73,74].

CONCLUSION

Within the limits of this review we can conclude that: Periodontitis is a highly prevalent infectious disease that relates to some systemic disorders, including diabetes mellitus.

Diabetes has been associated to different oral diseases such as: xerostomia, neuro-sensory disorders, several oral mucosa diseases, tooth decay and periodontal disease. It is well documented in the literature that periodontal disease is more prevalent and severe in diabetic individuals than in healthy subjects. However, it has to be kept in mind that the level of metabolic control and duration of diabetes appear to influence the risk for periodontal disease, with a significant heterogeneity among diabetic individuals.

Periodontal treatment is effective in diabetic patients, but more long-term recurrence can be expected when diabetes is not well controlled.

Severe periodontitis is more frequently found in diabetic subjects with high HbA1c levels and systemic diabetic complications; however, the influence of periodontal treatment on HbA1c is not that well established. The beneficial effects of periodontal treatment on HbA1c levels seem to be more apparent in type 2 diabetics and when antibiotics are associated to local periodontal therapy, although other reports did not find any improvement in diabetes control after periodontal treatment. More research on type 1 and type 2 diabetic subjects will be needed to know how periodontal treatment affects diabetes metabolic control. In those, it will be paramount to control other factors that may affect HbA1c levels, such as diabetic medication, diet and physical exercise.

HbA1c reduction after periodontal treatment is usually less than 0.5%. New studies are needed to evaluate the clinical significance of this improvement.

Additionally, it may be necessary to explore the effects of different modalities of periodontal therapy in patients with different types of diabetes and different degrees of metabolic control.

Further analysis of inflammatory mediators, such as CRP, may help to explain the relationship between diabetes and periodontal disease, and the individual variations detected in samples from different severities

of diabetes and periodontal disease.

Any improvement in the control of diabetes and/or periodontal disease has the potential to improve significantly the quality of life in diabetic subjects.

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Fetal programming of polycystic ovary syndrome

Esra Bahar Gur, Muammer Karadeniz, Guluzar Arzu Turan

Esra Bahar Gur, Guluzar Arzu Turan, Department of Obstetrics and Gynecology, Sifa University Faculty of Medicine, Izmir 35100, Turkey

Muammer Karadeniz, Department of Endocrinology, Faculty of Medicine, Sifa University, Bornova 35100, Turkey

Author contributions: Gur EB, Karadeniz M and Turan GA contributed to this paper.

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Correspondence to: Muammer Karadeniz, MD, Department of Endocrinology, Faculty of Medicine, Sifa University, Sanayi St. No. 7, Bornova 35100, Turkey. muammermd@hotmail.com
 Telephone: +90-232-3434445
 Fax: +90-232-3435656

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Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects up to 6.8% of reproductive age women. Experimental research and clinical observations suggest that PCOS may originate in the very early stages of development, possibly even during intrauterine life. This suggests that PCOS is either genetically-transmitted

or is due to epigenetic alterations that develop in the intrauterine microenvironment. Although familial cases support the role of genetic factors, no specific genetic pattern has been defined in PCOS. Several candidate genes have been implicated in its pathogenesis, but none can specifically be implicated in PCOS development. Hypotheses based on the impact of the intrauterine environment on PCOS development can be grouped into two categories. The first is the "thrifty" phenotype hypothesis, which states that intrauterine nutritional restriction in fetuses causes decreased insulin secretion and, as a compensatory mechanism, insulin resistance. Additionally, an impaired nutritional environment can affect the methylation of some specific genes, which can also trigger PCOS. The second hypothesis postulates that fetal exposure to excess androgen can induce changes in differentiating tissues, causing the PCOS phenotype to develop in adult life. This review aimed to examine the role of fetal programming in development of PCOS.

Key words: Polycystic ovary syndrome; Androgens; Fetal programming; Intrauterine growth retardation; Genetic

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Core tip: Polycystic ovary syndrome (PCOS) is a highly complex and heterogeneous disorder that is significantly influenced by genetic and environmental factors. There is some evidence that the development of PCOS may begin during the intrauterine period. Fetuses exposed to intrauterine nutritional restriction often have lowered insulin secretion and, as a compensatory mechanism, insulin resistance, which is known as the "thrifty" phenotype. Additionally, an impaired intrauterine nutritional environment can affect the methylation of some specific genes, which can trigger PCOS. The other hypothesis postulates that fetal exposure to excess androgen can induce changes in differentiating tissues, causing the PCOS phenotype and related disorders to develop in adult life.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex disorder characterized by defects in primary cellular control mechanisms that can result in hyperandrogenemia, hyperinsulinemia, insulin resistance, and chronic anovulation. PCOS is the most common endocrinologic disorder among women of reproductive age. Its prevalence typically ranges between 4% and 8% in diverse populations, but it has been reported to be as high as 25%^[1]. The variations in the reported prevalence of PCOS have been attributed to the use of different diagnostic criteria. Three main diagnostic criteria systems that are currently accepted for PCOS are those from the National Institutes of Health (NIH, 1990), Rotterdam (ASRM/ESHRE, 2003), and Androgen Excess Society (AES, 2006). According to the Rotterdam criteria, the diagnosis of PCOS is based on the presence of at least two of the following three clinical features: polycystic ovarian morphology, oligo/amenorrhea and hyperandrogenism. However, the NIH criteria require only oligo/amenorrhea and hyperandrogenism for a diagnosis, while the AES criteria require a combination of biochemical or clinical hyperandrogenism along with chronic anovulation or polycystic ovarian morphology^[1,2]. Although it is considered to be a disorder of reproductive age women (based on its classical symptoms of amenorrhea, hirsutism, and infertility), it can affect a woman any time during her life. Affected persons have a lifetime risk of disorders, including glucose metabolism, cardiovascular diseases, endometrial hyperplasia and/or cancer^[2].

The underlying causes of PCOS are not known. However, its signs and symptoms typically appear during or close to the onset of puberty. Signs of precocious pubarche and adolescent hyperandrogenemia with or without insulin resistance may indicate the early stages of PCOS^[3]. Further, epidemiologic studies have shown that adolescents with the aforementioned signs of PCOS had lower birth weights than those of controls^[4]. These results suggest the hypothesis that PCOS is a continuum of a process that begins during intrauterine life.

PCOS is also believed to be caused by several genetic and environmental factors. The prevalence of PCOS has risen in populations where the gene pool has been relatively constant, which indicates that environmental factors may be playing a more important role in its development^[5]. Further, obesity has been linked to the development of PCOS in susceptible individuals. A recent study revealed that, when compared with matched controls, non-obese women with PCOS had higher levels of glycotoxins, hyperandrogenemia, and advanced

glycation end products, which were positively correlated with insulin resistance indices^[6]. Some recent animal studies and observational human studies have suggested that impaired nutrition and steroidal environment during intrauterine life may play an important role in the development of PCOS^[7-9].

GENETICS OF PCOS

Although case reports indicate that PCOS clusters within families, genetic studies have been inconclusive^[10]. Twin studies have shown a heritability of 79% for PCOS with a correlation of 0.71 between monozygotic twins and 0.38 between dizygotic twins^[11]. The clinical presentation of PCOS varies widely and there is currently no consensus on its diagnostic criteria^[12,13]. Studies aimed at determining a genetic model of PCOS have produced different results when varying diagnostic criteria were used. For instance, some studies accepted hirsutism and ovaries with a polycystic appearance as diagnostic criteria (Rotterdam criteria) for the disease; these studies suggested that PCOS may have an autosomal dominant or X-linked simple Mendelian trait. Other studies using oligomenorrhea and hirsutism as the diagnostic criteria (NIH criteria) have reported lower genetic penetration rates^[14-17]. On the other hand, some other recent studies have shown that the genetic aspect of insulin resistance is more prominent than that of hyperandrogenism in PCOS patients^[18]. In conclusion, there is not yet a clearly established genetic model of PCOS. This is due to the diversity of both the diagnostic criteria and the clinical presentations of the disease, differences in its prevalence among various ethnic populations, and the limitations of some prior studies with respect to the number of subjects and statistical analyses used^[10].

While the etiology of PCOS remains unclear, intrinsic abnormalities in the synthesis and secretion of androgens, insulin and gonadotropins provide a plausible basis for the syndrome. Therefore, it has been suggested that specific primary enzyme abnormalities in these steroidogenic pathways may be an important cause of PCOS. Many different genes encoding these enzymes have been studied to determine the etiology of PCOS; these genes have altered expression, suggesting that the genetic abnormalities in PCOS affects signal transduction pathways controlling steroidogenesis, steroid hormone action, gonadotropin action and regulation, insulin action and secretion, energy homeostasis, chronic inflammation, and more (Table 1)^[19-27]. These genes may each contribute separately, or they might act collectively. Moreover, different variation in the same gene (allelic heterogeneity) and possible gene-environment interactions may have different effect on gene function. Data suggests that as of yet, there are no gene defects considered to be responsible for the etiology of PCOS; however, several studies have looked at many candidate genes and have suggested that alterations in these genes may contribute to the development of PCOS. Nevertheless, future studies are needed to determine

Table 1 Genetics of polycystic ovary syndrome

The genes associated with PCOS	Genetic mutation (specific enzyme, protein or receptor)
Genes involved in ovarian and adrenal steroidogenesis	CYP11A (P450 cytochrome) CYP21 (21-hydroxylase) CYP17 (17 α -hydroxylase and 17,20-lyase) CYP19 (the enzyme complex aromatase: cytochrome P450 aromatase, the NADPH cytochrome P450 reductase 30, and P450 arom)
Genes involved in steroid hormone actions	AR-VNTR polymorphism (the androgen receptors) 4-kb gene - A pentanucleotide repeat polymorphism (SHBG)
Genes involved in gonadotropin action and regulation	Trp8Arg and Ilg15Thr (the β -subunit of LH)
Genes involved in insulin action and secretion	INS -VNTR (insulin) INSR-SNP (insulin receptor) Gly972Arg for IRS1, Gly1057Asp for IRS2 (insulin receptor substrates) 112/121 haplotype of CAPN10 (calpain-10) ApaI; rs680-SNP (IGF-1, IGF-2)
Genes involved in energy homeostasis	T45G in exon 2 and G276T in intron 2 (adipocytokines)
Genes involved in chronic inflammation	Mutation 308 A alleles (TNF- α) TNFR2, IL-6 signal transducer gp 130, IL-6 receptor genes (type-2 TNF receptor, IL-6) Polymorphism 4G/5G (PAI-1)

PCOS: Polycystic ovary syndrome; PAI-1: Plasminogen activator inhibitor-1; IL-6: Interleukin-6; TNF- α : Tumor necrosis factor α ; IRS: Insulin receptor substrates; INSR: Insulin receptor.

which genes are the most appropriate PCOS biomarkers. In addition, more recent genetic approaches, namely genome-wide association studies, may begin a new era in PCOS research^[28].

EXPERIMENTAL ANIMAL STUDIES

Experimental evidence supports the hypothesis that the phenotypic expression of PCOS is strongly influenced by the intrauterine environment. Initial studies on rats have shown that elevated testosterone (T) levels early in intrauterine life are related to anovulatory sterility and ovarian polycystic changes in offspring^[29-31]. Prenatally T-treated monkeys and sheep serve as good models of PCOS because follicular differentiation in these species is similar to that in humans^[32]. The results of studies on prenatally androgenized rhesus monkeys are summarized as follows: (1) Following cessation of maternal testosterone treatment in the early period (beginning on days 40-44 of gestation; term 165 ± 10 d), fetuses of these mothers had irregular ovulatory menstrual cycles, ovarian hyperandrogenism, enlarged polyfollicular ovaries and luteinizing hormone (LH) hypersecretion, insulin resistance, diminished insulin secretion, increased incidence of type 2 diabetes, visceral adiposity, and hyperlipidemia. These conditions may be related to an increase in Gonadotropin-releasing hormone (GnRH) secretion, a reduced negative feedback effect of steroids on LH release and/or increased gonadotropin response to GnRH^[33,34]. Normally, healthy fetuses undergo a "critical hypothalamic hormonal period" during sexual differentiation. During this period, a sufficient androgenic stimulus in the brain allows for tonic gonadotropin release and contributes to male-type development; on the other hand, an insufficient androgenic stimulation level promotes the development of a female-type synaptology that is characterized by cyclic GnRH release^[35]. An

increased frequency and amplitude of GnRH release increases LH levels and impairs folliculogenesis, resulting in the anovulatory clinical picture that is characteristic of PCOS. Female offspring of monkeys that were androgenized during fetal life and have high LH levels have hormonal profiles similar to those of the normal male-type hormonal profile; (2) female monkeys similarly exposed to androgen excess during late gestation (100-110 gestation days) also exhibit an adult PCOS-like phenotype, but they do not have obvious abnormalities in LH and insulin secretion or in insulin action^[33]; (3) prenatally androgenized monkeys have high blood levels of androstenedione at birth and their androgens of adrenal origin continue to increase for a period of 4-25 mo after birth, suggesting that prenatal androgen exposure may permanently alter adrenal androgen production^[36]; (4) fetuses that are androgenized during the prenatal period have an increased number of primary, growing preantral, and small antral follicles and an accelerated proliferation of granulosa cells. In addition, an excess of prenatal androgen increases the mRNA expression of follicle-stimulating hormone receptor, insulin-like growth factor I (IGF- I) and the IGF- I receptor in granulosa cells. These morphological changes are similar to the increased follicular development from the primordial follicle pool that is seen in PCOS patients. Furthermore, prenatally androgenized fetuses have increased 5 α -reductase and decreased aromatase activities, which are similar to mechanisms involved in the impaired follicular maturation of PCOS patients^[37,38]; (5) prenatally androgenized female monkeys exhibit enhanced insulin secretion in both the fetal and infant zona reticularis. Therefore, an excess of fetal androgen may induce relative insulin hypersecretion in exposed female fetuses and infants, which in turn programs adrenal hyperandrogenism. In addition, the amelioration of impaired insulin action has beneficial

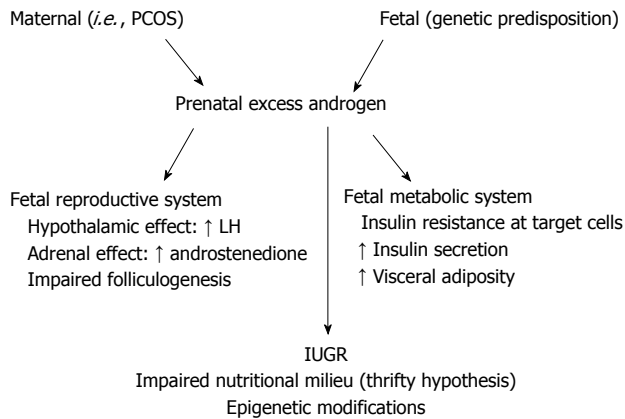


Figure 1 Possible effects of prenatal excess androgen on the fetus. PCOS: Polycystic ovary syndrome; LH: Luteinizing hormone; IUGR: Intrauterine growth restriction.

glucoregulatory effects in both PCOS patients and in prenatally androgenized female monkeys. Treatment with Pioglitazone (a thiazolidinedione-based insulin sensitizer) in prenatally androgenized female monkeys diminishes the aspects of adrenal androgen excess and normalizes menstrual cyclicity^[34]; (6) the hypothesis that metabolic disorders are programmed during the fetal stage is supported by the finding that, despite normal T levels after birth, prenatally androgenized male fetuses have insulin resistance and pancreatic beta-cell defects similar to those observed in females^[39]; and (7) T excess, when introduced prenatally, decreases birth weight in rodent and sheep offspring. In addition, in humans, impaired placental aromatization is accompanied by diminished uteroplacental perfusion and low infant birth weight^[40-44]. It has been suggested that maternal T excess may reduce fetal growth and birth weight *via* impaired placental function (Figure 1).

CLINICAL OBSERVATIONS FOR THE DEVELOPMENTAL ORIGIN OF PCOS

There is some evidence that female fetuses exposed to high androgen levels during the intrauterine period develop the clinical features of PCOS later in life. In humans, it is not possible to perform controlled studies to observe the fetal consequences of maternal androgens; this is left to animal research. However, some observations have been made in humans to support the validity of this hypothesis. Female fetuses having a congenital virilizing tumor or congenital adrenal hyperplasia due to 21-hydroxylase deficiency have been shown to display features of PCOS later in life, even after eliminating the hyperandrogenemia with postnatal therapies^[45]. Similarly, it has been reported that female fetuses of women with defects in the p-450 aromatase gene and sex hormone-binding globulin gene, which are rare conditions that cause androgenization, also develop PCOS later in life^[46]. Furthermore, it has been shown that exposure to androgen-like chemicals (e.g., Bisphenol A)

can lead to PCOS^[47,48].

Another study showed that the maternal androgen level is significantly higher in pregnant mothers with PCOS compared to that in healthy pregnant women^[49]. While the reason for elevated androgen levels in pregnant women with PCOS has not yet been clarified, it is hypothesized that it may be due to hCG-stimulated androgen production in maternal theca cells or the placenta. Under normal conditions, maternal androgens or fetal adrenal androgens are rapidly converted to estrogens by the activity of the placental enzyme aromatase. However, when the activity of this enzyme is inhibited, the availability of androgens may increase. Insulin has been shown to inhibit aromatase activity in human cytotrophoblasts and can stimulate 3-hydroxysteroid dehydrogenase activity^[50]. Therefore, hyperinsulinemia appears to coincide with elevated maternal androgen levels in the development of PCOS in offspring of pregnant women with the same disease. Furthermore, this hypothesis may be supported by the observation that the fetuses of diabetic mothers using insulin have increased levels of macrosomia and fetal pancreatic β -cell hyperplasia, as well as hirsutism, ovarian theca-lutein cysts, ovarian theca cell hyperplasia, and high T and hCG levels in the amniotic fluid^[32].

In addition to having increased androgen levels during pregnancy, women with PCOS may also deliver small-for-gestational age newborns at a higher prevalence than do normal control mothers^[51]. It is hypothesized that prenatal exposure to androgens in the offspring of women with PCOS may cause the development of the PCOS phenotype later in life, and it may also be the reason for low birth weight during the intrauterine period. With this in mind, recent studies in girls have shown that low birth weight is related to the development of premature pubarche followed by functional hyperandrogenism, insulin resistance with hyperinsulinism, and dyslipidemia during adolescence. It has been suggested that these manifestations may have a common early origin^[3,52]. A study in which the authors followed pregnant women during their entire pregnancies reported that pregnant women with PCOS had a progressive increase in both maternal androgens (testosterone and androstenedione) and insulin resistance during their pregnancies, and that these women were exposed to adverse pregnancy-related events significantly more often than those in the control group with a similar body mass index^[49]. Fetuses of mothers with PCOS can have developmental delay, which may be related to an elevated T level and insulin resistance. It has been shown that increased insulin resistance during pregnancy is related to adverse pregnancy outcomes including gestational diabetes, preeclampsia, preterm labor, and intrauterine growth restriction (IUGR)^[53,54]. This hypothesis is also supported by the fact that male children of mothers with PCOS also have increased prevalence of impaired glucose tolerance, insulin resistance, type-2 diabetes, dyslipidemia and pancreatic beta-cell defects later in life^[55].

Another possible mechanism related to the fetal

programming of PCOS involves an impaired intrauterine environment. Independent of elevated androgen levels, intrauterine nutritional insufficiency for any reason may lower insulin secretion and insulin resistance in target tissues as an adaptive mechanism (the thrifty hypothesis). The development of insulin resistance is believed to be directly related to the body "predicting" a life of starvation for the developing fetus. This fetus or infant will have retarded growth and will likely develop PCOS when exposed to nutritional surplus later in life. Epidemiologic studies have demonstrated that babies born with IUGR have an increased prevalence of metabolic syndrome, type-2 diabetes, and hypertension later in life^[48]. A recent study showed that urine from neonatal infants with IUGR contained significantly increased levels of metabolic syndrome-associated markers^[56]. Although conclusive evidence is lacking, it has been suggested that an impaired intrauterine nutritional environment causes epigenetic changes that trigger metabolic disorders in adult life. The best evidence for this is that there is hypomethylation in the 11p15 imprinting center region that is responsible for the etiology of Silver-Russell syndrome, which is characterized by severe IUGR, lack of catch-up after birth, and specific dysmorphisms^[57].

CONCLUSION

In conclusion, PCOS is a highly complex and heterogeneous disorder that is significantly influenced by both genetic and environmental factors. Environmental factors may play a role in the early stages of human development by helping to convert a predisposed genotype to the phenotypic expression of PCOS. In this review, the possible roles of intrauterine environmental factors in PCOS were summarized. Experimental animal studies suggest that maternal hyperandrogenism at a critical stage of fetal development may cause permanent changes in fetal physiology that can trigger PCOS development later in adult life. In humans, it is not possible to perform controlled studies to observe the fetal consequences of maternal androgens; however, some observations have been made in humans to support the validity of this hypothesis. In addition to having increased androgen levels during pregnancy may also deliver small-for-gestational age newborns at a higher prevalence that do normal control mothers. Furthermore, an insufficient intrauterine nutritional environment may also affect PCOS development by affecting cellular metabolism in target tissues or by causing epigenetic alterations to specific genes.

Mechanisms triggering PCOS may be eliminated by making improvements to the maternal hormonal environment and to the intrauterine nutritional environment. Future studies are necessary in order to determine whether insulin-sensitizing treatment of pregnant women with PCOS, or prenatally androgenized animals, will prevent postnatal PCOS in their daughters/female offspring. Results from such studies may help to identify a specific programming mechanism for PCOS.

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Contractile apparatus dysfunction early in the pathophysiology of diabetic cardiomyopathy

Mark T Waddingham, Amanda J Edgley, Hirotsugu Tsuchimochi, Darren J Kelly, Mikiyasu Shirai, James T Pearson

Mark T Waddingham, Amanda J Edgley, Darren J Kelly, Department of Medicine, St Vincent's Hospital, University of Melbourne, Melbourne, Victoria 3065, Australia

Amanda J Edgley, James T Pearson, Department of Physiology, Monash University, Clayton, Victoria 3800, Australia

Hirotsugu Tsuchimochi, Mikiyasu Shirai, Department of Cardiac Physiology, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka 565-8565, Japan

James T Pearson, Monash Biomedical Imaging Facility, Monash University, Clayton, Victoria 3168, Australia

James T Pearson, Australian Synchrotron, Clayton, Victoria 3168, Australia

Author contributions: Waddingham MT, Edgley AJ and Pearson JT wrote the paper; all authors provided intellectual input, edited and approved the final manuscript.

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Correspondence to: James T Pearson, PhD, Monash Biomedical Imaging Facility, Monash University, 770 Blackburn Road, Clayton, Victoria 3168, Australia. james.pearson@monash.edu
 Telephone: +61-3-99029783
 Fax: +61-3-99029500

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Abstract

Diabetes mellitus significantly increases the risk of cardiovascular disease and heart failure in patients. Independent of hypertension and coronary artery disease, diabetes is associated with a specific cardiomyopathy, known as diabetic cardiomyopathy (DCM). Four decades of research in experimental animal models and advances in clinical imaging techniques suggest that DCM is a progressive disease, beginning early after the onset of type 1 and type 2 diabetes, ahead of left ventricular remodeling and overt diastolic dysfunction. Although the molecular pathogenesis of early DCM still remains largely unclear, activation of protein kinase C appears to be central in driving the oxidative stress dependent and independent pathways in the development of contractile dysfunction. Multiple subcellular alterations to the cardiomyocyte are now being highlighted as critical events in the early changes to the rate of force development, relaxation and stability under pathophysiological stresses. These changes include perturbed calcium handling, suppressed activity of aerobic energy producing enzymes, altered transcriptional and posttranslational modification of membrane and sarcomeric cytoskeletal proteins, reduced actin-myosin cross-bridge cycling and dynamics, and changed myofilament calcium sensitivity. In this review, we will present and discuss novel aspects of the molecular pathogenesis of early DCM, with a special focus on the sarcomeric contractile apparatus.

Key words: Diabetes; Prediabetes; Insulin resistance; Myocardium; Sarcomere; Protein kinase C; Rho kinase

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Core tip: Pathological changes in cardiac muscle underlie diabetic cardiomyopathy (DCM) independent of hypertension and atherosclerosis. In advanced diabetes, fibrosis, hypertrophy and apoptosis, reduce myocardial compliance, which leads to increased diastolic filling pressures and overt left ventricular diastolic dysfunction. However, detrimental changes in sarcomeric and other cytoskeletal proteins in the cardiomyocytes of animal models of diabetes precede remodeling of the cardiac extracellular matrix, which has until now been considered the main contributor to diabetic diastolic dysfunction. An important target for preventing early DCM are the protein kinase C/rho-kinase pathways that drive oxidative stress and reduce myosin head cycling and prolong Ca^{2+} transients.

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INTRODUCTION

Diabetes mellitus is a rapidly escalating global epidemic with the International Diabetes Federation predicting that the incidence of diabetes will rise to 552 million people worldwide by 2030^[1]. Diabetes is a metabolic disorder characterized by hyperglycemia, insulin deficiency and or resistance. Type 1 diabetes mellitus (T1DM) is triggered by an autoimmune mechanism and accounts for approximately 5%-10% of diabetes cases. Type 2 diabetes mellitus (T2DM) represents 90%-95% of diabetes cases and is initiated by the interaction of genetic, environmental and lifestyle factors^[2].

Diabetes is associated with a number of complications such as retinopathy^[3], nephropathy^[4], peripheral neuropathy^[5] and cardiovascular disease, which are similar in their pathophysiological mechanisms in both T1DM and T2DM. Cardiovascular disease is the most common complication of diabetes and a leading cause of morbidity and mortality in patients^[6]. Several lines of evidence have established that chronic diabetes is associated with pathological changes to the cardiac muscle^[7-9], coronary vasculature^[10-12] and cardiac autonomic nerves^[13-15], all of which contribute to the increased risk of cardiovascular disease^[16,17].

Independent of other cardiovascular complications, a large body of evidence indicates that diabetes is associated with a specific cardiomyopathy known as diabetic cardiomyopathy (DCM)^[18-20]. In the long term,

people with diabetes more frequently have cardiovascular complications and double the mortality rate^[21]. Moreover, we have known for some time from the Framingham Heart study that diabetic men and women are 2 and 5 times more likely to develop heart failure respectively, independent of hypertension and coronary artery disease^[22].

Initially, DCM was recognized by impaired myocardial relaxation and left ventricle (LV) stiffening with progressive development of LV interstitial fibrosis and cardiomyocyte hypertrophy^[23]. Although the natural history of DCM has never been directly studied, extensive research utilizing experimental animal models of T1DM and T2DM and advances in clinical cardiac imaging over the past four decades support the notion that DCM is a progressive disease, beginning in the early time course of diabetes^[24].

Clinical investigations indicate that features of DCM are present early after the onset of T1DM. In T1DM patients, early DCM is identified by subtly reduced peak myocardial systolic velocity and early diastolic velocity preceding overt diastolic dysfunction^[25]. Early abnormalities in diastolic function are also apparent in patients with well-controlled T2DM in the absence of LV hypertrophy^[26]. Similarly, in one of the most common animal models, the streptozotocin (STZ) induced diabetic rat, depressed rates of pressure development and decay, reduced end systolic developed pressure and prolonged relaxation times are evident as early as 2-3 wk post diabetes induction^[27,28], prior to LV remodeling^[28].

In the advanced stages of diabetes, a myriad of cellular signaling pathways are chronically activated within the heart, leading to fibrosis, hypertrophy and apoptosis, resulting in inadequate myocardial compliance, increased diastolic filling pressures and the development of overt LV diastolic dysfunction^[24]. There is strong evidence from prevention and intervention studies using animal models that specifically targeting the structural manifestations of advanced DCM improves LV function^[29-31]. However, in the clinical setting, there is still uncertainty as to whether structural remodeling is a cause or consequence of DCM^[32]. Functional abnormalities in isovolumetric contraction and relaxation rates, as well as reduced systolic developed pressure are common to both early and advanced DCM. Moreover, it is not easy to diagnose when structural change has occurred. As these cardiac indices reflect the functional status of mechanisms regulating intracellular events within cardiomyocytes including the initiation and regulation of contraction and relaxation, there is sufficient evidence that demonstrates that subcellular changes to the cardiomyocytes can account for the functional abnormalities observed in early DCM.

In this review, we will focus on and discuss the multiple cellular biochemical derangements, intracellular signaling cascades as well as the structural and functional changes to the cardiomyocyte and sarcomere that establish the beginnings of contractile dysfunction in DCM ahead of myocardial remodeling and overt LV

diastolic dysfunction.

EARLY BIOCHEMICAL DYSFUNCTION AND THE CELLULAR SIGNALING PATHWAYS INVOLVED IN DCM

Diabetes is associated with hyperglycemia, hyperlipidemia, hyperinsulinemia and or insulin resistance. However, unlike T1DM, abnormal glucose homeostasis, hyperinsulinemia and insulin resistance frequently precede development of T2DM^[33]. Regardless of the etiology of the disease, diabetes results in multiple cellular metabolic derangements, including increased production and accumulation of advanced glycation end-products in the extracellular space, increased polyol and hexosamine pathway flux and activation of various protein kinase C (PKC) isoforms^[34] (Figure 1). In the myocardium, these biochemical derangements drive the generation of oxidative stress, a key contributor to the development of DCM^[35] (Figure 1A).

Oxidative stress

The development of oxidative stress has long been recognized as a cardinal molecular event in the initiation and progression of DCM. Importantly, in the long-term, diabetes and insulin resistance induce myocardial oxidative stress from a number of sources leading to enhanced production of reactive oxygen species (ROS) and reactive nitrogen species, whilst also diminishing the heart's antioxidant defense system (reviewed by^[36]). The specific mechanisms of how each of these sources generate oxidative stress are beyond the scope of this review and are described in detail by Huynh *et al.*^[37].

In the setting of chronic experimental diabetes, oxidative stress has been consistently implicated in myocardial remodeling and diastolic dysfunction. Studies conducted by our group and others demonstrate that elevated ROS expression and compromised antioxidant defense are associated with myocardial fibrosis, hypertrophy and diastolic dysfunction in STZ induced T1DM rats^[38,39]. Similar findings have been reported in genetic models of advanced T2DM including the db/db mouse^[40], the Goto Kakizaki (GK) rat^[38] and the Otsuka Long-Evans Tokushima Fatty (OLETF) rat^[41].

A role for oxidative stress in the initiation of DCM has also been shown to occur early after the induction of diabetes in animals. One source of myocardial oxidative stress that certainly plays a role in early DCM is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Inhibition of NADPH oxidase shortly after the onset of diabetes attenuates subsequent interstitial fibrosis and completely restores systolic and diastolic function in diabetic rats^[42]. Three to five weeks post induction of diabetes in rats, there is a significant increase in myocardial oxidative stress (nitrotyrosine and lipid peroxidation), which is associated with contractile dysfunction^[43,44]. Antioxidant treatment with N-acetylcysteine (NAC) significantly improved the prolonged rates of LV pressure decay, but not the rates of

pressure development^[43]. While myocardial remodeling was not directly assessed by Cheng *et al.*^[43], the improvements in LV diastolic indices they observed are most likely caused through actions on cardiomyocyte and sarcomeric function (Figure 1A).

Oxidative stress has been widely reported to induce posttranslational modifications of a large variety of contractile proteins within the sarcomere with redox modifications of such proteins playing a pivotal role in the evolution of heart failure (reviewed extensively by references^[45,46]). Therefore, in early DCM, cardiac contractile dysfunction is likely to arise in some part as a consequence of oxidative stress, through posttranslational modifications of contractile proteins as opposed to myocardial structural alterations derived from the increasing burden of chronic oxidative stress associated with advanced DCM. Two distinct cellular signaling cascades that drive generation of oxidative stress through NADPH production are the renin angiotensin aldosterone system (RAAS)^[47,48] and the PKC β_2 pathway^[49,50] (Figure 1A). Both have been implicated in the early pathogenesis of DCM.

RAAS

It has been known for many years that the RAAS is a key contributor to the development and progression of renal^[51], vascular^[52] and cardiac^[53] complications in diabetic patients, mainly by its effector molecule, angiotensin II (ANG II). Since systemic RAAS activity is unchanged or downregulated in diabetes^[54,55], local RAAS in the heart is thought to be responsible for the local production of ANG II. All the components of the classic RAAS have been identified in the heart including renin, angiotensinogen, ANG I, angiotensin converting enzyme (ACE), ANG II and ANG II type 1 receptors (AT₁R)^[56]. Traditionally, it is accepted that chronic activation of the RAAS in advanced T1DM and T2DM diabetes increases myocardial levels of ANG II. In addition to its potent vasoconstrictor actions, ANG II promotes myocardial remodeling by driving collagen production, extracellular matrix protein accumulation^[57], fibrosis, myocyte hypertrophy, apoptosis, impaired calcium handling, myofibrillar dysfunction and oxidative stress, all of which combine to evoke overt LV diastolic dysfunction^[48,58-65] (Figure 1B). Newer evidence suggests that in addition to the activation of classic RAAS, diabetes sequesters the activity of the cardiac ACE2-ANG-(1-7)-Mas receptor axis (Figure 1B), which opposes the pressor, fibrotic and hypertrophic effects of ANG II (reviewed in references^[66-68]). Although RAAS research in the context of DCM is in its infancy, the few studies conducted have reinforced the notion that diabetes suppresses cardiac ACE2-ANG-(1-7)-Mas receptor pathway in the hearts of rodents with chronic T1DM^[58,69] and T2DM^[62], resulting in myocardial remodeling and diastolic dysfunction in the long term (Figure 1B). Therefore, restoring the balance of the pathological and cardioprotective arms of local RAAS in chronic DCM has been proposed as a novel and more effective therapeutic strategy^[66,68].

In prediabetic, insulin resistant and early T1DM

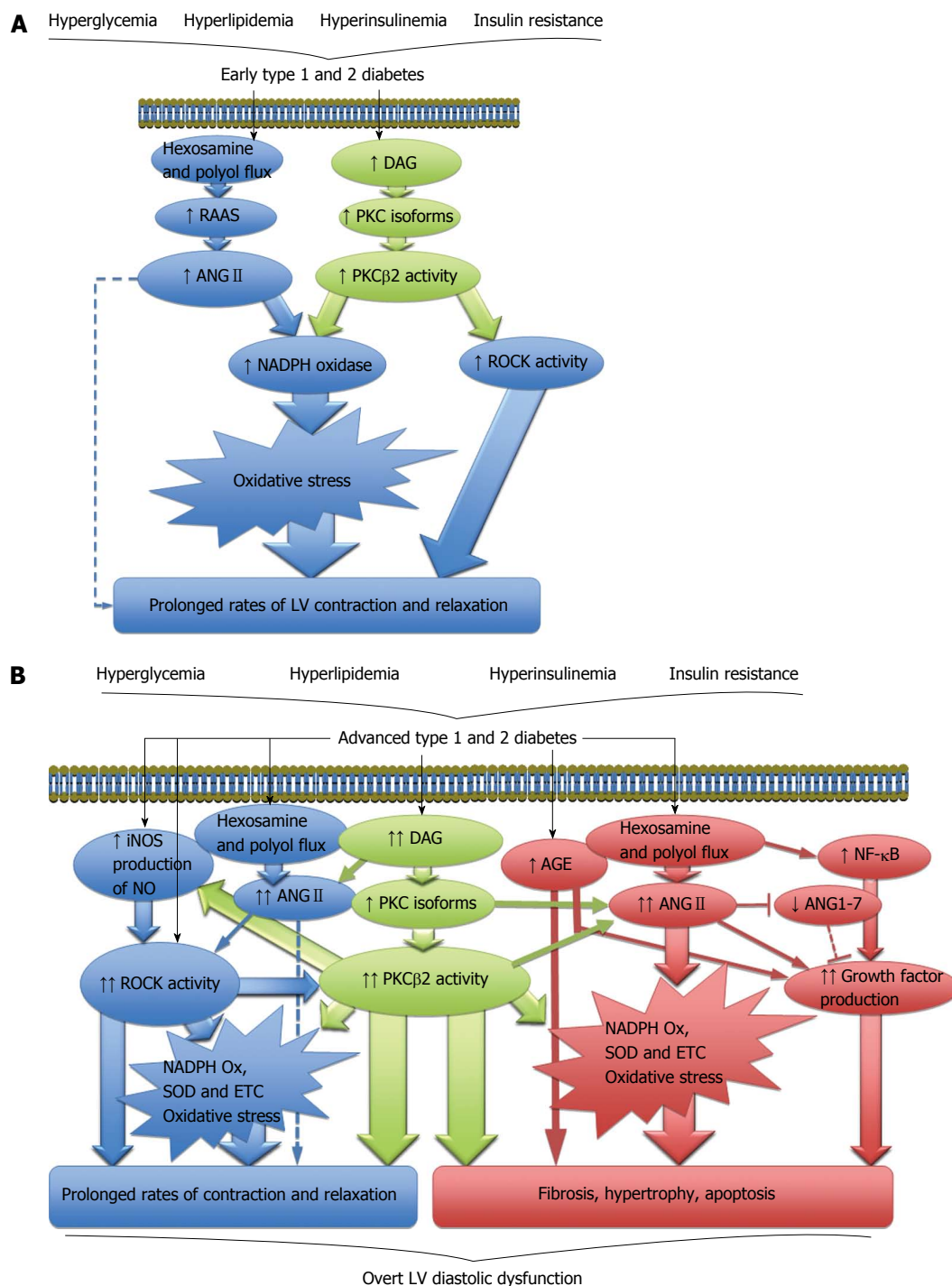


Figure 1 Cellular signaling pathways. A: Cellular signaling pathways driving contractile dysfunction in early diabetic cardiomyopathy; B: Cellular signaling pathways involved in the development of overt LV diastolic dysfunction in diabetes. ANG II: Angiotensin II; DAG: Diacylglycerol; NADPH: Nicotinamide adenine dinucleotide phosphate; PKC: Protein kinase C; RAAS: Renin angiotensin aldosterone system; ROCK: Rho kinase; AGE: Advanced glycation end products; ANG1-7: Angiotensin 1-7; ETC: Electron transport chain; iNOS: Inducible nitric oxide synthase; NF-κB: Nuclear factor-κB; NO: Nitric oxide; SOD: Superoxide dismutase; LV: Left ventricle.

animal models, there is also a potential role for elevated myocardial ANG II and activation of the classic RAAS arm in the initiation of cardiomyopathy. AT₁R-dependent NADPH oxidase-mediated oxidative stress is apparent in the hearts of insulin resistant, prediabetic OLETF rats^[70]. Normal myocardial nitric oxide production is restored after AT₁R blockade in a dietary induced rat model of obesity and insulin resistance^[71]. Moreover, one week of STZ diabetes in rats significantly increases

intracellular ANG II content in cardiomyocytes and is associated with myocardial oxidative stress and apoptosis^[72]. Unfortunately, as cardiac function was not assessed in these studies, it is not possible to say if the activation of the classic RAAS is responsible for LV contractile dysfunction documented in these early stages of diabetes. However, in support of this possibility, other studies have shown that increased RAAS activity in the hearts of diabetic animals is associated with impaired

calcium handling^[60,73] and reduced myofibrillar ATPase activity^[64]. Thus, there is a strong possibility for an early involvement of the RAAS in the pathogenesis of DCM as these subcellular alterations are often associated with early LV contractile dysfunction in diabetes (Figure 1A, broken line). Further studies are needed to clarify the involvement of classic RAAS and the ACE2-ANG-(1-7)-Mas receptor axis in contractile dysfunction at this time point, and if other angiotensin peptides^[74] and the AT₂R have any role in DCM development.

PKC β_2 pathway

There are multiple PKC isoforms in existence (α , β_1 , β_2 , γ , δ , ϵ , η , θ , ξ , ι/λ), which all regulate a large variety of cellular functions. In diabetes, hyperglycemia causes *de novo* synthesis of diacylglycerols, in turn activating several PKC isoforms in various tissues including the eye, kidney, vasculature and heart (reviewed extensively elsewhere^[75,76]) (Figure 1A). Of particular interest is the PKC β_2 isoform, which is found in the right and left ventricles of the heart^[77]. In rodent models of chronic STZ-induced T1DM, increased PKC β_2 expression and activity results in cardiomyocyte hypertrophy, fibrosis and impaired cardiomyocyte calcium handling capabilities, ultimately prolonging active diastolic relaxation and reducing passive compliance^[78-81] (Figure 1B). Elevated myocardial PKC β_2 expression levels are also reported in a rat model of dietary induced insulin resistance and T2DM^[82].

In the context of early DCM, one month after STZ induced diabetes in pigs, both cardiac PKC β_2 mRNA and protein expression is significantly increased compared to euglycemic controls^[83]. Consistent with this finding, two weeks post STZ diabetes induction, PKC β_2 protein activity is significantly increased in the hearts of rats^[84]. Furthermore, selective PKC β_2 inhibition from shortly after the onset of STZ diabetes prevented cardiomyocyte hypertrophy and NADPH oxidase mediated oxidative stress and thereby restored cardiac function to that of control rats^[85]. A significant finding of that study was antioxidant treatment with NAC had comparable effects to PKC β_2 inhibition in attenuating NADPH induced myocardial oxidative stress and hypertrophy. However, PKC β_2 inhibition was superior in preventing cardiac dysfunction by completely restoring isovolumetric relaxation times^[85]. This suggests that PKC β_2 also acts through oxidative stress independent pathways in the development of LV contractile dysfunction in early diabetes.

The RhoA/Rho kinase pathway axis - a possible mechanism for oxidative stress independent contractile dysfunction in early DCM

The Rho Kinases (ROCK), ROCK1 and ROCK2, are Rho-associated kinases activated by the small GTP-binding protein RhoA. ROCK1 is ubiquitously expressed, whereas ROCK2 appears to be brain and cardiac specific^[86]. The RhoA/ROCK pathway controls a diverse range of cellular processes in the cardiovascular system (see review^[87]), but primary actions of ROCK that are

pertinent to this review are the regulation of actin-myosin interactions^[88] and maintenance of cytoskeletal structure^[89].

A large body of evidence has established that ROCK is a key mediator of diastolic dysfunction in various rodent models of heart disease including hypertensive cardiomyopathy^[90-93], pressure-overload hypertrophy^[94] and myocardial infarction in mice^[95], as well as in patients with chronic heart failure^[96]. As a consequence, ROCK has gained attention as a promising therapeutic target for diabetic patients with diastolic dysfunction and preserved ejection fraction^[97]. In a small cohort of T2DM patients, daily administration of fasudil, a specific ROCK inhibitor, for two weeks significantly improved isovolumetric relaxation time, deceleration time, E/A wave ratio and E/e' wave ratio in comparison to baseline measures and placebo treated patients^[97]. Although not specifically identified in the aforementioned clinical study, elevated ROCK activity leads to diastolic dysfunction in chronic diabetes by inducing fibrosis, hypertrophy and cardiac ultrastructural remodeling^[98,99]. However, considering that ROCK is pivotal in regulating contractility at the level of the sarcomere and given the marked improvement in active diastolic indices in patients treated with fasudil, we speculate that elevated ROCK activity primarily drives diastolic dysfunction by modulation of sarcomeric proteins.

Early ROCK activation may also contribute to the initiation of DCM. Acute inhibition of ROCK in diabetic rats rapidly alleviates cardiac contractile dysfunction and increases developed force^[100], confirming ROCK has a direct interaction with the cardiomyocyte's contractile apparatus. Work conducted by our group demonstrates depressed actin-myosin dynamics and LV contractile dysfunction^[101] is associated with modestly elevated myocardial ROCK1 and ROCK2 expression^[102] suggesting that in the early diabetic rat heart, ROCK may drive contractile dysfunction by impairing actin-myosin interaction. Interestingly, we found all of these changes to occur largely independent of oxidative stress^[102]. Given the recent studies describing complex interactions between PKC β_2 and ROCK in chronic experimental DCM^[103,104] and the hypothesis that PKC β_2 is able to modulate cardiac function by oxidative stress independent mechanisms^[85], it is conceivable that PKC β_2 may indeed be acting through ROCK to prolong diastolic relaxation times (Figure 1A). Thus, there is strong evidence that implicates the PKC β_2 /RhoA/ROCK pathway in the pathogenesis and progression of DCM^[104]. Future investigations that examine the roles of ROCK at the level of the sarcomere in early diabetes may provide a clearer understanding of the initiating factors involved in DCM.

EARLY STRUCTURAL AND FUNCTIONAL CHANGES TO THE CONTRACTILE APPARATUS IN DCM

It has been widely held for some time that the diastolic

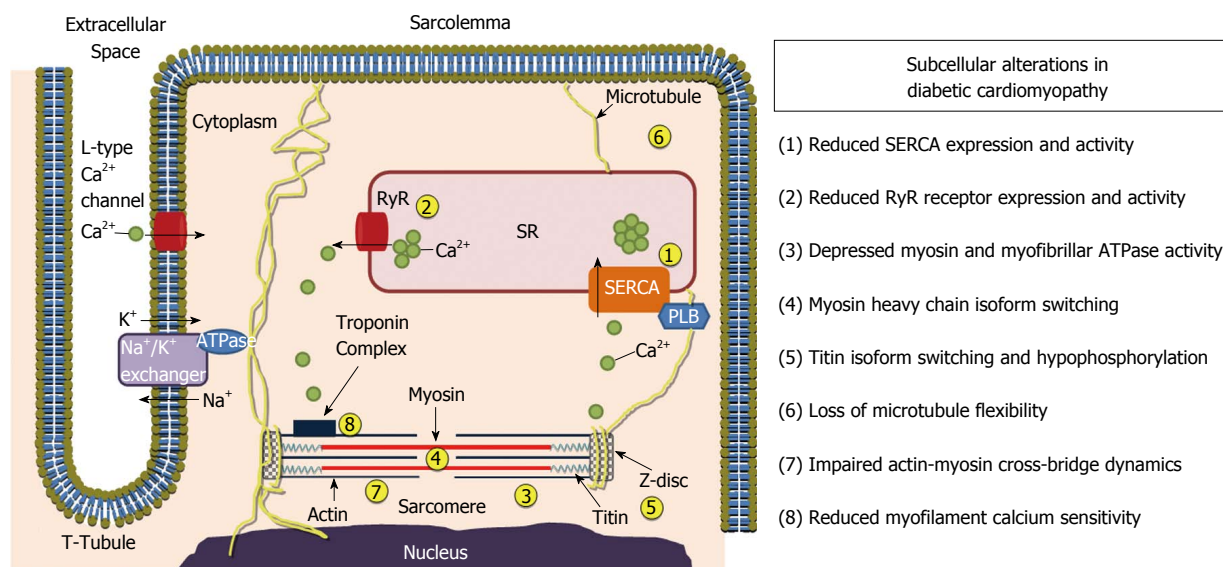


Figure 2 Subcellular alterations in various compartments of the cardiomyocyte in diabetic cardiomyopathy. PLB: Phospholamban; SERCA: Sarcoplasmic reticulum Ca^{2+} -ATPase; SR: Sarcoplasmic reticulum; RyR: Ryanodine receptor.

dysfunction associated with DCM is primarily attributed to increased LV fibrosis and hypertrophy, causing stiffening of the myocardium. However, a growing number of research findings provide evidence that insulin resistance and hyperglycemia also alter cardiomyocyte intracellular processes responsible for initiating and regulating cardiac contraction and force development on a beat-to-beat basis (see reviews^[105,106]). These include declines in cardiomyocyte calcium handling abilities, aerobic energy producing enzyme activity, integrity of cytoskeletal structures as well as sarcomeric dysfunction (Figure 2). In the following section we will discuss how all of these subcellular alterations contribute to contractile dysfunction in early DCM in both T1DM and T2DM (Table 1).

Alterations in calcium handling proteins and excitation-contraction coupling

Sufficient and timely transport of calcium ions (Ca^{2+}) in and out of the cardiomyocyte and sarcoplasmic reticulum (SR) is vital for the maintenance of normal cardiac function. During systole, Ca^{2+} enters through sarcolemma L-type Ca^{2+} channels and Ca^{2+} clusters are released spontaneously from the SR Ca^{2+} stores via the ryanodine receptor (RyR). The release of Ca^{2+} clusters causes Ca^{2+} sparks and initiates contraction by excitation-contraction coupling. In diastole, Ca^{2+} is pumped back into the SR via SR Ca^{2+} -ATPase (SERCA) and out of the cytosolic spaces by the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger, driving myocardial relaxation.

Systolic and diastolic Ca^{2+} handling impairment is characteristic of T1DM, even in the early stages. Three weeks post STZ, SR Ca^{2+} reuptake is prolonged and SERCA activity is decreased in comparison to the control group of normoglycemic rats^[107]. Slowed rates of contraction and relaxation in diabetic rats were attributed to prolonged Ca^{2+} transients and SR reuptake

in isolated papillary muscle preparations^[108]. In the whole heart, Takeda *et al.*^[109] also reported that prolonged myocardial relaxation in early diabetes was associated with depressed sarcolemma Ca^{2+} extrusion and SR Ca^{2+} reuptake, and reduced SERCA activity. More recently, it has been demonstrated that a progressive loss of cardiomyocyte Ca^{2+} handling abilities occurs ahead of LV contractile dysfunction in diabetic rats^[110].

Whether impaired Ca^{2+} handling is also an early event in the insulin resistant state and early T2DM is unclear. For example, in sucrose fed rats with insulin resistance^[111] and early T2DM^[112], impaired ventricular and cardiomyocyte relaxation is associated with decreases in SERCA and RyR expression and prolonged SR Ca^{2+} reuptake. However, in young prediabetic GK rats, appreciable changes in gene expression of Ca^{2+} handling proteins only resulted in moderate prolongation of shortening and a slower decay of Ca^{2+} transients^[113]. Similarly, in cardiomyocytes from young Zucker diabetic fatty (ZDF) rats, prolonged relaxation times cannot be fully explained by disruptions in Ca^{2+} handling^[114]. In stark contrast, Fredersdorf *et al.*^[115] have reported increased SERCA expression and Ca^{2+} sequestration in ZDF rats with early DCM, suggesting enhanced Ca^{2+} handling capabilities may be a compensatory mechanism to preserve cardiac function in the presence of hyperglycemia. Thus, subtle or moderately compromised cardiac contractile performance is not always attributable to changes in Ca^{2+} handling, except in early T1DM. Altered Ca^{2+} handling appears to be more important later in the course of diabetes.

Myosin heavy chain, myofibrillar/myosin ATPases and energy depletion

For cardiac contraction to occur, ATP bound to myosin must be hydrolysed to ADP and phosphate. Once the phosphate is released, ADP causes myosin to be strongly

Table 1 Reported subcellular alterations to cardiomyocyte structure and function in early type 1 and type 2 diabetes and gaps in our current knowledge

Cardiomyocyte function change		Type 1 diabetes	Type 2 diabetes
Excitation-contraction coupling, calcium release-reuptake and calcium sensitivity	(-) ↑ ↓	↑↑ cTnI phosphorylation ^[181] (-) cTnI phosphorylation, Ca ²⁺ sensitivity ^[123] ↑ PKCβ ₂ mediated AGE increase, Ca ²⁺ release ^[50] ↓ SERCA activity ^[107,109,110] ↓ SERCA and RyR expression ^[110] ↓ Diastolic Ca ²⁺ extrusion ^[109] ↑ SR Ca ²⁺ reuptake time ^[108,109]	↑ Ca ²⁺ transient times ^[113] ↑ SERCA expression, SR Ca ²⁺ reuptake ^[115] ↓ SERCA, RyR expression ^[111,112] ↑ SR Ca ²⁺ reuptake time ^[111,112]
Aerobic energy production	(-)	(-) ATP turnover ^[125]	?
Sarcomere organization		(-) Myofibrillar, sarcomeric order ^[130]	?
Contractile force development	(-) ↓	↓ Myosin, myofibrillar ATPase activity ^[117-120,143] ↓ CB cycling, myosin-actin proximity ^[101,123,124] ↓ MLC2 phosphorylation, (-) cMyBP-C phosphorylation ^[123] ↑ ROCK expression, (-) nitrosylation ^[102]	↑ Systolic LV pressure, CB formation ^[139] ?
Contractile force transmission, sliding velocity and compliance	↓	↑ V ₃ myosin isozyme expression ^[117,122-124] ↓ Myosin head extension ^[101] ↓ Relaxation rate ^[101,123,124] ↓ MLC2 phosphorylation, (-) cMyBP-C phosphorylation ^[123] ↑ Nitrosylation, lipid peroxidation, prolonged relaxation ^[43,44]	↑ Diastolic myosin head separation ^[139] ↑ Ventricular, myocyte relaxation ^[111,112] ↑ Shortening time ^[113] ↑ Relaxation time ^[114]
Cardiomyocyte hypertrophy and apoptosis	↑	↑ ANG II mediated oxidative stress ^[72] ↑ PKCβ ₂ mediated oxidative stress, apoptosis ^[50] ↑ PKCβ ₂ mediated oxidative stress, hypertrophy ^[85]	↑ ANG II mediated NADPH Ox ^[70]

References quoted as they appear in text. ¹Study conducted in humans; (-): No change compared to controls; AGE: Advanced glycation endproducts; ANG II: Angiotensin II; CB: Cross-bridge; cMyBP-C: Cardiac myosin binding protein C; cTnI: Cardiac troponin I; MLC2: Myosin light chain 2; NADPH Ox: Nicotinamide adenine dinucleotide phosphate oxidase; PKC: Protein kinase C; ROCK: Rho kinase; RyR: Ryanodine receptor; SERCA: Sarcoplasmic reticulum Ca²⁺-ATPase; SR: Sarcoplasmic reticulum; LV: Left ventricle.

bound to actin and induces the force-producing power stroke. Therefore, the rate-limiting step of cardiac contraction is ATP hydrolysis by myofibrillar and myosin ATPase^[116]. It has been known for several decades now that reduced myosin and myofibrillar ATPase activity is associated with contractile dysfunction in early diabetes. Three to four weeks post induction of T1DM, myosin and myofibrillar ATPase activities are significantly reduced in diabetic rats compared to normoglycemic, age-matched controls^[117,118]. Other studies have demonstrated that decreased myosin and myofibrillar ATPase activity is associated with *in vivo* contractile dysfunction in early diabetes in rats^[119] and reduced force development in isolated muscle preparations^[120]. Depression of myosin and myofibrillar ATPase activity in the diabetic heart is due to myosin isozyme switching from V₁ to V₃. Myosin isozyme expression is determined by the predominant myosin heavy chain (MHC) dimer^[121]. In the adult rodent heart, the V₁ (MHC_{αα} homodimer, α-MHC) is present^[105], however isozyme switching to the V₃ (MHC_{ββ} homodimer, β-MHC) is commonly exhibited in diabetic rodents. Such a V₃ isozyme shift has been reported along with depressed myosin ATPase activity in early diabetic rats, with some demonstrating a concomitant prolongation of tension development (without change in net tension development) and contraction times in isolated papillary muscle preparations^[117,122]. Notably, it has been shown that marked expression of β-MHC slows actin-myosin kinetics and thereby contributes to contractile dysfunction in early diabetes^[123,124].

Despite these convincing studies using animal

models, clinical experiments utilizing phosphorus magnetic resonance spectroscopy in T2DM patients with early DCM have found no association between diastolic dysfunction and ATP turnover^[125]. Thus, it is unclear if altered energetics of contraction is a cause or consequence of diabetes.

Changes in the structural compliance and order of the cardiomyocyte cytoskeleton network

The cardiomyocyte cytoskeleton is a highly organized and complex subcellular structure. The cytoskeleton can be divided into four main structures, this being the sarcomeric, extra-sarcomeric, membrane-sub-membrane and nuclear cytoskeleton^[126]. Although the cytoskeleton is considered a unified structure throughout the cardiomyocyte, there is a clear functional division between the contractile part, the sarcomere, and the non-contractile parts that transmit the developed power and ensures structural integrity of the cell and the functional syncytium^[127]. Arguably, the extra-sarcomeric cytoskeleton, comprising of microtubules, actin myofibrils and intermediate desmin filaments are as important for myocyte contraction as the sarcomeres, since these structures transfer the power produced by the sarcomeres through tethering of the sarcomere at the Z-disc to the submembrane skeleton and sarcolemma.

In the diabetic heart, there are inconsistent findings concerning the possible involvement of structural alterations to the sarcomere in the development of contractile dysfunction. One study using electron microscopy reported that hearts with advanced DCM

exhibited disarray of normal sarcomeric order, which was associated with contractile dysfunction^[128]. However, others have been unable to corroborate this finding. In contrast, ventricular ultrastructure, and especially the contractile apparatus, remained largely unchanged 8 mo post T1DM induction in rats^[129]. Jackson *et al.*^[130] demonstrated that in the initial stages of DCM sarcomeric and myofibrillar assembly remain unchanged from that of age-matched, normoglycemic controls, despite depressed contractile function. A unique feature of the sarcomeric cytoskeleton is the interaction of a myriad of proteins that provide exceptional stability. One of the major scaffolding proteins within the sarcomere is titin, a giant protein that spans half of the sarcomere from the Z-disc to the M-line, which provides spring-like recoil of the sarcomere to its relaxed length and facilitates power transmission of the sarcomere through the Z-disc. Titin exists in two isoforms; the stiffer N2B and the more elastic N2BA^[131]. The expression ratio and phosphorylation state of both isoforms are tightly controlled to regulate myocyte compliance and sarcomeric order^[132]. In the adult heart, the N2B isoform is predominantly expressed^[133], although a transition toward the N2BA isoform has been observed during the progression to heart failure^[134]. Indeed, titin isoform switching from N2B to N2BA has been demonstrated in the hearts of rats with advanced T1DM and diastolic dysfunction^[135]. Interestingly, in obese rats with T2DM, titin N2B hypophosphorylation contributes to contractile dysfunction in the absence of a change of isoform composition^[136]. Nevertheless, it is plausible that changes in composition and or phosphorylation of sarcomeric cytoskeletal proteins may be more responsible for contractile dysfunction in diabetes as opposed to overt disruption of sarcomeric order and organization.

Diabetes also affects microtubule function in the extra-sarcomeric compartment of the cytoskeleton. Microtubules are hollow protein cylinders created by the polymerization of α and β tubulin heterodimers. Amongst other functions, microtubules regulate the flexibility of the cytoskeleton and thus the contractile capacity^[127]. In the hearts of diabetic rats, microtubule flexibility is diminished^[137,138]. The lack of microtubule flexibility impedes adequate transfer of the power generated by the sarcomere and thus, contributes to contractile dysfunction^[137,138]. Polymerization of cytoskeletal actin filaments is increased in diabetes and essential for activation of the PKC β_2 /RhoA/ROCK pathways^[104]. The contribution of the extra-sarcomeric cytoskeleton is commonly overlooked in the pathogenesis of DCM and warrants further investigation, not only in areas of mechanical power translation but signal transduction between the sarcolemma, sarcomeres and the nucleus.

Actin-myosin cross-bridge dysregulation

The formation and dissociation of the actin-myosin cross-bridges (CBs) in the cardiac sarcomere is a pivotal determinant of force development and contractility. Various experimental approaches including X-ray diffraction^[139], simultaneous recording of heat generation^[140]

and the measurement of the work of isolated muscle^[141] have been used to investigate cardiac performance at the level of the sarcomere and cardiomyocyte. These approaches show that impaired cyclic transfer of myosin heads to actin filaments contributes to contractile dysfunction at the sarcomere level at the onset of diabetes. Moreover, CB dysregulation is also observed in the prediabetic state in the heart of insulin resistant animals.

A significant reduction in CB cycling has been reported in isolated cardiac muscle from early diabetic rats, due to a preferential shift to the less efficient β -MHC isoform^[123,124]. Indeed the time required for CB detachment is inversely related to the proportion of α -MHC expression in isolated ferret papillary muscle, attesting to a clear role for β -MHC content in prolonging CB kinetics^[142]. Joseph *et al.*^[143] demonstrated a reduced rate of CB attachment and detachment due to diabetes in *ex vivo* papillary muscle, as well as a significant reduction in the total number of CBs recruited, without a change in force developed per CB. Impaired CB dynamics in this preparation was attributed to a slower myosin ATPase turnover^[143]. Accordingly, T1DM is claimed to have no discernible effect on CB mechanical efficiency in isolated trabeculae from rats^[144]. Although it is important to recognize that in contrast to Joseph *et al.*^[143], the estimated number of CBs attached during contraction did not differ between control and diabetic rats in the study of Han *et al.*^[144]. Recent X-ray diffraction studies conducted by our group affirm these previous findings when CB dynamics are determined across the different layers of the ventricular muscle *in situ*, in the hearts of early diabetic^[101] and prediabetic insulin resistant rats^[139]. We demonstrated significant myosin head displacement from actin filaments throughout the cardiac cycle, but especially at end diastole, in the rat heart three weeks post STZ induction^[101]. Reduction in the proximity of myosin heads to actin filaments at end diastole was directly correlated with a prolonged LV pressure decay rate in diabetic rats. Further, a transmural gradient was found in actin-myosin separation, being most pronounced in the deeper subendocardial layer^[101]. However, we also found that the change in CB transfer to actin under β -adrenergic stimulation was comparable in control and diabetic rats suggesting that the impairment of the contractile apparatus precedes that of the β -adrenoceptor signaling^[101]. In young prediabetic GK rats, we also found significant myosin head displacement from actin at end diastole in the subendocardial layer of the LV wall^[139]. It is still unclear if these altered CB dynamics translate to significant LV dysfunction, but fiber shortening and Ca²⁺ transients are reported to be more prolonged in young GK rats^[113].

It is noteworthy that X-ray diffraction recordings from the *in situ* beating heart demonstrate in both STZ induced diabetic rats and young prediabetic GK rats that myosin-myosin separation (interfilament spacing) does not differ from that of controls, and therefore altered myosin head distribution cannot be explained by a

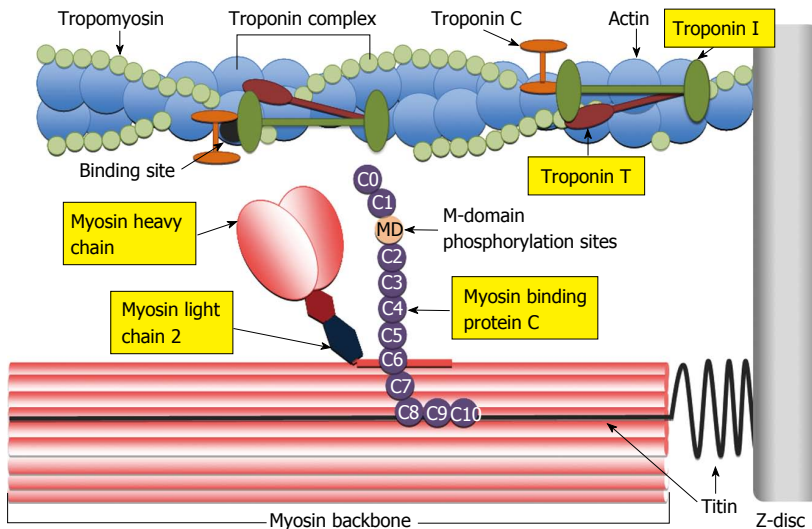


Figure 3 Illustration of the cardiac sarcomere indicating the location of actin thin-filament and myosin thick-filament accessory proteins involved in the regulation of actin-myosin cross-bridge dynamics and kinetics. C0-C10: Immunoglobulin-like and fibronectin-like domains of myosin binding protein C; MD: M-domain of myosin binding protein C containing protein kinase phosphorylation sites.

difference in sarcomere length^[101]. Since alterations in thin filament complex cannot explain changes in myosin head extension (despite being a critical determinant of Ca^{2+} sensitivity and CB cycling rates), this implies that modifications to thick filament proteins that regulate myosin head extension are likely to be involved in the impaired CB dynamics and contractile dysfunction in early DCM.

The thick filament accessory proteins - regulators of myosin head extension

Two major proteins reside within the myosin thick filament complex that regulate myosin head extension, namely myosin light chain-2 (MLC2), otherwise known as regulatory light chain and cardiac myosin binding protein-C (cMyBP-C) (Figure 3). Detailed information on the complex molecular structure and broader physiological functions of both these proteins is beyond the scope of this paper but has been reviewed extensively by others (see reviews^[145-149]).

It is well accepted that MLC2 and cMyBP-C regulate myosin head extension by means of kinase phosphorylation. In the case of MLC2, phosphorylation is tightly regulated by MLC kinase and Ca^{2+} /calmodulin-dependent protein kinase (CaMK), and is dephosphorylated by MLC phosphatase (see review^[150]). X-ray diffraction experiments conducted by Colson *et al.*^[151] reveal that the negative charge created on the myosin neck when MLC2 is phosphorylated, repels the negative charge of the myosin backbone and thus, subsequently the myosin heads are displaced toward the actin filament.

cMyBP-C is a multifunctional protein within the sarcomere that is primarily involved in the regulation of contractility^[152-155] and the organization and structural maintenance of the sarcomere^[156-158]. cMyBP-C prevents myosin head projection toward actin by a "tethering"

mechanism^[159], however, upon phosphorylation, the molecular tether mechanism is released and myosin heads are freed to be displaced towards actin^[160,161]. As protein kinase A (PKA) is the primary kinase that phosphorylates cMyBP-C, it is thought that under β -adrenergic stimulation, cMyBP-C works in cooperation with the troponin complex to maintain force development and adequate relaxation and LV filling at higher heart rates^[151]. In support of this proposed role, cMyBP-C phosphorylation by PKA evokes radial displacement of myosin heads toward actin in isolated cardiac muscle^[162]. To date, seventeen phosphorylation sites have been identified on cMyBP-C^[163], however three (Ser273, Ser282, Ser302)^[164] have been identified as crucial regulators of contractility, which are all located within the M-domain of cMyBP-C (Figure 3). CaMK is also able to phosphorylate cMyBP-C at these sites in a Ca^{2+} dependent manner and thereby play a potentially important role in regulating contractile function independent of changes in intracellular Ca^{2+} transients^[165,166].

Several studies have demonstrated diminished phosphorylation of cMyBP-C in experimental^[167,168] and human^[163,168,169] non-diabetic cardiac disease. Although there is paucity of information in the literature, some evidence suggests that MLC2 and cMyBP-C phosphorylation are also reduced in DCM. In the hearts of diabetic rats, a significant depression of MLC2 phosphorylation is reported, accompanied by prolonged LV pressure development and decay rates^[170]. Diabetic swine also exhibit significantly reduced cMyBP-C phosphorylation^[171]. Most recently, in a rat model of early DCM, MLC2 phosphorylation was significantly reduced in comparison to normoglycemic controls while cMyBP-C phosphorylation remained unchanged^[123].

It should also be appreciated that other kinases including $\text{PKC}\beta_2$ and ROCK can directly phosphorylate cMyBP-C, and thus, potentially impact on contractile

function, without appreciably altering pan phosphorylation state. In isolated rat cardiomyocytes, increased PKC β_2 expression did not change pan phosphorylation of cMyBP-C despite prolonging rates of shortening and relaxation, but phosphorylation of Ser302 in particular was significantly increased^[172]. Taken together with the finding that phosphorylation Ser302 on cMyBP-C modulates CB kinetics^[173], this suggests that the contractile dysfunction observed in the isolated cardiomyocytes may, in part, be a result of altered CB dynamics. Others have also demonstrated active ROCK directly phosphorylates cMyBP-C in cardiomyocytes^[174]. Therefore, it is possible that cMyBP-C phosphorylation by PKC β_2 and ROCK may not alter pan phosphorylation, but may indeed impair CB dynamics by displacing myosin heads from the thin filaments and slowing rates of CB attachment and detachment. At the very least, thick filament accessory proteins probably play a role in contractile dysfunction in early DCM, although this requires further investigation.

The actin thin filament troponin complex could changes in calcium sensitivity contribute to early DCM?

Much of our previous discussion has focused on diabetes impact on the myosin thick filament, however we cannot overlook the importance of the actin thin filament in modulating cardiac contractile properties. The heterotrimeric cardiac troponin (cTn) complex is comprised of cTnC (Ca²⁺ reception site), cTnT and cTnI, the latter two are involved in the transduction of the Ca²⁺-binding signal (Figure 3). The multiple interactions of cTnI and cTnT with actin and tropomyosin regulate CB kinetics and the number of recruited CBs (reviewed in^[175]) (Figure 3).

cTnI is widely recognized to be the principle regulator of CB kinetics and myofilament sensitivity of the thin filament. Under physiological conditions, increased cTnI phosphorylation at Ser23/24 by PKA (which usually occurs under β -adrenergic stimulation) leads to decreased myofilament Ca²⁺ sensitivity and accelerated CB dissociation, and thus hastened relaxation^[176]. However, under pathophysiological conditions when expression and activity of PKC isoforms are elevated, PKC rather than PKA predominantly phosphorylates cTnI^[177]. Ca²⁺ sensitivity is decreased by PKC phosphorylation at Ser23/24 of cTnI in a manner similar to physiological phosphorylation by PKA, but in contrast, phosphorylation of other sites (Ser44/45 and Thr144) slow CB cycling and sliding velocity and impair relaxation^[176]. Thus, PKC-mediated phosphorylation of cTnI may partly explain contractile dysfunction in advanced DCM. Many studies have demonstrated that depressed myofilament Ca²⁺ sensitivity and PKC-induced increases in cTnI phosphorylation are observed in the hearts of rats after eight to twelve weeks of T1DM^[178-180].

In early DCM however, there are some conflicting findings regarding the contribution of cTnI. One study found that cellular PKC ϵ translocation paralleled a 5-fold increase in cTnI phosphorylation^[181]. Importantly, neither

cardiomyocyte function nor myofilament Ca²⁺ sensitivity was examined in this study. Conversely, others reported no changes in cTnI phosphorylation or myofilament calcium sensitivity four weeks post T1DM induction^[123]. Therefore, there are some uncertainties as to the role of cTnI changes in early DCM. Given the strong evidence implicating cTnI in chronic diabetes models, this may only be a prominent driver of contractile dysfunction in the advanced stages of DCM.

Interestingly, ROCK is able to phosphorylate multiple sarcomeric proteins. Vahebi *et al.*^[174] revealed a direct role for ROCK activation in depressing myofilament tension development and ATPase activity *via* phosphorylation of cTnT of the thin filament complex, independent of MLC2 phosphorylation. A possible limitation of these findings is that the authors used constitutively active ROCK2, and whilst many other studies have demonstrated functional changes in diabetes with selective ROCK inhibition, not all have found an increase in ROCK phosphorylation^[102,182]. Nonetheless, there is evidence to suggest an increase in ROCK activation in diabetes could contribute to reduced CB cycling through modulation of the thin filament, as well as the thick filament.

OTHER EXACERBATING RISK FACTORS OF DCM

In the clinical setting, DCM is rarely diagnosed independent of any additional risk factors, however, the cumulative effects of multiple risk factors in insulin resistance and diabetes has been rarely considered in preclinical studies. Risk factors that have been shown to exacerbate these disease states include hypertension and obesity, both commonly present with T2DM in patients (reviewed by reference^[183]). Hypertension alone is a serious risk factor for the development of diastolic heart failure. Raised systolic blood pressure associated with hypertension evokes an increase in cardiac afterload and accordingly, vascular remodeling and LV hypertrophy ensue as pathophysiological adaptations to maintain cardiac output. Several decades earlier, Factor *et al.*^[184] showed that diabetes combined with hypertension increased microvessel tortuosity, microaneurysms and focal constrictions more than either disease state alone, which would increase the risk of myocardial ischemia. Activation of the RAAS and sympathetic nervous system (SNS) in the hypertensive state leads to fibrosis and cardiomyocyte hypertrophy, ultimately leading to diastolic dysfunction (see review^[185]). In the context of early DCM where LV structural remodeling is normally absent, the additive effect of hypertension may accelerate cardiomyocyte subcellular dysfunction. Jeong *et al.*^[186] have demonstrated that diastolic dysfunction in deoxycorticosterone acetate treated hypertensive mice is driven by oxidative stress modifications to cMyBP-C, which subsequently suppresses CB kinetics^[186]. Further, chronic activation of ROCK plays a central role in most forms of hypertension and diabetic coronary

dysfunction^[87,102]. Thus, hypertension may exacerbate the development of cardiac dysfunction in early DCM by redox modifications of various sarcomeric proteins, disturbed CB dynamics and increased oxidative stress.

Obesity (in particular abdominal obesity) is a risk factor for the development cardiac dysfunction and heart failure, independent of diabetes^[187]. Several metabolic and neurohormonal mechanisms have been postulated to contribute to obesity-induced cardiomyopathy (see review^[188]). Of particular interest, is the interplay of the SNS and the RAAS, which occurs during obesity^[189,190]. Classically, obesity leads to activation of the SNS, which in turn rapidly activates the production of renin from the renal juxtaglomerular apparatus as well as angiotensinogen from adipocytes and thus, promotes the excessive production of ANG II. Consequently, systemic hypertension ensues (reviewed in^[189]). Given our previous discussion on hypertension-induced modulation of sarcomeric proteins leading to diastolic dysfunction^[186], it is plausible that obesity may exacerbate DCM in patients *via* the promotion of systemic hypertension. On the other hand, obesity may also exacerbate DCM by directly increasing SNS outflow to the heart. In normotensive obese patients, increased LV mass correlates with elevated cardiac SNS activity^[191]. Considering the hypertrophic and fibrotic actions of ANG II, elevated local RAAS activity driven by activation of the cardiac SNS could, in part, explain increased LV mass in obese patients. In addition, one study has shown that a high fat diet induced mitochondrial lesions and depressed mitochondrial density in the hearts of GK rats^[192]. Therefore, obesity induced activation of the SNS in combination with diabetes may indeed exacerbate DCM.

Lastly, gender must also be considered as an additional risk factor for the development, progression and severity of DCM (reviewed in detail by^[193]). Indeed, the Framingham Heart study revealed that diabetic women had twice the frequency of heart failure in comparison to diabetic men^[22]. More recent clinical evidence suggests that although non-diabetic women appear to have more pronounced endothelium-mediated dilation (due to higher rates of nitric oxide production), T2DM abrogates these sex differences in vascular function^[194]. Female mice also lose this vascular protection with T2DM^[182]. Although, similar vascular dysfunction is observed in both sexes, the molecular mechanisms underlying this dysfunction in males appears to be elevated ROCK activity, however this does not appear to be the case in female mice^[182]. The impact of sex on myocardial function in diabetes is less clear. Early in the time course of experimental T1DM, it appears that male rats are more vulnerable to cardiac dysfunction. Five to six weeks of STZ induced diabetes in rats caused greater prolongation of the rates of pressure development and decay in diabetic male rats in comparison to their female counterparts^[195-197]. However, others have demonstrated that female rats after four weeks from STZ induction exhibit significantly

lower MLC2 phosphorylation compared to diabetic males despite equivalent impairment in cardiac muscle tension development in both male and female diabetic rats^[123]. Sex differences in the loss of cardiomyocytes may contribute to differential changes in contractile function in diabetes. Females respond to metabolic stresses with greater myocardial glycogen accumulation and elevated glycogen-induced autophagy than males^[198,199]. Thus, the underlying mechanisms of sex differences in DCM require a great deal of research to appreciate which of the factors we have identified in this review differ between sexes and how the pathophysiology of diabetes might be compounded by other risk factors in females.

CONCLUSION

In summary, DCM is a progressive disease beginning with contractile dysfunction ahead of LV remodeling. A number of subcellular alterations to the cardiomyocyte contribute to contractile dysfunction in early DCM. While changes in Ca²⁺ handling protein expressions are evident and likely contribute to the prolongation of systole and relaxation, diminished intracellular Ca²⁺ transients appear to be associated only with advanced DCM. Diastolic LV dysfunction has its origins in changes in sarcomeric and other cytoskeletal proteins, both due to transcription and posttranslation changes. Upregulation of PKC β 2 activity is proposed as a central factor in the development of DCM, promoting the activity of both oxidative stress dependent and independent pathways to drive contractile dysfunction in early DCM. Independent of oxidative stress we suggest that hyperglycemia alters modulation of the sarcomeric thick-thin filaments through PKC β 2/ROCK activation. It is not known if any of these pathological changes in sarcomere function are exacerbated by other risk factors such as hypertension and obesity.

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Peripheral artery disease in patients with diabetes: Epidemiology, mechanisms, and outcomes

Thejasvi Thiruvoipati, Caitlin E Kielhorn, Ehrin J Armstrong

Thejasvi Thiruvoipati, Caitlin E Kielhorn, Ehrin J Armstrong, Section of Cardiology, Denver VA Medical Center and University of Colorado School of Medicine, Denver, CO 80220, United States

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Correspondence to: Ehrin J Armstrong, MD, MSc, Section of Cardiology, Denver VA Medical Center and University of Colorado School of Medicine, Denver, CO 80220, United States. ehrin.armstrong@ucdenver.edu
 Telephone: +1-415-3122480

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Abstract

Peripheral artery disease (PAD) is the atherosclerosis of lower extremity arteries and is also associated with atherothrombosis of other vascular beds, including the cardiovascular and cerebrovascular systems. The presence of diabetes mellitus greatly increases the

risk of PAD, as well as accelerates its course, making these patients more susceptible to ischemic events and impaired functional status compared to patients without diabetes. To minimize these cardiovascular risks it is critical to understand the pathophysiology of atherosclerosis in diabetic patients. This, in turn, can offer insights into the therapeutic avenues available for these patients. This article provides an overview of the epidemiology of PAD in diabetic patients, followed by an analysis of the mechanisms by which altered metabolism in diabetes promotes atherosclerosis and plaque instability. Outcomes of PAD in diabetic patients are also discussed, with a focus on diabetic ulcers and critical limb ischemia.

Key words: Peripheral artery disease; Epidemiology; Pathophysiology; Outcomes; Diabetes

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Core tip: Diabetes mellitus (DM) is a major risk factor of peripheral artery disease (PAD), leading to increased morbidity and mortality as well as an accelerated disease course. As such, a more thorough understanding of the multi-factorial mechanisms underlying disease etiology for both DM and PAD is justified. This review provides clinical insight into the current state of research in the pathophysiology of PAD in diabetic patients, as well as highlights the progress of endovascular interventions for PAD, with a focus on techniques that have shown promise for treatment of critical lower limb ischemia.

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INTRODUCTION

Over 170 million people worldwide have diabetes mellitus (DM) and the worldwide burden is projected to increase to 366 million people by 2030^[1,2]. The major causes of DM include impaired insulin secretion or inadequate response to secreted insulin^[3]. DM is a major risk factor for atherosclerotic disease as well as cardiovascular mortality and morbidity^[3,4]. Atherosclerotic disease is not only increased in incidence in diabetic patients, but its course is also accelerated^[4], thereby accounting for as much as 44% of all-cause mortality^[5]. DM-associated atherosclerosis can lead to complications in all major of vascular beds, including the coronary arteries, carotid vessels, and lower extremity arteries^[5,6]. For example, a study by Haffner *et al.*^[7], estimated the 7-year incidence of a first-time myocardial infarction (MI) in diabetic patients at 20.2%, compared to 3.5% in nondiabetic patients.

Peripheral artery disease (PAD) is defined as atherosclerotic occlusive disease of lower extremities. PAD is associated with increased risk of lower extremity amputation and is also a marker for atherothrombosis in cardiovascular, cerebrovascular and renovascular beds. Patients with PAD therefore have an increased risk of MI, stroke and death^[8]. Additionally, PAD causes significant long-term disability in diabetic patients^[5,9]. The treatment of patients with PAD can therefore be expensive, owing to need for a variety of diagnostic tests, therapeutic procedures, and hospitalizations^[10].

The purpose of this article is to review the epidemiology and mechanisms that contribute to development of PAD in diabetic patients. The outcomes of PAD in diabetic patients are also compared to nondiabetics, with an emphasis on the prevention of major amputations among patients with DM who have severe PAD.

EPIDEMIOLOGY OF PAD IN PATIENTS WITH DIABETES

PAD affects 12 million people in United States. The most common symptom in PAD is claudication, characterized as a cramping, pain or aching in the calves, thighs or buttocks with exertion and relief with rest^[8]. However, many patients have atypical symptoms that may require formal testing with an ankle brachial index test to diagnose PAD^[11].

The strongest risk factors for PAD are DM and smoking, with an odds-ratio of 2.72 and 1.88, respectively^[12]. With decreased rates of smoking in Western countries, DM is projected to become an increasingly important contributor to the development and progression of PAD. Previous studies have shown that glucose intolerance is associated with a greater than 20% prevalence of an abnormal ankle-brachial index (ABI) relative to 7% in those with normal glucose tolerance^[4]. Moreover, 20%-30% of patients with PAD have DM, although this is likely underestimated by the asymptomatic nature of less severe PAD and the altered

pain perception in diabetic patients due to peripheral neuropathy^[5].

Age, duration of diabetes, and peripheral neuropathy are associated with an increased risk of PAD in patients with pre-existing DM^[8,12]. Using ABI to identify PAD, the prevalence of PAD in people with DM over 40 years of age has been estimated to be 20%^[13]. This prevalence increases to 29% in patients with DM over 50 years of age^[5,14]. The severity and duration of DM are important predictors of both the incidence and the extent of PAD, as observed in United Kingdom Prospective Diabetes Study, where each 1% increase in glycosylated hemoglobin was correlated with a 28% increase in incidence of PAD, and higher rates of death, microvascular complications and major amputation^[15,16]. This correlation is particularly strong in men with hypertension or active tobacco use^[5]. Patients with PAD who have DM also tend to stay longer in hospital, incur greater costs, and account for greater use of hospital resources compared to patients with PAD alone^[10,17].

DM is also associated with more severe below-the-knee PAD (e.g., popliteal, anterior tibial, peroneal and posterior tibial arteries), whereas risk factors such as smoking are associated with more proximal PAD in the aorto-ilio-femoral vessels^[8,16]. The prevalence of concomitant PAD and DM is especially high in those patients who have critical lower limb ischemia, with more than 50% of patients with critical limb ischemia (CLI) also having DM^[18].

In patients with PAD, the cardiovascular event rate over a 5-year period, including MI and stroke, is 20%, and the overall mortality rate is 30%^[19]. Among those with CLI, 30% undergo major amputation, and the 6-mo mortality rate is 20%^[20]. Diabetic patients comprise 25%-30% of patients undergoing coronary artery revascularization and up to 60% of patients presenting with acute MI^[21-23]. Cardiovascular and cerebrovascular event rates, both fatal and non-fatal, are increased in patients with PAD and DM relative to nondiabetic patients with PAD^[8].

Similar to the greater likelihood of diffuse and complex coronary artery disease in diabetic patients, patients with DM also tend to have more diffuse PAD, compared to the more focal disease observed in those without DM^[1,5,24]. Although patients with DM tend to present later in the course of disease progression, the incidence of intermittent claudication is also higher than in nondiabetics, as seen in Framingham study^[5,25]. In that cohort, the risk of claudication associated with DM was increased by 3.5 fold in men and 8.6 fold in women^[25]. Concomitant peripheral neuropathy, which diminishes sensory feedback and leads to a lack of symptoms from minimized pain perception, may predispose patients with DM and PAD to present with more advanced disease, such as an ischemic ulcer or gangrene, compared to patients without DM^[8]. The prevalence of major amputation in patients with DM is also higher than in nondiabetics, with rates ranging from 5 to 15 times greater in some studies^[8,16]. In a Medicare population, relative

to nondiabetic patients, the relative risk (RR) for lower extremity amputation was 12.7 in diabetic patients. The RR rose to 23.5 in a cohort aged 65–74 years^[4].

The risk relationship between PAD and DM is noted to be reciprocal: while DM is a risk factor for PAD, higher rates of PAD, up to 30%, have been found in diabetic patients^[26]. The Hoorn study further clarified the discrepancy in prevalence of PAD between diabetic and nondiabetic patients: glucose intolerance was associated with 20.9% prevalence of an ABI less than 0.9, relative to 7% in those with normal glucose tolerance^[27]. Moreover, the prevalence of PAD in diabetic patients is likely underestimated by the asymptomatic nature of the condition, lack of reporting by the patients, and the altered pain perception in diabetic patients due to peripheral neuropathy^[11,26].

MECHANISMS OF PAD IN PATIENTS WITH DIABETES

DM is characterized by hyperglycemia, dyslipidemia, and insulin resistance^[4,28–30]. These pathologic states foster development and progression of PAD through mechanisms similar to that in coronary or carotid artery disease^[31,32]. These mechanisms include derangements in the vessel wall through promotion of vascular inflammation and endothelial cell dysfunction; abnormalities in blood cells, including smooth muscle cells and platelets; and factors affecting hemostasis (Table 1). Such vascular abnormalities that cause atherosclerosis in DM patients are often prevalent prior to the diagnosis of DM, and their severity increases with worsening blood glucose control and duration of DM^[8,33]. Taken together, these mechanisms likely contribute to increased plaque burden, plaque instability, and greater complexity of vascular disease^[3,34–36].

Inflammation

Inflammation is a risk marker for atherothrombosis. Among biomarkers of inflammation, C-reactive protein (CRP) is associated with both the development of PAD and impaired glucose regulation^[37]. CRP may also play a direct pathophysiologic role by promoting production of procoagulant tissue factor, leukocyte adhesion molecules, and chemotactic substances. CRP causes derangement in vascular tone by inhibiting endothelial nitric oxide synthase (eNOS), which produces nitric oxide (NO) *via* phosphoinositol-3-kinase dependent pathway^[3,8,38]. Moreover, CRP impairs fibrinolysis *via* the production of substances such as plasminogen activator inhibitor (PAI)-1, which blocks the breakdown of plasminogen into plasmin, a fibrinolytic^[39]. All of these factors in diabetic patients increase the susceptibility of vascular walls to the development of atherosclerosis^[40].

DM is also associated with increased circulating levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin-6^[41,42]. These cytokines bind to endothelial cell surface receptors and activate nuclear

factor (NF)- κ B. This process promotes transcription of endothelial cell adhesion molecules, leading to increased binding of leukocytes and platelets to the endothelial surface, thereby fostering thrombogenesis. Plaque inflammation and instability may also be enhanced due to the increased leukocyte migration, which is associated with an increased risk of rupture and subsequent thrombus formation^[3,40,43].

Endothelial dysfunction

Endothelial cells mediate the interaction between blood cell elements and the vascular wall, thereby affecting blood flow, nutrient delivery, coagulation, and the balance between thrombosis and fibrinolysis^[8,44]. Endothelial cells also release substances that are critical for blood vessel function and structure, including NO, reactive oxygen species, and endothelin^[4,44]. Insulin is critical for the induction of phosphoinositol-3 kinase signaling, leading to production of NO and subsequent smooth muscle cell relaxation^[38,45]. NO also inhibits platelet activation and limits vascular smooth muscle cell (VSMC) migration and proliferation^[46–48]. By mediating the interaction between leukocytes and the vascular wall, NO also plays an important role in vasodilation and inflammation^[8,44].

Hyperglycemia, insulin resistance, and free fatty acid (FFA) production all reduce NO bioavailability in diabetic patients. Hyperglycemia impairs eNOS function, promoting oxidative stress by producing reactive oxygen species in endothelial and VSMCs^[38,49]. In turn, these factors inhibit endothelial vasodilation^[4,38,44]. Insulin resistance induces excess production of FFAs, which activate protein kinase C (PKC), inhibit phosphatidylinositol (PI)-3 kinase (an important agonist of eNOS), and produce reactive oxygen species^[24,28,50,51]. These mediators inhibit NO production and decrease its bioavailability, thereby causing endothelial dysfunction and leading to greater susceptibility of the vascular bed to atherosclerosis^[8,24,38,44,49–51].

DM is also associated with the enhanced production of advanced glycation end products (AGEs), which are formed by binding of reducing sugars to free amino groups *via* the Maillard reaction^[3,52–54]. The interaction of AGEs with their receptors can upregulate the synthesis of pro-inflammatory transcription factors such as NF- κ B and activator protein 1^[54]. In addition to decreased endothelial function and impaired NO formation, these factors also lead to increased leukocyte chemotaxis, adhesion, transmigration, and transformation into foam cells. The latter process is the first step in the formation of atheromatous plaque^[8].

VSMC

VSMC migration from the medial layer into the intimal layer is associated with deposition of complex extracellular matrix, thereby stabilizing the atheroma. This decreases the risk of plaque rupture associated with thrombosis^[4,48,55,56]. In diabetic patients, plaques have fewer VSMC, increasing the chance of rupture and

Table 1 Mechanisms of peripheral arterial disease in diabetes mellitus patients

Disease characteristics		Mechanisms of pathology	Disease characteristics	
DM	Hyperglycemia Dyslipidemia Insulin resistance ↑ FFA production	<p>→ Vascular inflammation</p> <p>CRP: promotes leukocyte adhesion, coagulation, and chemotaxis; inhibits eNOS; impairs fibrinolysis</p> <p>TNF-α and IL-6: activate NF-κB, leading to thrombogenesis; promote leukocyte migration and adhesion, increasing plaque instability/rupture</p> <p>Endothelial cell dysfunction</p> <p>Decreased NO production: inhibits vasodilation</p> <p>Increased reactive oxygen species: inhibits vasodilation</p> <p>Increased AGE production: is proinflammatory; induces leukocyte chemotaxis, adhesion, and transformation into foam cells</p> <p>Vascular smooth muscle cell derangement</p> <p>Tissue factor production: proatherogenic; procoagulation</p> <p>FGF and TGF-α: Extracellular matrix production</p> <p>Impaired synthesis of collagen: destabilizes plaque</p> <p>Apoptosis of VSMC: increases risk of plaque rupture and thrombosis</p> <p>Increased production of endothelin-1, angiotensin II, and prostanoids: leads to vasoconstriction</p> <p>Platelet dysfunction</p> <p>Enhanced uptake of glucose: increases oxidative stress; decreased NO production</p> <p>Upregulation of P-selectin, GP I b, and GP II b/IIIa receptors: promotes platelet adhesion and aggregation</p> <p>Calcium dysregulation: increases platelet aggregation</p> <p>Hypercoagulability</p> <p>Increased tissue factor and FVII production: enhances coagulability</p> <p>Decreased antithrombin and protein C synthesis: enhances coagulability</p> <p>Rheology</p> <p>Elevated blood viscosity</p> <p>Increased fibrinogen production</p> <p>Impaired arteriogenesis</p> <p>Inhibited sensing of shear stress</p> <p>Decreased monocyte and growth factor signaling</p>	<p>→ Atherosclerosis (Increased plaque burden)</p> <p>Atherothrombosis (Increased plaque instability/rupture)</p> <p>Restenosis (Increased complexity)</p>	PAD

Note that there is significant interplay between the different mechanisms: for example, impaired NO production can affect inflammation, endothelial cell function and arteriogenesis, while increased reactive oxygen species causes platelet and endothelial cell dysfunction. FFA: Free fatty acids; CRP: C-reactive protein; eNOS: Endothelial nitric oxide synthetase; TNF- α : Tumor necrosis factor- α ; IL-6: Interleukin-6; NF- κ B: Nuclear factor- κ B; NO: Nitric oxide; AGE: Advanced glycation end products; FGF: Fibroblast growth factor; TGF- α : Transforming growth factor- α ; VSMC: Vascular smooth muscle cell; GP I b: Glycoprotein I b; GP II b/IIIa: Glycoprotein II b/IIIa; FVII: Factor 7; DM: Diabetes mellitus; PAD: Peripheral artery disease.

thrombosis^[57]. Moreover, the lipid modifications noted in diabetic patients, such as glycated oxidized low-density lipoprotein, can promote apoptosis of VSMC^[4,46]. The metabolic syndrome that defines DM results in enhanced production of reactive oxygen species, inhibition of PI-3 kinase and upregulation of PKC, AGE receptors and NF- κ B, which in turn further promotes an atherogenic phenotype in VSMCs^[4,58]. These factors further contribute to the increased apoptosis of VSMC and upregulation of proatherogenic tissue factor in diabetic patients, while impairing synthesis of collagen, an important plaque-stabilizing compound^[8,59]. DM is also associated with increased matrix metalloproteinases, which further break down collagen, leading to plaque instability^[60]. Therefore, DM not only promotes atherosclerosis but also destabilizes plaques, triggering thrombus formation and impacting clinical outcomes^[8].

DM has also been found to promote upregulation and enhanced activity of endothelin-1, a protein that activates the endothelin-A receptor on VSMCs, leading to enhanced vascular tone^[61]. Such dysregulated hyperactivation

of endothelin-A receptor can cause pathological vasoconstriction^[62]. Endothelin-1 is also responsible for increasing salt and water retention, inducing the renin-angiotensin system, and causing vascular smooth muscle hypertrophy. Other vasoactive substances, such as vasoconstrictor prostanoids and angiotensin II, are also increased in production, further inducing vasoconstriction^[63].

Platelet function

Platelets mediate the interaction between vascular function and thrombosis. Hence, platelet dysfunction can accelerate atherosclerosis, as well as impact the destabilization of plaque and promote atherothrombosis^[8,64]. Platelets take up glucose independent of insulin, which in turn activates protein kinase-C and decreases NO production^[39]. Oxidative stress is also increased when platelets take up glucose, thus promoting platelet aggregation. Platelet adhesion is enhanced in diabetic patients due to upregulated expression of P-selectin on platelet surfaces^[3].

Diabetic patients also have upregulated expression of platelet receptors, such as glycoprotein I b (which binds to von Willebrand Factor) and II b/III a receptors (integral to platelet-fibrin interaction); these receptors mediate platelet adhesion and aggregation, thus inducing thrombosis^[39]. Intra-platelet calcium regulation, important for regulation of platelet shape change and aggregation, as well as for thromboxane production, is also deranged in diabetic patients, further contributing to atherosclerosis in this patient population^[4,39,65,66].

Coagulation

DM and hyperglycemic states promote hypercoagulability *via* upregulation of tissue factor by endothelial cells and VSMCs^[67,68]. These conditions also increase coagulation factor VIIA production and decrease anticoagulants, such as antithrombin and protein C production^[67,68]. DM also impairs fibrinolytic function and induces PAI-1 production^[69]. Taken together, these factors increase the risk of atherosclerotic plaque rupture and subsequent thrombus formation^[8,68,70].

Rheology

Elevated blood viscosity and fibrinogen production also occur in patients with DM. This is manifested *via* abnormal ABI in patients with PAD as well as development and complications of PAD^[8,71].

Restenosis after angioplasty

Acutely elevated glucose levels may induce inflammation, smooth muscle proliferation, abnormal matrix production, and inactivation of endothelium-derived relaxing factor^[72]. Additionally, hyperglycemia may impact expression of fibroblast growth factor and transforming growth factor- α , which in turn promotes proliferation of smooth muscle cells and extracellular matrix production. Increased TNF- α and CRP, as well as oxidative stress and endothelial dysfunction, may also play roles in explaining the restenosis rates in patients with higher blood glucose values at time of angioplasty^[73]. Acute hyperglycemia also induces production of monocyte chemoattractant-protein-1, which has been linked with a higher risk of restenosis^[73]. Restenosis among patients with DM can therefore be explained by the abnormal inflammatory state, oxidative stress, endothelial and platelet function in patients with acute hyperglycemia^[1].

Arteriogenesis

Outward remodeling of pre-existing arteries in response to obstruction of blood flow to restore blood flow distal to the occlusion is termed arteriogenesis^[74]. Endothelial shear stress, detected by the vessel wall through integrins, adhesion molecules, tyrosine kinases, and ion channels, is hypothesized to be the main trigger for arteriogenesis^[45,75,76]. DM limits the adaptive arteriogenesis response and collateral blood flow development by attenuating the remodeling process^[77,78]. Specifically, diabetes attenuates the sensing of shear

stress and increases the response to vasodilatory stimuli, which reduces the recruitment and dilation of collateral arteries. Additionally, DM impairs various other factors critical to remodeling, such as the downstream signaling of monocytes, growth factor signaling, and endothelial NO synthetase, thus inhibiting arteriogenesis and contributing to the severity of occlusive disease in these patients^[74].

OUTCOMES OF PATIENTS WITH PAD AND DM

The outcomes of patients with coexistent diabetes and PAD depend on the interplay between factors such as patient comorbidities, presence of infection, neuropathy, and immunologic factors^[79]. Poor glycemic control has been associated with a higher prevalence of PAD and risk of adverse outcomes, including need for lower extremity bypass surgery, amputation or death^[80]. Poor glycemic control is also associated with worse outcomes following vascular surgery or endovascular intervention^[80].

It is therefore important to identify therapies that can affect the multifactorial pathophysiologic mechanisms of DM in order to provide effective long-term treatments^[3]. Lifestyle interventions, such as weight loss, physical activity, and reduced cholesterol and fat intake, all help reduce the risk of progression from glucose intolerance to diabetes, as well as improve cardiovascular risk factors^[81]. Tobacco cessation is also critical and has been associated with improved outcomes after surgical and endovascular interventions. Such secondary risk factor reduction can help reduce the prevalence and severity of PAD in diabetic patients and also minimize adverse events post revascularization^[3].

Revascularization in patients with PAD and diabetes

Revascularization, either *via* a surgical or endovascular approach, is an important therapeutic option for treatment of symptomatic PAD in diabetic patients^[5]. Due to the greater prevalence of below-the knee disease in patients with DM, some studies have shown that endovascular interventions are associated with worse outcomes in diabetics, especially as distal runoff diminishes^[4]. Endovascular interventions were initially therefore considered more appropriate in patients with focal disease above the knee. Diabetic patients were also noted to have greater durability with surgical approach to revascularization, especially in the setting of tibial disease managed *via* bypass with autologous saphenous vein^[5]. However, recent studies have suggested that diabetic patients with adequate distal runoff appear to have patency rates comparable to that of nondiabetics^[4].

This association of glucose control and vessel patency has been investigated in a single-center retrospective study of outcomes after infrapopliteal balloon angioplasty among diabetic patients. Patients were divided based on median pre-procedure fasting blood glucose (FBG) values into two groups. At one-year follow-up, primary patency,

defined as freedom from restenosis or reintervention based on duplex ultrasound, was 16% for those with FBG values above the median and 46% for patients with below the median FBG values. Amputation rates also trended higher among patients with high pre-procedure FBG compared to low FBG. One-year major adverse limb event rates were significantly higher for patients with FBG values above the median, even after adjusting for insulin use and lesion-specific characteristics. No association between FBG values and overall mortality, amputation-free survival or rates of major adverse cardiovascular events was noted^[80]. When the FBG levels were divided into quartiles, a fivefold increase was noted in primary patency in the lowest quartile of FBG relative to those in the highest quartile of FBG. These results remained significant even after adjusting for baseline insulin use. These outcomes failed to show an association between HbA1C and restenosis, therefore implying that glycemic control at the time of intervention may be a better predictor of primary patency than overall glycemic control^[80]. Furthermore, these results suggest that the acute metabolic milieu at the time of intervention plays an important role in restenosis.

Treatment of critical limb ischemia

In patients with CLI, revascularization is usually required for successful limb preservation^[5]. The prevalence of DM in patients with CLI is extremely high, with some studies suggesting a prevalence of up to 76%^[18]. Disease severity at the time of presentation and progression of CLI in diabetics has also been noted to be worse^[82]. Current recommendations suggest arterial reconstruction in patients with CLI who have a predicted 1-year amputation-free survival of at least 75%^[18].

While patients with CLI may require multiple procedures and close follow-up, the choice of initial revascularization does not appear to influence success in diabetic patients with CLI^[18]. Whether chosen initially or subsequently, surgical and endovascular approaches both are associated with similar outcomes in terms of survival without major amputation or repeated target extremity revascularization (TER)^[18]. However, that study did confirm that repeat TER is more frequently required in diabetic patients. Despite the increased need for repeat revascularization, repeated procedures were associated with overall success rates comparable to that in nondiabetic patients^[18]. Immediate revascularization was also associated with improved outcomes relative to delayed revascularization in patients with CLI, regardless of diabetic status^[13]. Additional studies have also shown that an aggressive multidisciplinary approach in diabetic patients who present with CLI had similar limb salvage, 30-d mortality, cumulative survival, amputation-free survival, and major amputation rates, relative to nondiabetic patients^[83]. Revascularization rates do appear to be better in this patient population when both endovascular and bypass grafting procedures are available relative to one of the two approaches only^[84].

While most patients with CLI can be revascularized,

the presence of irreversible gangrene, the absence of a target vessel, and the lack of availability of an autologous vein can limit successful limb preservation. In these patients, amputation may be the best option^[5]. In general, however, medical management and use of multidisciplinary approach that includes revascularization can lead to reduced amputation rates in patients with DM^[79].

Diabetic foot ulcer is another complication in these patients that is associated with an increased risk of all-cause mortality^[79]. In those patients with PAD whose course is complicated by diabetic foot ulcer, similar outcomes in terms of limb salvage rates were seen with endovascular and open surgical approaches^[85]. It is important to note, however, that concomitant PAD in patients with diabetic foot ulcers is linked to greater failure rates of wound healing and need for amputation. This association is complex, and different studies have shown that successful revascularization and ulcer healing are not always correlated^[79].

CONCLUSION

DM is associated with greater severity and more diffuse PAD relative to nondiabetics. It also correlates to greater risk of mortality and impaired quality of life. The mechanisms by which diabetes induces atherosclerosis are multifactorial and include inflammatory processes, derangements of various cell types within the vascular wall, promotion of coagulation, and inhibition of fibrinolysis. These factors both increase the susceptibility of the vasculature to atherosclerosis, as well as the instability that makes plaque prone to rupture and thrombosis. Thus, it is important for different specialists, from cardiology and internal medicine to vascular surgery, to collaborate and use a multidisciplinary approach to improve the clinical outcomes in this patient population.

Although diabetics have a higher risk of adverse outcomes when compared to nondiabetics, the rates are improving thanks to recent advances in pharmacology and procedural techniques. Nonetheless, further work remains necessary. For instance, while trials such as TRITON-TIMI 38 and PLATO show better clinical outcomes with prasugrel or ticagrelor compared to clopidogrel after percutaneous coronary intervention, it is unclear if similar benefit is seen in DM patients with PAD^[3]. Further studies should also include the impact of biochemical factors found in central obesity, which are known to promote atherothrombosis^[86]. Better understanding of the mechanisms responsible for restenosis among diabetic patients will also ultimately improve the outcomes of surgical and endovascular procedures in these patients.

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Dyslipidaemia of diabetes and the intestine

Gerald H Tomkin, Daphne Owens

Gerald H Tomkin, Daphne Owens, Beacon Hospital Sandyford and Trinity College, Dublin 2, Ireland

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Correspondence to: Gerald H Tomkin, Professor, Beacon Hospital Sandyford and Trinity College, Clontra, Quinns Road, Shankill, Co Dublin, Dublin 2, Ireland. gerald.tomkin@tcd.ie
 Telephone: +353-1-2390658
 Fax: +353-1-2721395

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Abstract

Atherosclerosis is the major complication of diabetes and has become a major issue in the provision of medical care. In particular the economic burden is growing at an alarming rate in parallel with the increasing world-wide prevalence of diabetes. The major disturbance of lipid metabolism in diabetes relates to the effect of insulin on fat metabolism. Raised triglycerides being the hallmark of uncontrolled diabetes, *i.e.*, in the presence of hyperglycaemia. The explosion of type 2 diabetes has generated increasing interest on the aetiology of

atherosclerosis in diabetic patients. The importance of the atherogenic properties of triglyceride rich lipoproteins has only recently been recognised by the majority of diabetologists and cardiologists even though experimental evidence has been strong for many years. In the post-prandial phase 50% of triglyceride rich lipoproteins come from chylomicrons produced in the intestine. Recent evidence has secured the chylomicron as a major player in the atherogenic process. In diabetes chylomicron production is increased through disturbance in cholesterol absorption, in particular Niemann Pick C1-like1 activity is increased as is intestinal synthesis of cholesterol through 3-hydroxy-3-methyl glutaryl co enzyme A reductase. ATP binding cassette proteins G5 and G8 which regulate cholesterol in the intestine is reduced leading to chylomicronaemia. The chylomicron particle itself is atherogenic but the increase in the triglyceride-rich lipoproteins lead to an atherogenic low density lipoprotein and low high density lipoprotein. The various steps in the absorption process and the disturbance in chylomicron synthesis are discussed.

Key words: Triglyceride; Cholesterol chylomicrons; Microsomal triglyceride transfer protein; Niemann Pick C1-like1; Lipoproteins; Diabetes; ATP binding cassette proteins G5/G8

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Core tip: The explosion of type 2 diabetes has generated increasing interest on the aetiology of atherosclerosis in diabetic patients. Evidence is mounting on the importance of the atherogenic properties of triglyceride rich lipoproteins. In the post-prandial phase 50% of triglyceride rich lipoproteins come from chylomicrons produced in the intestine. Recent evidence has secured the chylomicron as a major player in the atherogenic process. In diabetes chylomicron production is increased through disturbance in cholesterol absorption. This paper reviews recent literature in relation to diabetes, the intestine and dyslipidaemia with a view to understanding new targets for treatment.

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INTRODUCTION

The increasing incidence and prevalence of diabetes is of concern to patients their relatives and the medical profession. Politicians who do not fit into the above groups are also concerned because of the health budget considerations. Management of chronic conditions is expensive! A major cost of diabetes care goes on the management of cardiovascular atherosclerotic complications which are so much more common in both diabetes and pre-diabetes. Although statins remain at the centre of dyslipidaemia treatment for diabetes, there is generally an unawareness of the importance of the chylomicron and triglyceride-rich proteins being atherogenic in their own right but also in forming atherogenic low density lipoprotein (LDL). This lack of appreciation of the importance of hypertriglyceridaemia has for example encouraged Tenenbaum *et al*^[1] to write an article entitled "Hypertriglyceridaemia: too long an unfairly neglected major cardiovascular risk factor". Another important milestone in bringing the intestine to the notice of diabetologists and cardiologists has been the validation of ezetimibe, a drug that inhibits the absorption of intestinal cholesterol, which has been shown beyond doubt in the IMPROVE-IT study, demonstrating significantly lower primary combined endpoints in moderate to high risk patients who stabilise following acute coronary syndrome^[2]. This article reviews dyslipidaemia in diabetes with a focus on the intestine as a dysfunctional regulator of cholesterol metabolism.

Insulin deficiency is associated with disturbance in both carbohydrate and fat metabolism. In fact even before diabetes becomes manifest, in the pre diabetes phase of the condition free fatty acids fail to be suppressed after a glucose load leading to the suggestion that diabetes should be defined as lipidus rather than mellitus^[3]. Indeed, had the serum rather than the urine been easily available many centuries ago, post prandial chylomicronaemia, as demonstrated by the milky serum, would have been preferred to the sweet taste of the urine as the diagnostic tool of choice!

The chylomicron is a particle containing protein fat and cholesterol. The protein, which is mostly apolipoprotein (apo) B48, is the solubilising protein which facilitates the transport of fatty acids, triglycerides and cholesterol. The chylomicron is assembled in the intestinal mucosa under the influence of microsomal triglyceride transfer protein (MTP) which is the rate limiting enzyme. Very LDL (VLDL) is the major triglyceride-containing particle assembled in the liver and, in the postprandial state, about 50% of triglyceride is carried on the VLDL particle and 50% on the chylomicron. Although the chylomicron

by definition is the triglyceride rich particle containing apo B48 and VLDL by definition is the triglyceride-rich particle containing apo B100, the term chylomicron is also used based on density following separation in the ultracentrifuge and thus is a mixture of both chylomicrons and large VLDL particles. Hence there is often confusion about what is meant by the term chylomicron.

CHYLOMICRON AS AN ATHEROGENIC PARTICLE

For many years triglycerides have taken a back seat in the perception and understanding of the aetiology of atherosclerosis. Part of the problem might have been that triglycerides have usually been taken fasting whereas the chylomicron is a post-prandial particle and the hepatic VLDL, the other major triglyceride containing particle, is also mostly produced in the postprandial state. Evidence that Apo B48 is found in the atherosclerotic plaque^[4-7] has been around for years confirming the atherogenicity of the chylomicron particle. Trials such as the FIELD Study^[8], failed to show cardiovascular benefit for reduction of triglycerides and had an adverse effect on the understanding of the atherosclerotic effect of the chylomicron. This has been rectified particularly by the Danish group who have shown that postprandial triglycerides are indeed associated with an increased in cardiovascular events^[9]. Further analysis of the FIELD Study, and in particular understanding that too many people with normal triglycerides were included in the study, has resulted in a number of post hoc analysis of that study showing that reduction in triglycerides did have cardiovascular benefit. An evaluation of the effect of fenofibrate by sex in the FIELD Study was recently reported^[10]. In that study the authors found that fenofibrate reduced LDL, non-high density lipoprotein (HDL) cholesterol and apo B more in women than in men irrespective of menopausal status. The prevention of total cardiovascular events was more in women (30% *viz* 13%). In Patients with high triglycerides and low HDL the cardiovascular reduction was less different between the sexes (30% *viz* 24%). In a recent review Varbo *et al*^[11] conclude that post hoc subgroup analysis of randomised trials using fibrates in individuals with raised triglycerides show a benefit in lowering triglycerides. Conversely low non fasting triglycerides have been shown to be associated with reduced all cause mortality^[12]. The authors examined individuals from the Copenhagen Heart study. Genetically derived low triglycerides were associated with a reduction in all cause mortality and the authors suggest probably due to a reduction in cholesterol in remnant particles. Apo C111 interferes with the uptake of triglyceride-rich apo E containing lipoproteins (both chylomicron and VLDL). Loss of function mutations are associated with lower triglycerides. The effect of these loss of function mutations on the risk of coronary heart disease (CHD) was examined^[13]. An aggregate of rare mutations in the gene encoding apo C111 was associated with lower plasma triglycerides.

Levels were 39% lower in carriers when compared to non-carriers and circulating levels of apo C111 were 46% lower than in non-carriers. The study found that the risk of CHD among carriers of any apo C111 mutation was 40% lower than the risk among the non carriers. An other important boost to the importance of triglycerides in CHD. Fasting blood sugar is of course a recognised cause of increased cardiovascular risk^[14] and it is very interesting to see that patients with polymorphisms of the various genes known to be associated with raised glucose such as the glucokinase gene (*GCK*) rs4607517 have been shown to be associated with an increased risk of ischaemic heart disease and myocardial infarction as compared to genotypes associated with lower levels^[15]. It is likely that the raised glucose is associated with the failure to suppress triglyceride.

Inflammation is a key factor in atherosclerosis progression and obesity is associated with an increase in inflammatory proteins such as tumour necrosis factor alpha and interleucine 6 (IL6). A study genotyping for variants affecting levels of non fasting remnant cholesterol, LDL cholesterol and C-reactive protein (CRP) by both CRP alleles and IL6 receptor alleles found that increasing non-fasting remnant cholesterol was associated with significantly higher CRP. This was not the case for LDL, suggesting that remnant cholesterol may in part cause acceleration of atherosclerosis through an inflammatory process^[16-18]. Endothelial dysfunction is a precursor of atherosclerosis. Post prandially, when triglycerides rise neutrophils increase with concomitant production of pro-inflammatory cytokines and oxidative stress suggesting a contributory cause of endothelial dysfunction^[19,20]. Further triglycerides have also been shown to increase leucocyte activation markers^[21,22].

The mechanisms whereby the postprandial lipoproteins might be pro-inflammatory and stimulate the progression of atherosclerosis has been investigated^[23]. One mechanism is through the activation of neutrophils and Klop *et al*^[24] have shown in healthy volunteers that post prandially changes occur in the white cell population which are similar to that shown in infection. A good review of post-prandial inflammation and the role of glucose and lipids has recently been published^[25].

MTP

Since chylomicronaemia is such an important finding in diabetes it is of interest to examine MTP function in diabetes.

Biosynthesis of lipoproteins requires apo B and MTP. MTP binds and chaperones lipoproteins to the nascent apo B. MTP is an endoplasmic reticulum resident heterodimeric complex. The liver and intestine are the major organs that express apo B and secrete apoB containing lipoproteins. There is good agreement between apoB levels and activity and in various animal models of diabetes in rats, rabbits and fructose fed hamsters diabetes MTP is up regulated^[26-29].

In human studies in type 2 diabetes we demonstrated

an increase in MTP mRNA in intestinal biopsies^[30,31]. Diabetic patients who were on statin therapy had lower MTP mRNA compared to those not on statins^[31]. We found positive correlations between MTP mRNA and chylomicron fraction cholesterol and apo B48^[31]. A novel intestinal specific inhibitor of MTP has been shown to ameliorate impaired glucose and lipid metabolism in Zucker diabetic fatty rats but whether this effect was due to impairment of food intake or to inhibition of fat absorption is not clear^[32]. The signals that upregulate chylomicron formation to cope with excess fat in the diet are slowly being elucidated. Another non-specific inhibitor of MTP, which reduced serum levels of triglycerides by more than 70%, was also associated with significant improvements in glucose tolerance and insulin sensitivity in Zucker fatty rats^[33]. Hepatic MTP mRNA expression is negatively regulated by insulin and it is suggested that insulin might also directly inhibit apo B48 secretion independently of MTP even though it is probable that up-regulation of MTP stimulates apo B secretion^[34]. The membrane glycoprotein CD36 binds long chain fatty acids. CD 36 deficiency reduces chylomicron production^[35]. It has recently been shown that binding of lipid by CD36 upregulates apo B48 and MTP through CD 36 signalling *via* the ERK 1/2 pathway^[36]. Interestingly polymorphisms of MTP which have been associated with differences in serum lipids appear to alter cholesterol absorption but not synthesis in women^[37].

ATP BINDING CASSETTE PROTEINS G5/ G8

Once cholesterol has been transported across the brush border membrane it faces another regulatory process and may be excreted back into the intestinal lumen rather than being further processed for absorption into the lymphatic circulation. ATP binding cassette proteins G5/G8 (ABC G5/G8) are heterodimers which are mostly confined to the human small intestine and liver^[38]. These two proteins act in tandem to re-excrete both cholesterol, and in particular non-cholesterol sterols from the body. Much of the understanding of ABC G5/G8 comes from the rare mutations that cause a defect in ABC G5 and G8 and result in high levels of sitosterol in the blood. Sitosterolemia, is a condition which manifests itself in children as tendon xanthomas or in young adults as severe CHD with massive accumulation of sterols and stanols in monocyte derived macrophages^[39]. Ma *et al*^[40] found in an animal model, that dietary calcium had a beneficial effect on lipoprotein profile by up-regulating the mRNA levels of intestinal ABC G5/8 and cholesterol-7 α -hydroxylase (CYP7A1), whereas it down-regulated the intestinal NPC1L1 and MTP due to enhanced biliary cholesterol excretion. Méndez-González *et al*^[41] investigated the effect of ABC G5 and G8 deficiency on lipoproteins in mice. They found that postprandial triglycerides were 5 fold higher in the ABCG5/G8^{-/-} mice due to a lower fractional catabolic rate with lower post heparin lipoprotein lipase activities. They also showed

that liver triglyceride secretion and intestinal triglyceride secretion were higher and there was a relationship between this and the HOMA index as a measure of insulin resistance. Rats with induced diabetes (streptozotocin) had impaired expression of ABC G5/8. Treatment with insulin partially reversed this effect^[42]. This trend in impairment was found in Zucker diabetic rats^[43,44] and the Psamonas Obesus (sand rat) was found to have the same intestinal impairment^[45,46]. Intestinal G5/G8 mRNA in type 2 diabetic subjects produced similar findings^[30].

ABC 5/8 genetic variants have been associated with susceptibility to CHD. One polymorphism in particular was shown to be associated with increased triglycerides with a significant gene - tobacco smoking interaction^[47]. Another study has shown that ABC G5/8 regulate cholesterol available for chylomicron production. It is interesting to read that ABC G5/8 genotypes that are associated with low LDL cholesterol are protective against myocardial infarction but increase risk of symptomatic gall stone disease^[48].

NIEMANN PICK C1-LIKE 1

The first step in cholesterol absorption in the intestine appears to be through the multi transmembrane protein Niemann Pick C1-like1 (NPC1L1) which is highly expressed in the jejunum^[49]. In humans it is localised to the brush borders of the enterocytes and acts as a unidirectional transporter of cholesterol and non-cholesterol sterols^[50]. Zhang *et al.*^[51] discovered that it is the N-terminal domain of NPC1L1 that binds cholesterol. Twenty rare NPC1L1 alleles have been found in the low cholesterol absorbers and appear to impair NPC1L1 cholesterol uptake through various mechanism^[52,53], for review see Calandera^[54]. It has been shown that the effectiveness of ezetimibe, which blocks NPC1L1 and inhibits cholesterol absorption, depends on the NPC1L1 genotype.

Cholesterol absorption has been shown to be increased in both animal and human diabetes^[55] due to an increase in NPC1-L1^[43,44,55]. In an animal model of type 2 diabetes, Sammons Obesus, the opposite was found even though these animals have an increase in apo B48^[45,46]. Ezetimibe inhibits cholesterol absorption through inhibition of NPC1L1 (for review see^[56]). NPC1L1 activity appears to be governed by dietary cholesterol^[57]. The mechanism of this control is through the nuclear receptor, peroxisome proliferator-activated receptor (PPAR) δ/β ^[58]. Fenofibrate, a PPAR α agonist has been shown to inhibit cholesterol absorption, the mechanism has been shown to be through NPC1L1 transcription by binding to a PPAR α response element upstream of the human *NPC1L1* gene^[59]. In a human construct Iwayanagi *et al.*^[60] showed that PPAR α positively regulated human NPC1L1 transcription and Valasek *et al.*^[59] showed that Fenofibrate reduced intestinal cholesterol absorption by PPAR α modulation of NPC1L1. HMGCoA reductase inhibition (Atorvastatin) has been shown to increase cholesterol absorption in the intestine and downregulation

of NPC1L1^[61] in the intestine. Ezetimibe has been shown to improve biomarkers of inflammation and platelet activity^[62] as stated above the IMPROVE-IT trial has been presented at the American Heart 2014 but not yet published. Ciriacks *et al.*^[63] have examined the addition of Ezetimibe to simvastatin in type 1 and type 2 diabetes. The study demonstrated that ezetimibe was at least as effective in lowering cholesterol as simvastatin among type 1 diabetics. Some studies have suggested that there may be a difference in cholesterol absorption rates between type 1 and type 2 diabetic patients^[64].

OTHER TRANSPORTERS OF CHOLESTEROL

There are other transporters of cholesterol for example scavenger receptor class B type 1 (SR-B1) which is located both in the apical and basolateral membranes of the enterocyte^[65]. SR are cell surface proteins that can bind and internalise modified lipoproteins. SR-B1, which is involved in cholesterol uptake in the intestine, may play an important part in intestinal chylomicron production^[66]. The fatty acid transporter CD36 which is also involved in the uptake of oxidised LDL, is another member of the class B scavenger receptor family^[66]. Hayashi *et al.*^[67] investigated gene expression of key proteins involved in the active absorption of dietary fat and cholesterol in response to the development of insulin resistance. They used 2 models of diet induced insulin resistance, the fructose fed hamster and the high fat fed mouse. Expression of SR-B1 was increased in both the fructose fed hamster and the high fat fed mouse models of insulin resistance. In CaCo2 cells SR-B1 over expression increased apo B100 and apo B48 secretion. The authors conclude that apical or basolateral SR-B1 may have an important role in cholesterol absorption and may play a part in cholesterol over absorption in insulin resistant states. SR-B1 in the intestine may play an important role in chylomicron production. CdC42, a member of the Rho family of small Guanine triphosphatases with numerous functions, has been shown by Xie *et al.*^[68] to interact with NPC1L1 and to control its movement from endocytic recycling compartment to plasma membrane in a cholesterol dependent manner. Glucose stimulated CDc42 signalling appears to be essential for second stage insulin secretion^[69]. It is probable that in insulin resistance the signalling of NPC1L1 is disturbed through this pathway but we have been unable to find any studies in the intestine that have explored the pathway in diabetes/insulin resistance.

THE EFFECT OF HIGH GLUCOSE ON CHOLESTEROL ABSORPTION

Ravid *et al.*^[70] have shown that high glucose increases intestinal absorption of cholesterol through an increase in the protein expression of NPC1L1. The same group later showed that the effect was through the basolateral

domain suggesting glucose in the circulation rather than in the lumen to be the stimulus^[71]. The reason for the up-regulation of cholesterol through NPC1L1 in diabetes has been explored by Malhotra *et al*^[72]. Using CaCo2 cells they showed that removal of glucose from the culture medium significantly decreased NPC1L1 mRNA protein expression as well as pro-motor activity. Glucose replenishment significantly increased the promoter activity of NPC1L1 in a dose dependant manner. The authors concluded their experiments by examining mouse jejunum after 24 h fasting which confirmed the CaCo2 cell results.

The role of cholecystokinin on intestinal absorption has been reported by Zhou *et al*^[73]. They found that in mice cholecystokinin (CCK) increased cholesterol absorption and increased cell surface associated NPC1L1. Previously Irwin *et al*^[74] have shown that an CCK-8 analogue improves insulin sensitivity and triglyceride deposition in liver and muscle but with reduction in weight gain and food intake. The effect therefore on lipid metabolism might be very dependant on dietary intake and the inhibition of apatite and improvement in insulin sensitivity with improvement in glucose tolerance with CCK makes analogues of CCK of interest as a possible treatment in diabetes and the metabolic syndrome.

Interest in bile salt binding drugs for treatment of dyslipidaemia, such as cholestyramine, went out of fashion because of their poor cholesterol lowering effects and their unacceptable side effects. A new formulation colsevelam, is interesting in that not only that it lowers cholesterol and apo B but also lowers blood sugar. A recent study in type 2 diabetic patients demonstrated a 0.32 drop in HbA1c vs placebo at 24 wk and a reduction of cholesterol of 6.5%^[75,76]. The majority of adverse effects were mild or moderate, the authors concluding that the drug was well tolerated. Thus another option for patients who are near to but not on target with current medication.

CHOLESTEROL SYNTHESIS AND 3-HYDROXY-3-METHYLGLUTARYL CO-ENZYME A REDUCTASE

Cholesterol synthesis in the intestine makes up 25% of *de novo* cholesterol synthesis. Cholesterol synthesis is regulated by 3-hydroxy-3-methylglutaryl co-enzyme A (HMGCo A) reductase the rate limiting enzyme in the synthetic pathway. HMGCo A reductase activity has been shown to be reduced by insulin in the rat hepatocyte^[77]. It has been suggested that in type 1 diabetes improved glycaemic control will increase cholesterol synthesis. HMGCoA reductase inhibition has been shown to increase cholesterol absorption through a lowering of ABC G5/G8 and an increase in NPC1L1^[78].

HEPATIC STEATOSIS

Hepatic steatosis is common in diabetes, insulin resis-

tance and obesity. Inflammatory stress is present in these conditions and is also associated with obesity insulin resistance and diabetes. It is therefore of interest to read that Zhao *et al*^[79] demonstrated that IL1b and IL6 stimulation in Hep G2 cells increased SREBP2 and HMGCoA mRNA. Further high fat loading in mice or LDL loading in HepG2 cells suppressed the above genes but this suppression could be over ridden by the above inflammatory proteins. Severe calorie restriction in patients with steatosis results in rapid reduction of liver fat, insulin resistance and improvement in diabetes control^[80]. On the other side of the coin insulin resistance and the accompanying hyperinsulinaemia are associated with an upregulation of SREPB-2 through extracellular signal regulated pathways involving the kinases ERK-1 and 2 another example of the interaction between fat and carbohydrate metabolism, for review see Van Rooyen *et al*^[81].

In conclusion, vascular disease in diabetes is complex as would be expected with a condition that impacts on so many metabolic pathways. Examination of the intestine in the search for abnormalities in cholesterol absorption and chylomicron formation has been rewarding. Statins have been very effective in reducing the burden of atherosclerosis in patients with diabetes but even so a large proportion of patients still succumb to events. Reduction in triglycerides is now accepted as being important in those patients who have raised triglycerides and in particular the importance of postprandial disturbance in triglyceride metabolism and its impact on atherosclerosis is now accepted as being an important issue in management of diabetes and in the prevention of macrovascular complications.

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Serum hepcidin concentrations and type 2 diabetes

Alex Aregbesola, Sari Voutilainen, Jyrki K Virtanen, Adeola Aregbesola, Tomi-Pekka Tuomainen

Alex Aregbesola, Sari Voutilainen, Jyrki K Virtanen, Adeola Aregbesola, Tomi-Pekka Tuomainen, Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Yliopistonranta 1C, FI70211 Kuopio, Finland

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Correspondence to: Alex Aregbesola, MD, MScPH, Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Yliopistonranta 1C, PO Box 1627, FI70211 Kuopio, Finland. alex.aregbesola@uef.fi
Telephone: +358-44-9788099
Fax: +358-17-162936

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Abstract

Hepcidin is a peptide hormone with both paracrine and endocrine functions that help in maintaining body iron stores. Type 2 diabetes (T2D) is one of the sequelae of excess body iron stores; thus, iron regulatory hormone hepcidin may have a direct or at least an indirect role in the aetiopathogenesis of T2D. Both human and animal studies at molecular and genetic levels have attempted

to establish a role for hepcidin in the development of T2D, and a few epidemiologic studies have also showed a link between hepcidin and T2D at population level, but the findings are still inconclusive. Recent data have suggested different pathways in which hepcidin could be associated with T2D with much emphasis on its primary or secondary role in insulin resistance. Some of the suggested pathways are *via* transcription modulator of hepcidin (STAT3); ferroportin 1 expression on the cells involved in iron transport; transmembrane protease 6 enzyme; and pro-inflammatory cytokines, interleukin (IL)-1, IL-6, tumor necrosis factor- α and IL-10. This review briefly reports the existing evidence on the possible links between hepcidin and T2D and concludes that more data are needed to confirm or refute hepcidin's role in the development of T2D. Examining this role could provide a further evidence base for iron in the aetiopathogenesis of T2D.

Key words: Serum hepcidin; Body iron; Diabetes; Type 2 diabetes; Insulin resistance

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Core tip: Excess body iron has been demonstrated as an independent risk factor of type 2 diabetes (T2D). Lately, manipulation of serum hepcidin concentrations through the use of hepcidin agonist is being suggested in the management of iron overload diseases, of which T2D is one. However, little is known about the role of hepcidin in the development of T2D; hence, the need for a review of the existing evidence linking hepcidin and T2D. We discuss some of the main mechanisms through which hepcidin could be associated with T2D.

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INTRODUCTION

Recently, attention has been shifting towards the iron-regulatory hormone hepcidin and its possible role in the aetiopathogenesis of type 2 diabetes (T2D). Hepcidin is primarily a hepatic peptide synthesized as a preprohepcidin, which is a 84-amino acid peptide. It undergoes enzymatic cleavage into a 60- to 64-residue prohepcidin peptide and finally into a biologically active 25-amino acid peptide hormone, hepcidin^[1-3]. Tissues of the kidney^[4], pancreatic beta cell^[5], macrophages^[6] and adipocytes^[7] have also been reported to produce hepcidin, but the role of this extra hepatic contribution to serum hepcidin is still unclear.

Hepcidin's role is to maintain iron homeostasis^[8,9]. It performs this action by regulating the expression and function of cell membrane-embedded ferroportin (FPN)^[10], the cellular iron exporter in iron-transporting cells^[8,9]. In response to the level of body iron stores, hepcidin regulates dietary iron absorption from the intestine and iron release from macrophages, by decreasing the cell surface expression of FPN.

Among the known diseases associated with the iron overload syndrome is T2D^[11,12]. Even mildly elevated body iron has been demonstrated as a risk factor of T2D^[13]. Some recent epidemiologic studies have tried to explore the association between serum hepcidin and T2D, with inconsistent findings^[14-16]. Also, serum glucose concentration has been shown to regulate serum hepcidin^[17]. As the underlying mechanisms between body iron stores and T2D still need further clarifications, hepcidin could provide some answers. Therefore, we reviewed the emerging links between hepcidin and T2D.

ROLE OF HEPCIDIN IN IRON METABOLISM

Iron is a transition metal that is predominantly absorbed in the duodenum and upper jejunum^[18]. The intestinal absorption of iron is in two waves of uptake of iron from intestinal lumen into the intestinal mucosa and from mucosa into the blood^[19]. The two known forms of dietary iron are heme and non-heme iron. The heme iron is well absorbed by the body while the non heme iron predominantly in ferric form Fe^{3+} is reduced to ferrous Fe^{2+} form by the duodenal cytochrome b reductase^[20]. This is transported by the divalent-cation transporter 1, a member of the natural-resistance-associated macrophage protein family, across the intestinal apical membrane^[21], and later stored in the cytosol of ferritin or exported from the basolateral membrane of the enterocytes *via* FPN1. Because of its numerous health effects due to excess or deficiency, there is a need to strictly regulate iron within the physiological range.

Following a feedback mechanism, hepcidin is either up- or down-regulated by hepatocytes, depending on the level of body iron. Genetically, the mRNA of the gene (*HEPC*) coding for hepcidin increases with increase in

body iron stores. At molecular level, hepcidin regulates iron transport from iron-exporting tissue into plasma by inhibiting intestinal iron absorption^[9], release of iron from macrophages^[22] and the placental passage of iron^[23]. It performs these functions by binding to FPN1, which is expressed on the duodenal enterocytes, hepatocytes, placental syncytiotrophoblasts and the reticuloendothelia macrophages. It later internalizes and degrades FPN1 leading to a reduction in the ability of these cells to export iron into the plasma^[8]. Conversely, there is re-expression of FPN1 on these iron-exporting cells in iron deficiency. However, it must be noted that body iron is not the only stimulus for hepcidin release. Hepcidin also responds to inflammation, infection, or both; as such, hepcidin appears to be a link between iron and inflammation. The antimicrobial properties of hepcidin would require conditions inconsistent with those observed in the serum, further emphasizing its iron-regulatory role rather than its broad-spectrum antibiotic activity^[24].

MECHANISMS LINKING HEPCIDIN AND T2D

Insulin resistance is a feature of T2D, and the relationship between iron metabolism and insulin resistance has been suggested to be bidirectional^[25]. However, the association between hepcidin and insulin resistance remains vague. Molecular studies have showed that insulin stimulates hepcidin *via* STAT3, which is a novel transcription modulator of hepcidin^[26]. A study by Wang *et al.*^[26], in which they induced diabetes in rats using streptozotocin with or without high-fat diet, showed a significant reduction in hepcidin expression in the liver, mediated by STAT3, causing abnormal elevation of FPN in the intestine, leading to serum iron elevation. Le Guenno *et al.*^[27] showed a 3.5-fold reduction in hepcidin mRNA in the group with higher insulin resistance when compared to the control group. Some previous studies^[15,28,29] also have shown reduced hepcidin and prohepcidin concentrations in T2D subjects, suggesting insulin signal loss among T2D subjects with elevated iron stores.

Another mechanistic link between hepcidin and insulin is through glucose stimulation. One of the extrahepatic sources of serum hepcidin is the beta cell of the pancreas^[5]. Insulin and hepcidin release have also been localized to beta cell granules where hepcidin may evoke its paracrine function. Thus, as glucose stimulates insulin release, there is a concomitant production of hepcidin. In the trial arm of a study by Aigner *et al.*^[17] in which they assessed the effect of glucose on serum iron and hepcidin, they found an increase in serum hepcidin in glucose-treated subjects compared to the control group treated with only water.

To further strengthen the association between hepcidin and T2D, a genome-wide association study (GWAS) by Gan *et al.*^[30] evaluated the association of transmembrane protease serine 6 (TMPRSS6) variants

Table 1 Studies linking hepcidin/prohepcidin and type 2 diabetes (n = 6)

Ref.	Subject/study design	Measurement of hepcidin/prohepcidin	Assessment of T2D	Results
Sam <i>et al</i> ^[15]	British men and women/case-control	Active serum hepcidin-25 and serum hepcidin-ferritin ratio measured using RIA	HbA1c = 73.17 ± 4.12, mean ± SEM	Student's <i>t</i> -test/Mann-Whitney <i>U</i> -test to compare hepcidin (ng/mL) and hepcidin-ferritin ratio in T2D <i>vs</i> control showed: 20.00 (10.00-41.00) <i>vs</i> 33.00 (18.05-54.00), <i>P</i> < 0.05 and 0.22 (0.15-0.32) <i>vs</i> 0.45 (0.26-0.58), <i>P</i> < 0.01
Guo <i>et al</i> ^[16]	Chinese men and women/case-control	Serum hepcidin measured using ELISA	Fasting plasma glucose > 7 mmol/L	Wilcoxon rank test to compare hepcidin (ng/mL) in T2D <i>vs</i> control showed: 34.44 ± 26.98 <i>vs</i> 32.34 ± 22.75, <i>P</i> = 0.72. Logistic regression analysis showed no significant association between serum hepcidin concentrations and onset of T2D: OR = 1.03, 95% CI: 0.87-1.22, <i>P</i> = 0.75
Gan <i>et al</i> ^[30]	Chinese men and women/prospective cohort	Two TMPRSS6 SNPs [rs855791 (V736A) and rs4820268 (D521D)] of hepcidin by DNA genotyping	WHO 1999 criteria or previous history of T2D. Fasting plasma glucose < 5.6 mmol/L = normoglycemia	Logistic regression analysis showed both 2 TMPRSS6 SNPs to be significantly associated with decreased risk of T2D:OR _{rs855791 (V736A)} = 0.801, 95% CI: 0.654-0.98, <i>P</i> = 0.0314 and OR _{rs4820268 (D521D)} = 0.802, 95% CI: 0.656-0.98, <i>P</i> = 0.0311
Jiang <i>et al</i> ^[14]	Chinese men and women/case-control	Serum hepcidin measured using ELISA	Fasting glucose ≥ 7.0 mmol/L, non-fasting glucose ≥ 11.1 mmol/L, use of diabetes medication, or a self-reported physician diagnosis	Kruskal-Wallis test/student's <i>t</i> -test to compare hepcidin (μg/L) in T2D <i>vs</i> control showed: 778.91 ± 175.22 <i>vs</i> 513.44 ± 281.73, <i>P</i> < 0.001
Aso <i>et al</i> ^[29]	Japanese men and women/case-control	Serum prohepcidin measured using ELISA	Not reported	Mann-Whitney <i>U</i> test to compare prohepcidin (ng/mL) in T2D <i>vs</i> control showed: 141 ± 42.6 <i>vs</i> 198.1 ± 36.7, <i>P</i> < 0.0001
Fernández-Real <i>et al</i> ^[28]	Spanish men/ cross-sectional and intervention	Serum prohepcidin measured using ELISA	Oral glucose tolerance test	Pearson's test showed significant correlation between circulating prohepcidin and fasting glucose (<i>r</i> = 0.27; <i>P</i> = 0.002) and HbA1c (<i>r</i> = 0.31; <i>P</i> < 0.0001). After phlebotomy, prohepcidin decreased significantly in T2D (<i>P</i> = 0.04) and in <i>HFE</i> gene mutation carrier (0.03) with a negative correlation between serum prohepcidin and insulin sensitivity (<i>r</i> = -0.50, <i>P</i> = 0.04)

HbA1c: Glycated hemoglobin; T2D: Type 2 diabetes; RIA: Radioimmunoassay; ELISA: Enzyme linked immunosorbent assay; TMPRSS6: Transmembrane protease serine 6; SNPs: Single nucleotide polymorphisms; WHO: World Health Organization.

with risk of T2D. TMPRSS6 is an enzyme that inhibits the expression of hepcidin and its iron-lowering variants were used in their study. They found a reduced risk between the two TMPRSS6 variants and T2D (*P* = 0.0277). It is at least plausible to report that lower hepcidin concentration exacerbates insulin resistance seen in T2D, if causal relationship is yet to be fully established.

T2D is a dysmetabolic state characterized by low-grade chronic inflammation, and some authors have suggested a role for pro-inflammatory cytokines such as interleukins (IL)-1, IL-6 and tumor necrosis factor- α , in the development of insulin resistance^[31], and in addition, IL-10 in T2D^[32]. Increased circulating concentrations of these pro-inflammatory cytokines have also been observed in T2D subjects^[33]. IL-6 is the most important inflammatory cytokine regulating hepcidin, and it performs this function during STAT3 phosphorylation, thus activating STAT3 for hepcidin gene expression^[24]. STAT3 has been reported as necessary and sufficient for IL-6 responsiveness of the hepcidin promoter^[34]. Thus, a low hepcidin concentration could stimulate IL-6, thereby enhancing its role in the development of T2D. A population-based study by Spranger *et al*^[35] showed that IL-1 β and IL-6 concentrations predict the risk of T2D. It is therefore enticing to speculate from the available evidence that hepcidin has a role in insulin resistance, the hallmark of T2D, through iron regulation from interrelated signals of STAT3, pro-inflammatory cytokines and the TMPRSS6 enzyme.

STUDIES LINKING HEPCIDIN AND T2D

Existing data across different populations suggest a link between hepcidin and T2D (Table 1). There are four case-control studies^[14-16,29]. In Sam *et al*^[15] study, the authors measured serum hepcidin and serum hepcidin:ferritin ratio, which has been suggested as a marker of adequate hepcidin production for a particular iron dosage^[36]. Aso *et al*^[29] also measured serum ferritin, prohepcidin and adiponectin, and both studies showed decrease in serum hepcidin/prohepcidin in T2D subjects when compared with the healthy control^[15,29]. Because of the confounding effect of obesity and renal status in serum hepcidin measurement, Sam *et al*^[15] matched their control subjects for body mass index (BMI) and serum creatinine. Aso *et al*^[29] assessed the correlation between adiponectin and prohepcidin in T2D subjects on the basis that adiponectin has a beneficial role in

glucose homeostasis^[37]; hence, in glucose dysregulation observed in T2D, adiponectin and prohepcidin were expected to be low. In keeping with their hypothesis, Aso *et al.*^[29] found low concentrations of adiponectin and prohepcidin and a positive correlation between them in T2D subjects. The other two case-control studies^[14,16] showed increased hepcidin/prohepcidin in T2D subjects when compared with the control group. In Jiang *et al.*^[14] study, T2D subjects had higher BMI and creatinine than the controls, thus suggesting obesity and renal impairment as possible reasons for the elevated serum hepcidin. Further, inflammatory markers, *i.e.*, IL-6, C-reactive protein and white cell counts, were elevated in T2D subjects compared to the controls, speculating inflammatory signals as the cause of the elevated hepcidin. One factor that could be responsible for the contradictory findings in the hepcidin-T2D association study is the wide range of assays with varying degrees of limitation that were used in evaluating serum hepcidin and serum prohepcidin. The lack of an accurate assay to evaluate serum hepcidin in the past may have influenced some investigators to choose prohepcidin, which is thought to be easier to measure due to its higher immunogenicity. Although some studies have shown that prohepcidin does not accurately reflect iron status and iron absorption^[38], others have claimed that it is an indicator of endogenous hepcidin levels^[39] in healthy subjects. This could be the reason why some studies used hepcidin while others used prohepcidin in examining the association between hepcidin and T2D. This is of particular interest in chronic renal disease patients in end-stage renal failure, where there is cross-reactivity in serum hepcidin-25 measurement with that of other hepcidins, *i.e.*, hepcidin-20 and hepcidin-22^[40]. The question then arises whether the concentration of hepcidin in T2D subjects is primary or secondary to elevated body iron stores. The GWAS with a prospective design by Gan *et al.*^[30] at least provided further insight into the role of hepcidin as they showed a decreased risk in developing T2D with the iron-lowering variants of TMPRSS6. Also, in the trial arm of the three-in-one study by Fernández-Real *et al.*^[28], a negative correlation was observed between prohepcidin and insulin sensitivity after phlebotomy to reduce body iron stores, suggesting an association between prohepcidin and body iron in insulin sensitivity.

CONCLUSION

In conclusion, we have briefly reviewed the emerging evidence pertaining to the role of hepcidin in T2D. Although the causative role of body iron in insulin resistance and T2D has been documented in both observational^[41] and interventional studies^[42], the role of hepcidin in this process is still uncertain. However, in addition to the regulatory role in body iron stores, serum hepcidin concentrations have been linked to pro-inflammatory cytokines, STAT3, and TMPRSS6, all of which have been associated with T2D. Data gathered

in this review showed that hepcidin concentrations vary in different populations with T2D. Further, hepcidin has either a primary or secondary role in insulin resistance which characterizes T2D. However, it is still inconclusive from these accumulated data that serum hepcidin is an independent risk factor in the aetiopathogenesis of T2D. Thus, more experimental and clinical studies are needed to confirm or refute the claim that hepcidin has a role in T2D.

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Observational Study

CD36 expression and lipid metabolism following an oral glucose challenge in South Asians

Jeetesh V Patel, Amitava Banerjee, Silvia Montoro-Garcia, Eduard Shantsila, Mushfique Alam, Paul Flinders, Kathleen AL Houlton, Elizabeth A Hughes, Gregory YH Lip, Paramjit S Gill

Jeetesh V Patel, Amitava Banerjee, Silvia Montoro-Garcia, Eduard Shantsila, Elizabeth A Hughes, Gregory YH Lip, University of Birmingham Centre for Cardiovascular Sciences, Sandwell and West Birmingham Hospitals NHS Trust, B71 4HJ West Midlands, United Kingdom

Jeetesh V Patel, Sandwell Medical Research Unit, Sandwell General Hospital, Lyndon, B71 4HJ West Bromwich, United Kingdom

Mushfique Alam, Paramjit S Gill, Primary Care Clinical Sciences, University of Birmingham, B15 2TT West Midlands, United Kingdom

Paul Flinders, Kathleen AL Houlton, Medical School, University of Nottingham, NG7 2UH West Midlands, United Kingdom

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Correspondence to: Dr. Jeetesh V Patel, Sandwell Medical Research Unit, Sandwell General Hospital, Lyndon, B71 4HJ West Bromwich, United Kingdom. jeeteshp@gmail.com
Telephone: +44-121-5073971
Fax: +44-121-5073216

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Abstract

AIM: To investigate lipid metabolism and the relationship with monocyte expression of the fatty acid translocase CD36 in South Asians.

METHODS: An observational study of South Asians whom as an ethnic group have - a higher risk of developing diabetes. The susceptibility to diabetes is coupled with an earlier and more rapid progression of micro-, and macro-vascular complications. Twenty-nine healthy South Asian participants [mean age 34.6 (8.9) years, 76.2% male, mean body-mass index 25.0 (5.2) kg/m²] were recruited from an urban residential area of central Birmingham (United Kingdom). The main outcomes measured were post prandial (30 min) and post absorptive (120 min) changes from fasting (0 min) in circulating lipoproteins, lipids and hormones, and

monocyte expression of CD36 post injection of a 75 g oral glucose challenge. The inducements of variations of monocyte CD36 expression were analysed.

RESULTS: Our results showed evident changes in monocyte CD36 expression following the glucose challenge ($P < 0.001$). Non-esterified fatty acids (NEFA) levels decreased progressively during the challenge ($P < 0.001$), in contrast to increased cholesterol (but not triglyceride) concentrations within very low density lipoprotein (VLDL) and low density lipoprotein subfractions ($P < 0.01$). Levels of, glucose, serum triglycerides and high density lipoprotein cholesterol remained largely unchanged. Variations of monocyte CD36 were negatively ($r = -0.47$, $P = 0.04$) associated to fat from the diet and positively to carbohydrate from the diet ($r = 0.65$, $P < 0.001$).

CONCLUSION: These data suggest that the initiation of VLDL genesis follows the consumption of glucose within this population, inferring that the sequestration of NEFA from these particles happens due to the increased availability of CD36 receptors. While these are preliminary results, it would appear that lifestyle exposures have a role in moderating the expression of CD36.

Key words: CD36; Lipoprotein; Glucose; South Asians; Diabetes; Micro-vascular; Macro-vascular

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Core tip: This study investigated the relationship between the expression of fatty acid translocase CD36 on monocytes and lipid and lipoprotein metabolism in South Asians. Post prandial and post absorptive changes from fasting in circulating lipids, lipoproteins, hormones and monocyte expression of CD36 were recorded subsequent to an oral glucose challenge. Our results showed discernible changes in monocyte CD36 expression post glucose administration. These data suggest that the production of very low density lipoprotein occurs subsequent to the ingestion of glucose within this population. It is presumed that the sequestration of non-esterified fatty acids from these particles happens due to an increase in availability of CD36 receptors.

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INTRODUCTION

The prevalence of diabetes is increased on the Indian

subcontinent^[1]. Globally dispersed migrant populations of "South Asians" also have high rates of diabetes, which is evident when compared to the various indigenous populations of where they migrated^[2-6]. Nephropathy and retinopathy develop earlier and are more progressive in South Asian diabetics compared to White diabetics^[7-11]. The incidence of vascular disease is also higher in South Asians compared to the general White population^[12].

Established risk factors such as obesity and urbanised lifestyle "operate" amongst South Asians but it remains unclear as to how far they explain an increased susceptibility to diabetes for this group. Rates of glucose intolerance amongst Indians living in rural village settings appear to be no different to their migrant contemporaries living overseas in Western countries^[5,6]. We and others have observed that high glucose excursions in South Asians are underpinned by unregulated non-esterified fatty acids (NEFA) metabolism^[13,14]. This phenomenon is likely to be complex and multifactorial, and we were interested to investigate the role of the fatty acid translocase CD36^[15], which is the major facilitator of NEFA sequestration from the blood^[16]. Specifically, CD36 allows the transport of NEFA that are generated from the lipolysis of triglyceride-rich lipoproteins such as very low density lipoprotein (VLDL) across plasma membranes, and into cells (*e.g.*, monocytes, cardiomyocytes and adipocytes) for fatty acid oxidation and lipid deposition^[17]. Cardiomyocytes use glucose in addition to fatty acids for cellular respiration, and the expression of glucose transporter 4 (GLUT4) and the fatty acid transporter CD36 is stimulated by raised insulin concentrations and an increase in cardiac work^[18].

Given that abnormalities of increased CD36 expression and NEFA uptake may represent a common cause for diabetes and the progression of its complications^[19-21], we measured changes in the expression of CD36 on circulating monocytes and its association with direct indices of NEFA metabolism amongst South Asians during an oral glucose challenge.

MATERIALS AND METHODS

Participants

Healthy South Asian volunteers were recruited between Jan and July 2011 for diabetes research Diabetes Health, Residence and Metabolism in Asians (DHRMA): the DHRMA study - a blinded, randomised, placebo controlled trial at Sandwell and West Birmingham Hospitals NHS Trust (Birmingham United Kingdom) (described in detail elsewhere^[22]). All the participants were aged 18 years and over and were of self reported South Asian ethnicity with no known cardiovascular disease or associated medications, body-mass index (BMI) less than 30 kg/m², and normal glucose tolerance. The study design was approved by the Local Research Ethics Committee. Written informed consent was collected from all participants.

Table 1 Characteristics of South Asian volunteers

Characteristics of 29 South Asian volunteers	Mean (SD)
Age (yr)	34.6 (8.9)
Weight (kg)	69.6 (18.4)
Body-mass index (kg/m ²)	25 (5.2)
Waist circumference (cm)	86.8 (16.3)
Hip circumference (cm)	101 (10)
Hip to waist ratio	0.892 (0.144)
Systolic BP (mmHg)	119 (13)
Diastolic BP (mmHg)	75.8 (8.8)
Energy from carbohydrates (%)	45 (6.3)
Energy from fats (%)	40.8 (5.8)
Energy from protein (%)	9.4 (6.8)
Energy from sugars (%)	12.6 (7.5)
Energy from starch (%)	32 (8.5)
Energy from saturated fat (%)	9.7 (5.8)
Energy from monounsaturated fat (%)	10.7 (5.2)
Energy from polyunsaturated fat (%)	6.8 (2.9)
Energy from alcohol (%)	0.5 (2.2)

BP: Blood pressure.

Variables

All patients and controls attended a baseline assessment for the DHRMA study at Sandwell and West Birmingham Hospitals. The assessment incorporated an interview-administered medical history questionnaire, and a dietary assessment (24-h food recall) scrutinised using the WISP (Weighed Intake Software Program) nutritional package (version 3, Tinuviel Software, Llanfechell, United Kingdom)^[6], and anthropometric measurements, which were measured using Seca scales and stadiometer (Seca Ltd, Birmingham). Waist measurements were recorded from the narrowest circumference above the umbilicus and below the rib, and the hip was recorded as the widest circumference at the buttocks. Both girths were measured in duplicate, and repeated where there were differences more than 2%. Blood pressure (BP) measurement was repeated three times, 1 min apart (analysing the mean) using a semi automated BP monitor, the OMRON 705CP (Omron Healthcare Europe, Mannheim, Germany) in combination with suitable cuff sizes for each participant, after at least five minutes in the sitting position. During this assessment venepuncture was performed to collect blood (as serum, fluoride oxalate plasma and EDTA plasma) at fasting, 30 min post administration of a 75 g oral glucose load (Maxijul; SHS Supplies, Liverpool, United Kingdom). Blood collected with fluoride oxalate was analysed for glucose (glucose oxidase method) within 2 h of venepuncture using the Cobas Integra 400 auto analyser (Roche Diagnostics, United Kingdom). EDTA plasma was stored at 4°C was analysed by: (1) Flow cytometry was recorded using the becton dickinson (BD) FACSCalibur flow cytometer (BD, Oxford, United Kingdom) (described elsewhere^[23]). Absolute counts of monocytes analysed for human CD36 antibodies (Miltenyi Biotec GmbH, Germany). Monocyte CD36 was also assessed in subsets of monocytes by order of their co-expression of CD14, CD16 and CCR2, defined as Mon1: CD14(+)-CD16(-)-CCR2(+),

Mon2: CD14(+)-CD16(+)-CCR2(+), and Mon3: CD14(low)-CD16(+)-CCR2(-) (as described previously^[23]); and (2) Density gradient ultracentrifugation (described elsewhere^[24]) was used to separate VLDL, low density lipoprotein (LDL), high density lipoprotein (HDL)₂ and HDL₃ subfractions using the Optima TLX Ultracentrifuge (Beckman Coulter, High Wycombe UK). Briefly, VLDL subfractions were extracted at a density 1.006 kg/L, LDL at 1.063 kg/L, HDL₂ at 1.123 kg/L and HDL₃ at 1.21 kg/L. Concentrations of cholesterol and triglyceride were measured on these separated lipoprotein subfractions on the Cobas Integra 400.

Blood collected as serum was separated and stored at -70°C for batch analysis using commercially available (1) colourimetric assays for NEFA (Acyl CoA synthase/oxidase method, Randox Laboratories, Co Antrim, United Kingdom); (2) ELISA for Insulin (Abcam, Cambridge, United Kingdom), adiponectin (R and D systems, Abingdon, United Kingdom), soluble CD36 (Adipo Bioscience Inc., Santa Clara, United States); and (3) automated biochemistry assays for total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein AI, apolipoprotein B on the Cobas Integra 400.

Statistical analysis

Statistical review of the study was performed by a biomedical statistician. Data were analysed and validated using SPSS version 16.0 (SPSS Inc., Chicago, IL, United States). The parametric distribution of variables was scrutinised against Kolmogorov-Smirnov plots. Normally distributed data was analysed using ANOVA. The central tendencies of the data were presented as mean and variation by SD. Non-parametrically distributed data were analysed using the Friedman test for related measures, and data were presented as both median and interquartile ranges. A two-tailed bivariate correlation analysis was performed using Spearman's correlation coefficient. Categorical data were analysed using χ^2 tests. A *P* value < 0.05 was accepted as statistically significant.

Statistical review was performed by Dr. Andrew Blann, a biomedical statistician.

RESULTS

A total of 29 volunteers were consecutively recruited for this study. The characteristics of the cohort are shown in Table 1. South Asians were typically of Indian origin (72.4%) and subscribed to diets where the fat intake was 40% of the total energy intake. Their mean age was 34.6 (8.9) years, 16 were male (76.2%) and mean levels of BMI, fasting blood glucose, fasting serum lipids, and BP were reflective of a healthy cohort (Table 2). Soluble levels of CD36 were unrelated to monocyte expression of CD36. Analysing variables reported in Tables 1 and 2, variations in the percentage expression of monocyte CD36 was associated with anthropometry and dietary intake (Table 3). These correlations were specific to monocyte subsets, where Mon1 was positively

Table 2 Fasting metabolic indices and monocyte CD36 expression amongst South Asian volunteers

Characteristics of 29 South Asian volunteers Median (interquartile range)	
Plasma glucose (mmol/L)	5.09 (4.50, 5.47)
Serum NEFA (mmol/L)	0.416 (0.264, 0.514)
Serum cholesterol (mmol/L)	3.41 (2.95, 3.96)
Serum triglycerides (mmol/L)	0.9 (0.67, 1.44)
HDL cholesterol (mmol/L)	1.27 (0.98, 1.48)
LDL cholesterol (mmol/L)	2.03 (1.56, 2.44)
Apolipoprotein AI (g/L)	1.21 (1.10, 1.43)
Apolipoprotein B (g/L)	0.58 (0.46, 0.77)
VLDL subfraction cholesterol (mmol/L)	1.26 (0.97, 1.44)
LDL subfraction cholesterol (mmol/L)	1.3 (0.80, 1.65)
HDL ₂ subfraction cholesterol (mmol/L)	0.58 (0.41, 0.76)
HDL ₃ subfraction cholesterol (mmol/L)	0.37 (0.25, 0.51)
VLDL subfraction triglyceride (mmol/L)	0.34 (0.24, 0.43)
LDL subfraction triglyceride (mmol/L)	0.27 (0.17, 0.55)
HDL ₂ subfraction triglyceride (mmol/L)	0.08 (0.06, 0.15)
HDL ₃ subfraction triglyceride (mmol/L)	0.06 (0.04, 0.08)
Insulin (mU/L)	4.64 (2.60, 8.23)
Adiponectin (ng/mL)	1.79 (0.65, 2.65)
Plasma soluble CD36 (mmol/L)	108 (0, 249)
Total monocyte CD36 (Mon CD36 ⁺ per μ L)	361 (301, 432)
Monocyte CD36 on Mon1 (%)	81.8 (75.1, 85.8)
Monocyte CD36 on Mon2 (%)	7.2 (5.0, 10.3)
Monocyte CD36 on Mon3 (%)	10.5 (8.7, 16.6)

Monocyte subset. Mon1: CD14(+)CD16(-)CCR2(+); Mon2: CD14(+)-CD16(+)-CCR2(+); Mon3: CD14(low)CD16(+)-CCR2(-); NEFA: Non-esterified fatty acids; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein.

Table 3 Correlation between characteristics and fasting metabolic indices against percentage monocyte CD36 expression

Characteristics and fasting metabolic indices	Spearman correlation coefficient (P)		
	Mon1	Mon2	Mon3
Body-mass index	-0.438 (0.02)	0.156 (0.43)	0.418 (0.027)
Hip circumference	-0.633 (0.002)	0.419 (0.06)	0.483 (0.027)
Serum NEFA	-0.349 (0.06)	0.151 (0.43)	0.478 (0.009)
Apolipoprotein AI	-0.282 (0.14)	0.475 (0.009)	0.149 (0.44)
Energy from total fat (%)	-0.472 (0.036)	0.399 (0.08)	0.458 (0.04)
Energy from monounsaturated fat (%)	-0.691 (< 0.001)	0.724 (< 0.001)	0.230 (0.33)
Energy from carbohydrates (%)	0.645 (0.002)	-0.496 (0.026)	-0.533 (0.016)

Monocyte subset. Mon1: CD14(+)CD16(-)CCR2(+); Mon2: CD14(+)-CD16(+)-CCR2(+); Mon3: CD14(low)CD16(+)-CCR2(-); NEFA: Non-esterified fatty acids.

associated with carbohydrate intake, and negatively with fat intake and BMI, while such trends appeared reversed in Mon2 and Mon3 subsets, which were additionally associated with apolipoprotein AI and NEFA (Table 3).

On analysis of serial measures of monocyte CD36 expression, there were evident changes in receptor concentrations for all monocytes ($P < 0.001$), as well as across subsets Mon1 ($P < 0.001$), Mon2 ($P = 0.011$) and Mon3 ($P = 0.03$) (Table 4). The profile of monocyte subset changes reflected a decrease post-prandially (30 min after the glucose challenge) and higher levels post-absorptively (after 120 min) in Mon1 and Mon2

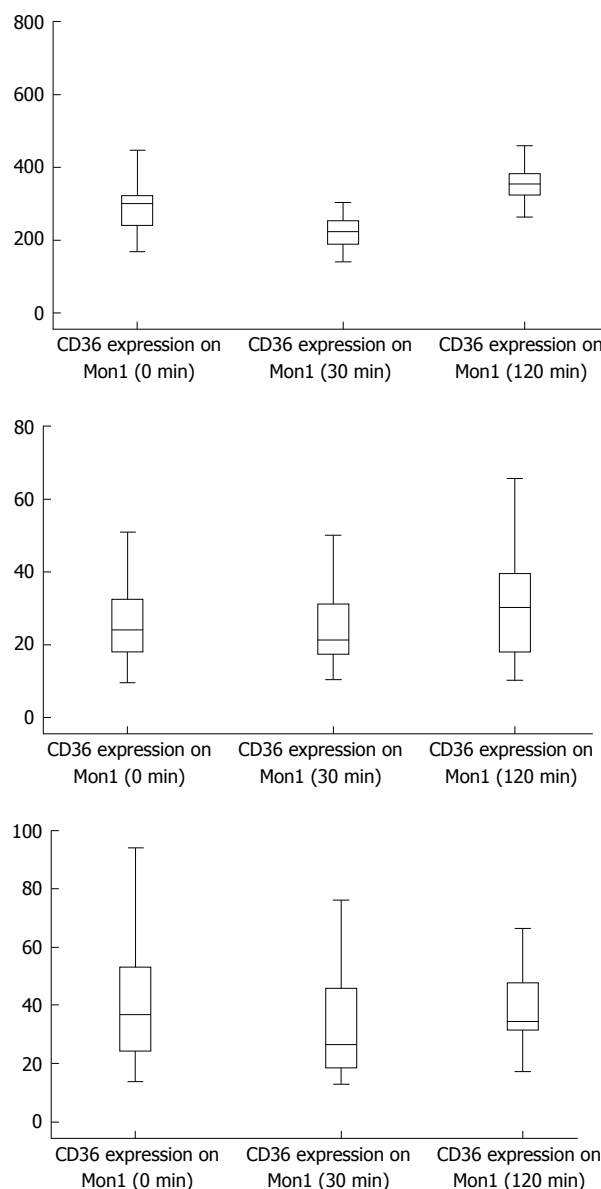


Figure 1 Changes in monocyte subset CD36 expression (Mon CD36⁺ per μ L) pre and post administration of oral glucose. Monocyte subsets. Mon1: CD14(+)CD16(-)CCR2(+); Mon2: CD14(+)-CD16(+)-CCR2(+); Mon3: CD14(low)CD16(+)-CCR2(-).

(Figure 1). NEFA levels progressively decreased during the glucose challenge ($P < 0.001$), whereas cholesterol concentrations within VLDL and LDL subfractions increased ($P < 0.001$). The levels of triglyceride within VLDL particles and HDL particles appeared to decrease during the glucose challenge, but these changes were not significant. Levels of serum triglycerides, glucose, HDL subfraction lipids were largely unchanged following the glucose load. Similarly, there were no significant changes in percentage expression of monocyte CD36 on Mon1, Mon2 or Mon3.

DISCUSSION

Greater CD36 expression following a glucose challenge in healthy South Asians, could reflect a physiological

Table 4 Changes in metabolic factors absolute monocyte CD36 expression pre and post administration of oral glucose

	Circulating concentrations following the administration of glucose			P
	0 min	30 min	120 min	
Monocyte CD36 on Mon1 (Mon CD36 ⁺ per μ L)	300 (240, 324)	223 (187, 254)	353 (323, 382)	< 0.001
Monocyte CD36 on Mon2 (Mon CD36 ⁺ per μ L)	23.9 (17.9, 32.3)	21.3 (17.2, 30.8)	29.9 (18.1, 39.6)	0.011
Monocyte CD36 on Mon3 (Mon CD36 ⁺ per μ L)	36.7 (24.3, 53.2)	26.1 (18.4, 45.8)	34.4 (31.3, 48.7)	0.03
Total monocyte CD36 (Mon CD36 ⁺ per μ L)	361 (308, 432)	278 (240, 324)	422 (394, 458)	< 0.001
Plasma glucose (mmol/L)	5.09 (4.5, 5.47)	6.68 (4.93, 8.12)	5.2 (4.64, 6.07)	0.08
Serum NEFA (mmol/L)	0.416 (0.264, 0.514)	0.215 (0.098, 0.326)	0.094 (0.071, 0.207)	< 0.001
Serum cholesterol (mmol/L)	3.41 (2.95, 3.96)	3.29 (2.95, 3.95)	3.44 (3.06, 3.80)	0.52
Serum triglycerides (mmol/L)	0.9 (0.67, 1.44)	0.83 (0.55, 1.19)	1.01 (0.54, 1.35)	0.26
VLDL cholesterol (mmol/L) ¹	1.26 (0.97, 1.44)	1.31 (0.94, 1.50)	1.42 (1.27, 1.62)	0.001
LDL cholesterol (mmol/L) ¹	1.39 (0.89, 1.71)	1.61 (0.8, 1.82)	1.92 (1.58, 2.24)	0.003
HDL ₂ cholesterol (mmol/L) ¹	0.600 (0.425, 0.735)	0.470 (0.360, 0.725)	0.540 (0.415, 0.820)	0.19
HDL ₃ cholesterol (mmol/L) ¹	0.395 (0.285, 0.505)	0.330 (0.205, 0.450)	0.325 (0.250, 0.570)	0.43
VLDL triglyceride (mmol/L) ¹	0.338 (0.223, 0.440)	0.263 (0.213, 0.365)	0.265 (0.105, 0.353)	0.37
LDL triglyceride (mmol/L) ¹	0.270 (0.170, 0.505)	0.250 (0.170, 0.360)	0.275 (0.125, 0.385)	0.16
HDL ₂ triglyceride (mmol/L) ¹	0.080 (0.055, 0.138)	0.070 (0.043, 0.093)	0.060 (0.028, 0.100)	0.09
HDL ₃ triglyceride (mmol/L) ¹	0.058 (0.035, 0.083)	0.035 (0.013, 0.045)	0.038 (0.025, 0.065)	0.12

¹Subfraction lipids. Data are median (interquartile range). NEFA: Non-esterified fatty acids; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein.

response to counteract the toxicity of excessive plasma glucose. Monocytes have been shown to present an intracellular CD36 pool^[25], and the transient expression of CD36 in monocytes during glucose challenge, may serve as a critical process in dictating the functional activity of CD36 during diabetic conditions and perhaps, atherogenesis. These increases in absolute monocyte CD36 concentrations occurred in parallel to an exponential decrease in NEFA, a rise in levels of VLDL and LDL cholesterol, and no changes in plasma glucose, serum triglycerides or HDL cholesterol. It is possible that the oral glucose challenge in South Asians results in the generation of VLDL particles, and the increased availability and action of CD36 results in the liberation of triglyceride from these particles. The expression of CD36 on monocytes is associated with factors such as dietary fat and carbohydrate, and we are tempted to speculate that lifestyle exposures have a role in moderating the expression of CD36.

An increase in the expression of CD36 is seen as a dysfunctional event, and in diabetics it is associated with a down regulation of the GLUT4 receptor and a reduction in glycogen synthesis^[18]. In South Asians, this response to increase CD36 expression following a glucose load may reflect a homeostatic process to up-regulate those mechanisms that preferentially store energy as fat. In animal models and cellular models, CD36 is shown to lower circulating NEFA concentrations and to promote the efflux of triglyceride from VLDL^[16].

The reasons this "ethnic" preference to process energy in this way are complex. One interesting aspect of CD36 measurement in South Asians is in its dual role in the clearance of red blood cells infected with *Plasmodium falciparum*. The interaction between malaria and CD36 receptors is complex and the subject of debate^[26]. South Asians have evolved from a part of the world where malaria was endemic^[27], and this may have

conferred a survival benefit^[28,29].

Hepcidin is upregulated in malarial infected individuals by interleukin-6^[29], a protein that supports hepatic gluconeogenesis, by a process that is dysregulated in diabetics. Further research is required to fully understand the link between exposure to malaria and a subsequent susceptibility to diabetes.

Monocytes and macrophages play a fundamental role in the pathogenesis of atherosclerosis, and various subtypes of monocytes are associated with cardiovascular diseases^[30]. Functional differences of three Mon1, Mon2 and Mon3 monocyte subsets have been described^[23]. Each monocyte subset responds differently to distinctive immunological stimuli. For example, Mon2 and 3 monocytes predominate during inflammatory states and Mon1 in response to *Candida albicans*^[29]. The production of cytokines such as tumor necrosis factor- α , associated with diabetes, also varies with stimulus^[31]. However, responses in CD36 expression following the glucose challenge were largely similar across these subsets, and as such the metabolic significance of these subclassifications are unclear. The increased surface expression of CD36 on Mon1 was associated with lower BMI and a higher intake of carbohydrate, where as the converse appeared true for Mon1 and Mon2, which were positively associated with dietary fat. Such findings suggest that the expression of CD36 on monocytes can be moderated by diet.

Due to the low numbers of subjects in this study, we only had sufficient statistical power to detect significant associations (α at 0.02) where the correlation coefficient was ≥ 0.55 . We found levels of soluble CD36 to be unrelated to monocytes and metabolic measures. However, elsewhere, soluble CD36 has been shown to reflect several aspects of insulin resistance in humans^[32]. The isolation of lipoprotein subfractions by ultracentrifugation is prone to losses during the

extraction process. Further work would need to be undertaken to analyse the generalisability of the data generated here. There is considerable diversity within the South Asian ethnic category, it encompasses over 22 different languages and more than 6 different religions. The dietary intake of fat in this cohort was similar to that we have measured in a larger cohort of South Asians Living in the United Kingdom^[33]. Nonetheless, the dietary intake of fat in these groups is much greater than that seen amongst South Asians living in rural India^[28].

In summary, these data describe changes in lipid metabolism following the oral ingestion of glucose in South Asians which includes the generation of VLDL, and an increase in monocytes expressing CD36. We presume that triglycerides from these particles are cleared by an increase in the availability of CD36 receptors, and it would appear that lifestyle exposures may influence this process.

COMMENTS

Background

The incidence of diabetes in South Asian populations, including those living in the Indian Subcontinent, and those who have migrated away, is significant. Indeed it is higher than in comparable Caucasian populations and the resulting complications of diabetes such as nephropathy and retinopathy occur both earlier and more severely. Despite extensive investigation, the underlying pathophysiological processes explaining this phenomenon remain elusive. For its part, this study investigated lipid and lipoprotein metabolism and its relationship with the expression of the fatty acid translocase CD36 on monocytes in South Asians.

Research frontiers

Gene variants in CD36, a macrophage scavenger receptor, have been implicated in the pathogenesis of type 2 diabetes and its complications.

Innovations and breakthroughs

This study found that following the administration of a glucose load to South Asian individuals, an upregulation of CD36 positive monocytes was demonstrated which has not been previously seen. In keeping with animal models, it is presumed this increased CD36 expression facilitates the sequestration of triglycerides from very low density lipoprotein particles.

Terminology

This finding suggests that CD36 may represent an as yet unexploited target for therapeutic interventions addressing both diabetes and dyslipidaemia.

Peer-review

This is a very interesting study and all the sections of the manuscript are complete; results have been well described in the text and in the Tables and Figure.

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Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

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Metabolic surgery: A paradigm shift in type 2 diabetes management

Joseph M Pappachan, Ananth K Viswanath

Joseph M Pappachan, Ananth K Viswanath, Department of Endocrinology and Diabetes, New Cross Hospital, the Royal Wolverhampton Hospital NHS Trust, Wolverhampton WV10 0QP, United Kingdom

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Correspondence to: Dr. Joseph M Pappachan, MD, MRCP (London), Department of Endocrinology and Diabetes, New Cross Hospital, the Royal Wolverhampton Hospital NHS Trust, Wolverhampton Road, Wolverhampton WV10 0QP, United Kingdom. drpappachan@yahoo.co.in
Telephone: +44-1922-721172
Fax: +44-1922-721172

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Abstract

Obesity and type 2 diabetes mellitus (T2DM) are major public health issues globally over the past few decades. Despite dietary interventions, lifestyle modifications and the availability of several pharmaceutical agents, management of T2DM with obesity is a major challenge to clinicians. Metabolic surgery is emerging as a promising treatment option for the management of T2DM in the obese population in recent years. Several observational studies and a few randomised controlled trials have shown clear benefits of various bariatric procedures in obese individuals in terms of improvement or remission of T2DM and multiple other health benefits such as improvement of hypertension, obstructive sleep apnoea, osteoarthritis and non-alcoholic fatty liver disease. Uncertainties about the long-term implications of metabolic surgery such as relapse of T2DM after initial remission, nutritional and psychosocial complications and the optimal body mass index for different ethnic groups exist. The article discusses the major paradigm shift in recent years in the management of T2DM after the introduction of metabolic surgery.

Key words: Metabolic surgery; Bariatric procedures; Type 2 diabetes mellitus; Body mass index; Diabetes remission

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Core tip: Metabolic surgery or bariatric surgery has revolutionised the 21st century management of type 2 diabetes mellitus (T2DM) in obese patients. Marked reduction of body weight following the bariatric procedures results in improvement or remission of T2DM in a significant number of patients along with improvement of other diseases associated with obesity

such as hypertension, obstructive sleep apnoea, osteoarthritis and non-alcoholic fatty liver disease. Uncertainty exists about the long-term outcomes in terms of diabetes relapse, nutritional and psychosocial complications. However, the marked benefits of metabolic surgery outweigh the risks related to the procedure that has resulted in a major paradigm shift in the management of obese population with T2DM in recent years which is the topic of discussion of this paper.

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METABOLIC SURGERY: A PARADIGM SHIFT IN TYPE 2 DIABETES MANAGEMENT

Obesity has become a global pandemic in recent years that affects more than 600 million adults worldwide^[1]. World Health Organization estimated that 39% of adults aged 18 years and over were overweight [body mass index (BMI) ≥ 25 kg/m²; more than 1.8 billion persons], and 13% were obese (BMI ≥ 30 kg/m²) in the year 2014. A majority of these individuals reside in the developed countries although obesity and overweight are major public health issues even in developing nations. The National Health and Nutrition Examination Survey 2011-2012 regarding population prevalence in obesity revealed that 34.9% of the adults were obese and 33.6% were overweight in the United States^[2]. Although data on the prevalence of obesity is scant from most other countries, comparable figures probably exist in many developed countries.

Obesity is a major risk factor for type 2 diabetes mellitus (T2DM). By 2014 diabetes affected 387 million people worldwide and 4.9 million deaths in 2014 alone were directly related to diabetes^[3]. The global diabetes disease burden is mainly from T2DM and a majority of these cases are related to obesity. Despite diet and lifestyle interventions and the availability of pharmaceutical agents with weight losing properties the long-term management of obesity with these measures are disappointing. Different gastric bypass procedures collectively termed as bariatric surgery/metabolic surgery have emerged as very promising methods to treat obesity in the past 3 decades that can improve and potentially cure diabetes and many other diseases related to obesity. Through this paper we discuss the major paradigm shift in the management of T2DM in recent years after the introduction of metabolic surgery. We also discuss the long-term health benefits, adverse complications and emerging research questions related

to metabolic surgery.

TYPES AND EFFICACY OF DIFFERENT BARIATRIC PROCEDURES

Although there are a multitude of bariatric procedures developed over the past 50 years the common techniques used in present day clinical practice are adjustable gastric banding (AGB), sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB) and bilio-pancreatic diversion (BPD). The different surgical procedures are depicted in the Figure 1.

AGB and SG are predominantly restrictive procedures whereas RYGB and BPD are mainly mal-absorptive procedures that reduce effective area of nutrient absorption in the intestinal mucosa. Food passes through the alimentary limb in RYGB and BPD with gastrointestinal secretions in the bilio-pancreatic limb mixing with the nutrients where both limbs form the common channel.

In various randomised controlled trials (RCTs), the reported mean percentage (%) excess body weight loss (with 95%CI in parenthesis) achieved at one year after AGB, SG and RYGB were 33.39 (22.57-44.21), 69.70 (41.09-98.32) and 72.32 (64.60-80.04) respectively^[4]. The % excess weight loss (%EWL) reported with BPD was 76.89 ± 1.53 that is significantly higher than RYGB (67.17 ± 1.43 ; $P = 0.0004$)^[5]. AGB procedures are losing popularity in the recent years because of inferior efficacy and the necessity for repeated surgery in a higher proportion of cases years after the initial surgery^[6]. Although BPD is associated with a significantly higher %EWL and T2DM remission compared to other bariatric procedures the post-operative complication rates are higher^[7] making this a less preferred operation.

BARIATRIC SURGERY IN T2DM

The major RCTs reporting the effects of metabolic surgery on T2DM are summarised in Table 1. The total number of patients in these RCTs is relatively small for a common condition like T2DM and the duration of follow-up is limited to 12-24 mo. However there are cohort studies and non-randomized trials reporting benefits of metabolic surgery from different regions of the world. There are also a few systematic reviews and meta-analyses reporting the beneficial effects of bariatric procedures in T2DM.

In a meta-analysis of weight loss and remission of T2DM evaluated in RCTs and observational studies (OBS) of bariatric surgery vs conventional medical therapy over a 17 mo period the mean excess weight loss (EWL) for the bariatric surgery and the conventional treatment groups were 75.3% and 11.3% respectively; the corresponding T2DM remission rates were 63.5% and 15.6%^[12]. The limitation of the meta-analysis was that many short-term OBS were included and surgery was not always compared directly to more vigorous medical weight loss interventions. There was lack of

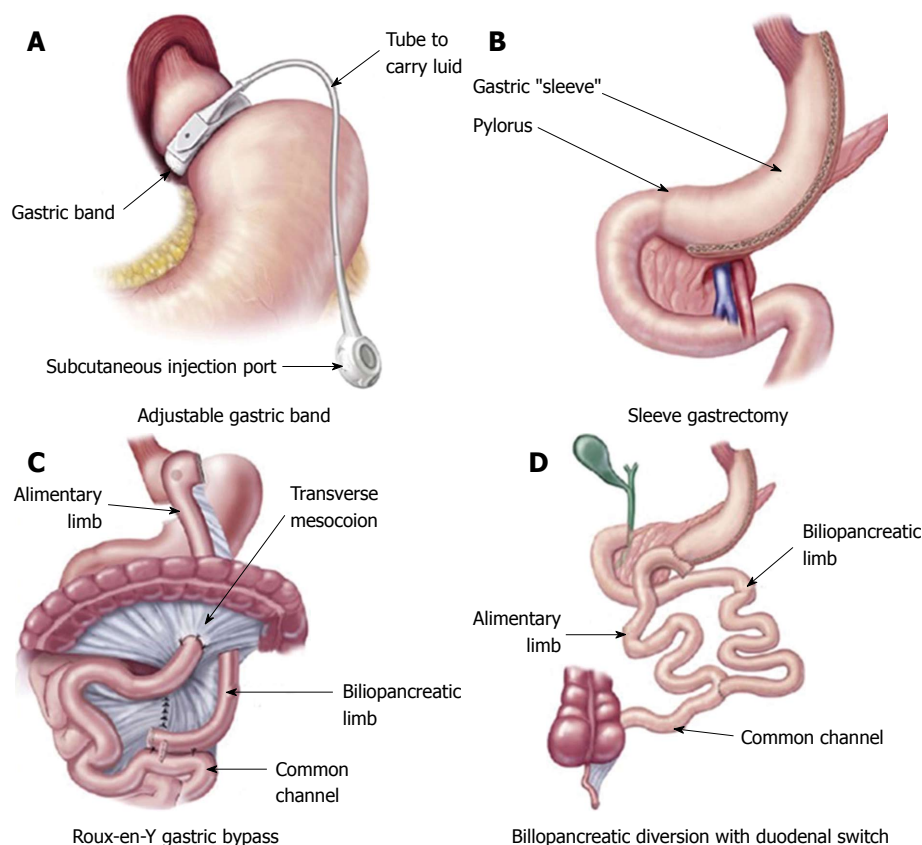


Figure 1 The diagrammatic representation of different bariatric surgical procedures. A: Adjustable gastric band; B: Sleeve gastrectomy; C: Roux-en-Y gastric bypass; D: Bilio-pancreatic diversion. Figure reproduced with permission from John Wiley and Sons. *Obesity* (Silver Spring) 2013 Mar; 21 Suppl 1: S1-27.

standardization in the definition of diabetes remission and it was unclear how specific bariatric procedures were chosen or what criteria were used for performing bariatric surgery.

A comparison of the mean changes in BMI and haemoglobin A1c (HbA1c) achieved with individual procedures^[12] are shown in the Table 2.

A few long-term cohort studies give us insight on the beneficial effects of metabolic surgery in T2DM^[16]. For example, the Swedish Obese Subjects (SOS) study, started in 1987 is a prospective case-control study with 2010 obese subjects who underwent bariatric procedures (predominantly vertical banded gastrostomy which is considered to be less effective and no longer undertaken)^[16,17]. A 72% remission of T2DM after two years and 36% durable remission after 10 years were observed in obese subjects in the SOS study^[16,18]. Compared to control the risk of developing T2DM was reduced by 96%, 84%, and 78% after 2, 10, and 15 years in obese subjects without T2DM at baseline^[19].

Utah obesity study retrospectively compared 7925 obese subjects who underwent RYGB with the same number of age, sex and weight matched controls and after an average follow up period of 7.1 years, found a 40% reduction in all cause mortality, 49% reduction of mortality from cardiovascular diseases and 92% reduction of death related to T2DM^[20]. The Longitudinal

Assessment of Bariatric Surgery (LABS-2) study is an ongoing multi-center cohort study that showed T2DM remission in 67% and 28% of those who underwent RYGB and AGB respectively after 3 years of follow up^[21].

MECHANISMS OF DIABETES IMPROVEMENT/REMISSION FOLLOWING METABOLIC SURGERY

Reduction in calorie intake

Typically, patients are put on a calorie-restricted diet after bariatric procedures. The average caloric intake ranges from 400-800 kcal/d in the first month^[22]. Calorie restriction has a significant impact on hyperglycemia with reduction of blood glucose levels. In fact, in a recent case-control study^[23], post-bariatric diet implemented before metabolic surgery resulted in more profound glucose reduction in T2DM patients than in the post-surgical period, indicating the role of calorie restriction in glycemic control after bariatric procedures. Very low calorie diet alone resulted in improvement of insulin sensitivity and β -cell function in obese T2DM cases (comparable to those who had bariatric surgery) in the short-term^[24]. Generally, a calorie-restricted diet should be maintained in patients following metabolic surgery on a long-term basis.

Table 1 Major randomised controlled trials reporting effects of metabolic surgical procedure on type 2 diabetes mellitus

RCT	Study details	Outcome	Diabetes remission
Dixon <i>et al</i> ^[8]	Un-blinded RCT. N = 60 Obese patients with recent onset T2DM Conventional therapy <i>vs</i> LAGB Follow-up: 2 yr	Weight loss $-1.7\% \pm 5.2\%$ in conventional group and $-20.7\% \pm 8.6\%$ in the surgical group	73% in surgical group 13% in conventional group (Remission of T2DM defined as FBS < 7.0 mmol/L and HbA1c < 6.2%)
Mingrone <i>et al</i> ^[7]	Single centre non-blinded RCT. N = 60. Severely obese patients. T2DM of at least 5 yr duration and HbA1c > 7.0%. Conventional medical therapy <i>vs</i> RYGB or BPD Follow-up: 2 yr	Weight loss $-4.7\% \pm 6.3\%$ with medical therapy, $-33.3\% \pm 7.8\%$ with RYGB and $-33.8\% \pm 10.1\%$ with BPD	75% in RYGB group; 95% in BPD group. None in the conventional group (Remission defined as FBS < 5.6 mmol/L and HbA1c < 6.5%)
Schauer <i>et al</i> ^[9]	Single centre non-blinded RCT in obese uncontrolled T2DM. N = 150 Intensive medical therapy <i>vs</i> RYGB or SG Follow-up: 12 mo	Weight loss -5.4 ± 8.0 kg in medical therapy group, -29.4 ± 9.0 kg in RYGB group and -25.1 ± 8.5 kg in SG group	12% medical therapy group 42% in RYGB group 37% SG group (Remission/primary outcome defined as HbA1c of 6% or less)
Ikramuddin <i>et al</i> ^[10]	Un-blinded RCT in obese T2DM with HbA1c over 8% with average duration of 9 yr. N = 120 Intensive medical therapy <i>vs</i> RYGB Follow-up: 12 mo	Difference in weight loss between surgical and medical group -17.1 ± 5.6 kg. % weight change in medical <i>vs</i> Surgical group -7.9 ± 2 <i>vs</i> -26 ± 2	75% RYGB group 32% in medical group (Remission defined as HbA1c < 7%)
Liang <i>et al</i> ^[11]	RCT in obese T2DM. N = 108. RYGB compared with standard care with or without Exenatide therapy Follow-up: 12 mo	Reduction in BMI (kg/m^2) in standard <i>vs</i> Exenatide <i>vs</i> RYGB: -0.56 ± 1.66 <i>vs</i> -3.44 ± 1.21 <i>vs</i> -5.97 ± 0.91	90% in RYGB group None in patients receiving standard care with or without Exenatide (Remission defined as HbA1c < 6.5%)

RCTs: Randomised controlled trials; T2DM: Type 2 diabetes mellitus; N: Number of subjects; LAGB: Laparoscopic gastric banding; FBS: Fasting blood sugar; RYGB: Roux-en-Y gastric bypass; SG: Sleeve gastrectomy; BPD: Bilio-pancreatic diversion.

Table 2 The mean changes in body mass index and haemoglobin A1c achieved with different gastric bypass procedures

Bariatric procedure	Body mass index (kg/m^2)			HbA1c (%)		
	Pre-surgery	Post-surgery	Mean reduction (95%CI)	Pre-surgery	Post-surgery	Mean reduction (95%CI)
AGB	37	29.5	7.5 (5.9-9.1)	7.8	6	1.8 (1.3-2.3)
SG	41.3	28.3	13.0 (10.1-15.9)	7.9	6	1.9 (1.0-2.8)
RYGB	34.6	25.8	8.8 (5.2-12.4)	8.2	6.1	2.1 (1.3-2.9)
BPD	50.5	34.6	15.9 (11.8-20.0)	8	5.2	2.8 (2.1-3.5)

AGB: Adjustable gastric banding; SG: Sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; BPD: Bilio-pancreatic diversion; HbA1c: Haemoglobin A1c.

Alterations in gut hormones

The acceleration of gastrointestinal transit time after bariatric procedures results in augmented secretion of gut hormones such as glucagon-like peptide-1 (GLP-1), Glucose-dependent insulintropic peptide (GIP) peptide-YY (PYY) and oxyntomodulin (OXM) that alter energy and glucose metabolism^[25]. The anorexiant and weight losing properties of GLP-1 is well established in experimental and clinical models. Several studies showed significant elevation of GLP-1 levels during oral glucose challenge and with meals after bariatric procedures such as SG^[26], RYGB^[27] and BPD^[28]. Elevated GLP-1 levels following metabolic surgery was also shown to reverse the obesity-induced endothelial dysfunction conferring cardiovascular protection in the obese^[29].

Increased PYY levels following bariatric procedures result in weight loss in obese subjects. PYY administration resulted in a 30% reduction in the caloric value of a meal consumed 2 h after PYY infusion and a 33% reduction in food consumption over 24 h period in human beings^[30]. Similarly, OXM administration has been shown

to reduce appetite, amount of food ingested and body weight^[31]. Ghrelin (a gut-derived peptide hormone that stimulates hunger) level was found to be low after RYGB, whereas the level was high in diet-induced weight loss, indicating the impact of suppression of hunger signals in subjects following bariatric surgery^[32]. Hypergastrinemia following SG has been recently reported in rat models of T2DM, although its impact on metabolic pathways and body weight changes have been unclear^[33]. The alteration in these gastrointestinal hormonal factors together contributes to significant improvement in T2DM and the body weight of the individual.

Pancreatic β -cell function

Improvement in insulin sensitivity and pancreatic β -cell function are important factors that contribute to improvement/remission of T2DM in obese subjects. Increase in secretion of incretin hormones (GLP-1 and GIP) and proliferation of the β -cell mass have been demonstrated following bariatric procedures in human beings and experimental animals^[34]. Although these

factors clearly contribute to T2DM control, there may be other mechanisms which are not yet clear.

Hepatic and peripheral insulin sensitivity

Significant improvement of hepatic insulin sensitivity is observed within few days of bariatric procedures much earlier and before significant weight loss occurs. Reduction of energy intake from the post-bariatric diet may contribute significantly to the improvement in hepatic insulin sensitivity. Although peripheral insulin sensitivity is not altered in the immediate post-operative period, delayed improvement is observed in patients^[25,35]. Reduced hepatic fat content and body weight loss account for the sustained improvements in hepatic and peripheral insulin sensitivity during long-term follow up of patients who had metabolic surgery^[25].

Role of bile acids

Bariatric procedures were shown to increase the plasma levels of bile acids that improved glucose and lipid metabolism^[25,36]. However, the exact mechanisms by which bile acids improve glycemic control and body weight remain elusive.

Gut microbiota

Major changes in the gut microbiota have been demonstrated following bariatric procedures in animal models and human beings^[37]. Increase in numbers of some of these intestinal microbial flora, and the related changes in the gut biochemical environment, may affect glucose and lipid metabolism that contribute to weight loss and diabetes improvement^[25,37]. More research is necessary on the role of gut flora in glucose and fat metabolism following bariatric procedures.

Body weight loss and diabetes remission

The major mechanism by which improvement and/or remission of T2DM occurs in obese subjects following metabolic surgery is the significant weight loss after the procedure. Analysis of participants in the Look AHEAD (Action For Health in Diabetes) study clearly showed a progressive increase in odds ratios for HbA1c reduction with higher proportions of weight loss^[38]. The odds ratios for % weight reduction (in parenthesis) were: 1.80 (≥ 2 to < 5), 3.52 (≥ 5 to < 10), 5.44 (≥ 10 to < 15) and 10.02 ($\geq 15\%$) respectively for HbA1c reduction of 0.05% in the study subjects. Substantial loss of body weight post-bariatric surgery therefore would explain the remarkable improvements in glycemic control and even remission of T2DM in the majority of patients.

Overall, a multitude of physiological, behavioural and anatomical alterations following the bariatric procedure result in significant improvements in metabolic and glycemic parameters that may even result in potential cure of T2DM in a good number of patients after the metabolic surgery.

COST BENEFITS AND OTHER HEALTH-RELATED OUTCOMES

Data from the National Bariatric Surgery Registry (NBSR) of the United Kingdom that included 18283 cases from 2010 to 2013 clearly showed compelling evidence of the cost effectiveness of bariatric surgery as a treatment option for severely obese T2DM patients^[39]. NBSR data showed that 61% of patients with obstructive sleep apnoea could come off their treatment after surgery 65% of patients with T2DM could stop their diabetic medications. A recent systematic review and meta-analysis revealed important cardioprotective effects of metabolic surgery in terms of regression of left ventricular hypertrophy, improvement of diastolic function and reduction of left atrial size^[40]. Improvement of hypertension, hypercholesterolemia, gastro-esophageal reflux disease, and arthritis are some of the other reported major beneficial effects of bariatric surgery^[41].

IMMEDIATE AND LONG-TERM COMPLICATIONS

The main complications in the immediate post-operative period are pulmonary complications, vomiting, wound infections, bleeding and anastomotic leak^[16,17]. In a recent systematic review and meta-analysis the peri-operative and post-operative mortality rates were 0.08% and 0.31% respectively in RCTs and 0.22% and 0.35% respectively in OBS^[4]. The overall complication rates were 17% in RCTs and 10% in OBS, and the reoperation rates were 7% and 6% respectively.

The most common long-term complications were iron deficiency anaemia in up to 15% of cases and re-operations in up to 8%^[14,16]. Psychological issues are emerging as an important complication on follow up of the cases, and alcohol overconsumption and substance misuse are increasingly being reported in patients on long-term follow up^[16,42]. For unknown reasons, the suicidal rates were found to be higher in patients who underwent bariatric surgery^[16,20]. Nutritional deficiencies including deficiencies of calcium, vitamin D, iron, zinc, and copper, are common after bariatric surgery^[16,43]. Periodic checking for deficiencies and nutritional supplements are indicated in patients.

Massive weight loss after the surgery may result in abnormal body contour because of extensive skin folds that may affect the psychological well being of many patients. Body contouring surgery improves this problem and may help improvement of physical and mental well being in these patients although financial cost may become an issue in many healthcare systems^[44]. Post-prandial hypoglycaemia is a common problem in many patients after metabolic surgery. Rapid transit of contents from stomach with smaller capacity could be a reason in many cases that can be treated with small frequent

meals and complex carbohydrates. However, severe hypoglycaemic episodes caused by hyperinsulinemia from pancreatic islet cell hyperplasia (nesidioblastosis) can sometimes be crippling necessitating pancreas resection, reversal of gastric bypass and restriction of gastric pouch in extreme cases^[45].

Diabetic microvascular complications such as retinopathy, neuropathy and nephropathy can sometimes get worse if there is rapid (abrupt) improvement of longstanding severe dysglycemia in patients with poorly controlled diabetes. A similar situation could be expected following metabolic surgery with acute improvement in glycaemic control. There is some evidence of worsening of pre-existing diabetic retinopathy in a proportion of cases following bariatric procedures, although improvement of the disease is also noted in some others^[46,47]. Therefore, counselling about this potential complication before surgery and close monitoring after the procedure are advisable. Though the data is insufficient, there is some evidence for improvement of diabetic neuropathy^[48] and nephropathy^[49] after bariatric surgery. There are no reports of worsening of these conditions after improvement of T2DM following bariatric procedures.

Pregnancies after bariatric surgery were found to be associated with significantly lower risk of gestational diabetes [odds ratio (OR): 0.25; 95%CI: 0.13–0.47], large-for-gestational-age infants (OR: 0.33; 95%CI: 0.24–0.44) and shorter gestation (mean difference: –4.5 d; 95%CI: –2.9 to –6 d; $P < 0.001$)^[50]. However, there were significantly higher risk of small-for gestational-age infants (OR: 2.20; 95%CI: 1.64–2.95; $P < 0.001$). The risk of stillbirth or neonatal death post-bariatric pregnancies appeared to be higher [1.7% vs 0.7% (OR: 2.39; 95%CI: 0.98–5.85; $P = 0.06$)] although this risk did not reach statistical significance.

APPROPRIATE PATIENT CATEGORY FOR METABOLIC SURGERY

There is no clear and uniform consensus from different international bodies about the minimum BMI cut off for consideration of the bariatric procedure in obese individuals. The National Institute for Health and Clinical Care Excellence (NICE) of the United Kingdom recently recommended bariatric surgery for people with a BMI of $> 40 \text{ kg/m}^2$ and $> 35 \text{ kg/m}^2$ in the presence of co-morbidities such as T2DM or hypertension^[51]. For people of Asian family origin a lower BMI threshold should be considered. NICE also recommends expedited assessment for Bariatric surgery to people with BMI of 30–34.9 kg/m^2 who have recent onset T2DM. This is based on the evidence that earlier intervention can improve the chances of remission following bariatric surgery^[39].

The Canadian Diabetes Association Clinical Practice Guidelines (2013) recommend bariatric surgery for people with class III obesity (BMI $\geq 40.0 \text{ kg/m}^2$) or class II obesity (BMI = 35.0 to 39.9 kg/m^2) in the presence of co-morbidities, with an inability to maintain

weight loss following adequate trial of health behaviour intervention^[52].

The American Association of Clinical Endocrinologists, the Obesity Society and the American Society for Metabolic and Bariatric Surgery (2013) recommend metabolic surgery in obese individuals with a BMI of $\geq 40 \text{ kg/m}^2$ without coexisting medical problems and surgical risk^[53]. For patients with BMI $\geq 35 \text{ kg/m}^2$, surgery may be offered if one or more severe obesity-related co-morbidities exist including T2D, hypertension, hyperlipidemia, obstructive sleep apnea (OSA), obesity-hypoventilation syndrome (OHS), Pickwickian syndrome (a combination of OSA and OHS), nonalcoholic fatty liver disease or nonalcoholic steatohepatitis, pseudotumor cerebri, gastroesophageal reflux disease, asthma, venous stasis disease, severe urinary incontinence, debilitating arthritis, or considerably impaired quality of life. Patients with BMI of 30–34.9 kg/m^2 and T2DM or metabolic syndrome may also be offered a bariatric procedure, although evidence for this recommendation is inadequate with the unavailability of long-term data^[53].

With robust clinical and epidemiological data emerging from all continents of the world, a global consensus on the appropriate patient categories that get definite benefits from metabolic surgery is expected to emerge in the near future.

AREAS OF UNCERTAINTY

Although there is accumulated experience from different regions of the world on the excellent outcomes of bariatric procedures, there is not enough data from resource poor nations of Asia, Arabian Peninsula, Africa and South America to generalise the recommendations of metabolic surgery, even though obesity epidemic is becoming a public health issue in these regions. Moreover, the BMI cut off for obesity in Asians is different from that of the western populations. For example BMI of $\geq 25 \text{ kg/m}^2$ is considered as obesity in India^[54] and $\geq 27 \text{ kg/m}^2$ in Taiwan^[55]. Diabesity (diabetes caused by overweight or obesity) is different for populations of Asian and Afro-Caribbean ethnic background owing to the difference in abdominal adiposity in these groups compared to other races, making generalisation of BMI cut offs metabolic surgery inappropriate.

For a common condition like diabetes there are only a handful of RCT's of short duration comparing bariatric surgery to medical therapy which ranged from standard care to intensive medical intervention. There are a few prospective studies available on long term outcomes of bariatric surgery. However, T2DM being a lifelong multisystem disease with almost all organs of the body involved, more data based on lifelong follow up of cases is necessary to understand the true impact of bariatric procedures. This requires maintenance of nationwide bariatric registries globally along the lines of NBSR in the UK which can provide valuable information.

Though there are some studies on the impact of bariatric surgery on the psychosocial, nutritional and

mineral metabolic status of patients, long-term data on these areas are still inadequate. Similarly the optimal management of post-bariatric nesidioblastosis that emerged as a challenging clinical problem is not yet clear.

Different multicentre on-going prospective clinical trials would be expected to answer these unresolved questions.

CONCLUSION

Metabolic surgery is emerging as a major paradigm shift in the 21st Century management of T2DM, and has revolutionised the care of diabetes. Massive weight loss with remission of T2DM in a significant proportion of cases along with improvement of most other obesity-related ailments makes the treatment a very attractive option for clinicians and patients. A recent analysis of the NBSR data clearly showed its cost effectiveness. Appropriate patient selection and long-term follow up of cases are necessary to optimise the outcomes and reduce the complications.

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Impact of new technologies on diabetes care

Elisa Giani, Andrea Enzo Scaramuzza, Gian Vincenzo Zuccotti

Elisa Giani, Gian Vincenzo Zuccotti, Department of Pediatrics, Ospedale dei Bambini-V. Buzzi, Università degli Studi di Milano, 20154 Milan, Italy

Andrea Enzo Scaramuzza, Department of Pediatrics, Ospedale L. Sacco, 20157 Milan, Italy

Gian Vincenzo Zuccotti, Center for Research in Nutrition (CURN), Biomedical and Clinical Science Department, Università degli Studi di Milano, 20154 Milan, Italy

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Correspondence to: Gian Vincenzo Zuccotti, MD, Full Professor and Chairman, Department of Pediatrics, Ospedale dei Bambini-V. Buzzi, Università degli Studi di Milano, 32, Via Castelvetro, 20154 Milan, Italy. gianvincenzo.zuccotti@unimi.it
 Telephone: +39-02-57995322
 Fax: +39-02-57995132

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Abstract

Technologies for diabetes management, such as continuous subcutaneous insulin infusion (CSII) and continuous glucose monitoring (CGM) systems, have improved remarkably over the last decades. These developments are impacting the capacity to achieve recommended hemoglobin A1c levels and assisting in preventing the development and progression of micro- and macro vascular complications. While improvements in metabolic control and decreases in risk of severe and moderate hypoglycemia have been described with use of these technologies, large epidemiological international studies show that many patients are still unable to meet their glycemic goals, even when these technologies are used. This editorial will review the impact of technology on glycemic control, hypoglycemia and quality of life in children and youth with type 1 diabetes. Technologies reviewed include CSII, CGM systems and sensor-augmented insulin pumps. In addition, the usefulness of advanced functions such as bolus profiles, bolus calculators and threshold-suspend features will be also discussed. Moreover, the current editorial will explore the challenges of using these technologies. Indeed, despite the evidence currently available of the potential benefits of using advanced technologies in diabetes management, many patients still report barriers to using them. Finally this article will highlight the importance of future studies tailored toward overcome these barriers to optimizing glycemic control and avoiding severe hypoglycemia.

Key words: Diabetes; Technology; Glycemic control; Quality of life; Outcomes; Management

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Core tip: There have been many advances in the technologies associated with diabetes care in the last few years, which have resulted in new opportunities

in the treatment of diabetes. Despite the encouraging results and the prospect of a fully automated closed loop system in the near future, metabolic control remains suboptimal in most patients with type 1 diabetes. Data from registries has recently shown that a large proportion of children with type 1 diabetes does not meet the age associated A1c targets across all countries, especially in the youth age. This editorial discusses the impact of these technologies on glycemic control and quality of life and attempts to address how to overcome barriers using these technologies to achieve improved metabolic control.

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Recently, data related to the safety and effectiveness of a bionic pancreas under unrestricted outpatient conditions were published by Russel *et al.*^[11], reporting that “as compared with an insulin pump, a wearable, automated, bi-hormonal, bionic pancreas improved mean glycemic levels, with less frequent hypoglycemic episodes” in both adults and adolescents with type 1 diabetes, in outpatient settings. While the device is still imperfect, (difficulty with wireless connectivity, poor stability of glucagon, need for faster insulin analogues, risk of hypoglycemia and need for restrictions in food and alcohol intake) these results marked an important step toward a fully automated closed-loop system.

Currently, at least 20 research groups are working worldwide on glucose-sensor-controlled automated insulin delivery systems (closed loop pumps), and during the last years, great progress was reported in closed-loop system in outpatients settings, with a particular focus on overnight glycemic control, whereas postprandial and post-exercise glucose control remains a challenge^[2-5].

These promising studies bring the artificial pancreas closer to public use, which is possible due to the recent improvements in technology for diabetes care. Nonetheless, many patients spend the majority of their day outside the recommended glycemic ranges. As a result glycemic control remains suboptimal for many patients with type 1 diabetes^[6].

It has now been 10 years since the Epidemiology of Diabetes Interventions and Complications study confirmed the need to optimize glycemic control as early as possible to sustain risk reduction for micro- and macro vascular complications^[7,8]. Since then, many national and international diabetes associations [e.g., the American Diabetes Association and the International Society for Pediatric and Adolescent Diabetes (ISPAD)] revised their guidelines for type 1 diabetes management and now recommend a target glycated hemoglobin (A1c) of 6.5%-7.5% (48-59 mmol/mol) for most people with type 1 diabetes (T1D)^[9,10]. However, recently published

data by McKnight *et al.*^[11], reported that only 30% of males and 29% of females aged < 15 years, 24% of males and 20% of females aged 15-24 years, and 30% of males and 28% of females aged > 25 years achieved these recommended A1c levels (< 7.5% or < 59 mmol/mol). These data confirmed that this target is not easily achieved in many people with type 1 diabetes and also that A1c levels are higher in those aged 15-24 years than among other age groups across many countries^[11]. It is clear that there is still a gap between patients' glycemic control outcomes and what can be achieved with newer therapeutic improvements, even if technological key advances as the continuous subcutaneous insulin infusion (CSII) and the continuous glucose monitoring (CGM) have been shown to greatly improve diabetes care.

Focusing on the effectiveness of new technologies and the limitations of the use of such technologies in the real world may help find a way to achieve the A1c goals for many patients. In addition, it could give us greater insight into barriers to sustain the use of these therapeutic advances and how to overcome them. Several recently published review studies and meta-analyses addressed these topics^[12-14]. Deeb *et al.*^[15] assessed the association between how insulin pumps were used and blood glucose control to determine if the use of advanced pump features improved glycemic control. Indeed, over the last 15 years, it has been shown that the increasing use of insulin pump can result in many health benefits and an improvement of overall treatment satisfaction^[16,17]. Thus, it would be expected to improve long-time metabolic outcome in patients using this treatment. Although randomized controlled studies and systematic reviews of pediatric cohorts using CSII showed only modest benefits (in the range of 0%-0.9%^[18]) in terms of mean A1c compared to multiple daily injections (MDI), many prospective and retrospective case-control studies, clinic-based series and registries, reported that pediatric insulin pump users have a lower A1c when compared to patients using MDI, and that they are more likely to achieve A1c targets than those on injections. Recently, Olsen *et al.*^[19] showed a significantly lower mean A1c ($P < 0.0001$) in 1493 children and youth using CSII vs 1846 using MDI therapy over a 5 year period in all age groups. In the T1D Exchange clinic registry, A1c was shown to be lower in CSII users vs MDI users (7.9% vs 8.5%, $P < 0.001$); in the longitudinal analysis, one year after initiation of CSII therapy, A1c decreased by 0.2% on average ($P < 0.001$), with no difference in frequency of severe hypoglycemic events ($P = 0.2$)^[20]. Similar data have been reported in the national pediatric diabetes audit of England and Wales and in the DPV initiative of Germany and Austria at the last ISPAD meeting^[21]. What is more, in their meta-analysis, Pickup and Sutton reported patients on CSII had less hyperglycemia and less severe hypoglycemia^[22]. Other meta-analyses showed that the frequency of severe hypoglycemia was significantly higher with multiple daily insulin injections

than with insulin-pump therapy [odds ratio (OR), OR: 4.19; 95%CI: 2.86-6.13]. The greatest reduction was seen among patients who had had the greatest number of episodes of severe hypoglycemia while they were receiving injection therapy. Among these patients, the rate of severe hypoglycemia was higher by a factor of about 30 with multiple daily insulin injections than with insulin-pump therapy^[16].

Finally, CSII has been associated with an improved quality of life^[23,24]: CSII use is related to reduced frequency and intensity of parent stress, decreased fear of hypoglycemia, increased flexibility in quantity and timing of meals and sleep schedule, improvement in diabetes self-efficacy and independence^[23,25].

However, not all children benefit from CSII. This discrepancy allows us to determine predictors for improvement of glycemic control on pump. For example, Olsen *et al*^[19] showed that achievement of target A1c was significantly associated with lower A1c before insulin pump therapy initiation, younger age (< 12 years), shorter diabetes duration, higher number of daily boluses and more frequent daily self-blood glucose monitoring. Thus, patient characteristics are critical factors in deciding whether or not it is appropriate to prescribe an insulin pump to an individual.

Similar results are seen with continuous glucose monitors (CGM) use, and data from the T1D Exchange Clinic Registry showed that only a small proportion of patients with type 1 diabetes are using CGM daily in clinical practice, especially in the pediatric age range^[26]. The accuracy and usability of CGM has gradually improved over the past decade so that the overall accuracy of the latest sensor generations measured as the mean relative absolute difference vs a given laboratory standard is in the 8%-15% range^[27]. Despite this, CGM is still far from perfect. For example, more accurate evaluation of interstitial glucose levels during hypoglycemic events are necessary as CGM performs poorly in the hypoglycemic range, and the lag time between interstitial glucose and blood glucose, increased sensor sensitivity and inappropriate calibration require improvement^[28].

Several studies have showed that CGM is associated with a significant reduction in A1c^[29]. In two recent meta-analyses of randomized controlled trials, CGM was shown to be superior to self-monitoring of blood glucose alone in reducing A1c by almost 0.4% in both children and adults^[30,31]. In a JDRF-sponsored multicenter trial, there was a larger percentage of subjects 8-14 years old using CGM who achieved at least a 10% decrease in A1c and a target A1c < 7% (59 mmol/mol), compared with children using capillary blood monitoring (SMBG)^[32]. In a Cochrane meta-analysis, the largest improvement in glycemic control was observed in poorly controlled diabetes patients using CGM and CSII (sensor-augmented pump - SAP). There was no increase in risk of severe hypoglycemia or ketoacidosis in this evaluation.

Although the impact of CGM use on hypoglycemia is less clear, Floyd *et al*^[31] found a significant decrease in the duration of time in both mild and severe hypoglycemia

ranges and an increase in the time "in range" (70-180 mg/dL) in patient using CGM^[31].

In the last few years, several studies evaluated the impact of SAP on metabolic control compared to either MDI or SMBG^[33] or CSII and SMBG^[34-36]. SAP therapy was demonstrated to be effective at lowering mean A1c in both adult and pediatric patients^[33-36]. Switching to SAP therapy helped patients using MDI to lower their A1c levels to the same extent as the patients originally allocated to the SAP arm of the study. Benefits persisted through the entire 12-mo study phase (STAR 3 Study)^[33], as well as its follow up phase^[34]. Patients using SAP therapy were more likely to meet age-appropriate A1c target^[33].

However, studies investigating the effectiveness of SAP in patients already using the insulin pump showed conflicting results, ranging from no significant benefit to significantly improved glycemic control^[35-37].

SAP therapy was also associated with decreased time spent in hypoglycemia compared to MDI or CSII, but few significant results were found in the rate of severe hypoglycemic events.

Although current standards for diabetes management reflect the need to avoid diabetes complications, in the pediatric clinical setting, the fear of hypoglycemic events is a common barrier to achieving optimal metabolic control.

It has been reported that the most severe hypoglycemic events in children occur at night, and account for 75% of all hypoglycemic seizures^[38]. Thus, children may represent a group of patients that can benefit greatly from SAP therapy, especially when a low-glucose suspend (LGS) feature is implemented (*i.e.*, the feature that automatically suspends insulin delivery when the blood glucose is less than a pre-selected value, typically 70 mg/dL). LGS and predictive low-glucose suspend (PLGS) are the first steps toward the artificial pancreas, and can help reduce family stress related to glucose management, especially overnight. LGS systems have been demonstrated to be effective in reducing the rate, severity and duration of hypoglycemia, without an increase in A1c^[39]. In particular, this feature was shown to be most effective in patients with more frequent and severe hypoglycemia and in those with hypoglycemia unawareness^[39].

In a study from Ly *et al*^[40] the incidence of hypoglycemia after 6 mo decreased from 34.2/100 patient-months in the insulin pump group to 9.5/100 patient-months in the SAP plus LGS group, with the rate of severe hypoglycemia reduced to zero (0) in the SAP plus LGS group^[39,40].

In the PLGS system, a predictive algorithm stops insulin delivery prior to reaching a predetermined threshold. Only a few outpatient studies using PLGS have been published to date, but it was shown that a further reduction of the severity of hypoglycemia as compared with SAP plus LGS alone is possible^[41,42].

Despite all these encouraging results, CGM use is still difficult in youth with type 1 diabetes of all ages^[43]. It is

now clear that CGM can greatly help to improve glycemic control only in patients with type 1 diabetes who use the sensor for the majority of time (more than 70%)^[29,31,32], and works best when used on a near-daily basis. For this reason, physical, socioeconomic and educational factors that could impact the use of this technology are an area of current research, as are predictors of pump and sensor use^[44].

There are a number of barriers that may inhibit youth from wearing CGM. CGM use requires significant patient input (sensor insertion, calibration, response to sensor alarms and glucose trends) and ongoing SMBG for insulin dosing. The Juvenile Diabetes Research Foundation CGM trial on CGM satisfaction reported pain in sensor insertion, frustration with sensor alarms, skin reaction, and issues related to discomfort with wearing the device or technical problems as barriers to CGM wear^[44]. In the T1D Exchange registry, CGM use was more likely in subjects with higher educational level, higher income, private insurance, longer diabetes duration and those on insulin pump^[26]. In addition, recent data showed that most patients using CGM may not receive the full benefits of this technology, either because they do not use it enough or because they do not regularly download it and retrospectively review the data from the device^[45].

Lack of a proper education, diminished motivation, deliberate insulin omission, and behavioral attitude can affect patients' compliance. Ensuring long-term follow-up with intensifying education and involving behavioral therapy in training might improve adherence and enhance treatment satisfaction, leading to a better glycemic control^[26].

Beside technology by itself, great improvement has been observed also in immune-suppressor drugs or other drugs, useful to improve type 1 diabetes management^[46].

In conclusion, since most of the recently reported epidemiological data demonstrates that a large proportion of type 1 diabetes patients do not achieve A1c targets, we consider increased education on diabetes care as a good option to improve glycemic control. New technologies may have positive outcomes, but can underperform if the technology is not used as expected^[16,42-45].

While the hope for a fully automated artificial pancreas available in the near future remains, it is crucial to develop approaches for implementing and sustaining the use of technological advances that are currently available (*e.g.*, beside CSII and CGM). In addition, we need to continue our patient/family education efforts.

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Importance of telemedicine in diabetes care: Relationships between family physicians and ophthalmologists

Pedro Romero-Aroca, Ramon Sagarra-Alamo, Alicia Pareja-Rios, Maribel López

Pedro Romero-Aroca, Department of Ophthalmology, University Hospital Sant Joan, University Rovira i Virgili, Institut de Investigació Sanitària Pere Virgili, 43202 Reus, Spain

Ramon Sagarra-Alamo, ABS Reus-1, Retinography non-mydratic Unit, CAP Sant Pere, 43202 Reus, Spain

Alicia Pareja-Rios, Department of Ophthalmology, Retina section, Hospital Universitario de Canarias, 38320 Tenerife, Spain

Maribel López, Department of Ophthalmology, University Hospital Valladolid, Ocular Diabetes Unit of IOBA, 47001 Valladolid, Spain

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Correspondence to: Pedro Romero-Aroca, MD, PhD, Department of Ophthalmology, University Hospital Sant Joan, University Rovira i Virgili, Institut de Investigació Sanitària Pere Virgili, Avda, Doctor Josep Laporte 2, 43202 Reus, Spain. romeropere@gmail.com
Telephone: +34-977-310300
Fax: +34-977-32375

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Abstract

Diabetic retinopathy (DR) is the worldwide leading cause of legal blindness. In 2010, 1.9% of diabetes mellitus (DM) patients were legally blind and 10.2% had visual impairment. The control of DM parameters (glycemia, arterial tension and lipids) is the gold standard for preventing DR complications, although, unfortunately, DR still appeared in a 25% to 35% of patients. The stages of severe vision threatening DR, include proliferative DR (6.96%) and diabetic macular edema (6.81%). This review aims to update our knowledge on DR screening using telemedicine, the different techniques, the problems, and the inclusion of different professionals such as family physicians in care programs.

Key words: Diabetic retinopathy; Telemedicine; Family physicians; Clinical decisions support system; Diabetic retinopathy screening

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Core tip: If telemedicine is especially suited for a particular medical specialisation, that specialisation is undoubtedly ophthalmology. The enormous healthcare pressure derived from the general population's high demand for vision control and the prevalence of certain diseases which affect the eyes, such as diabetes mellitus, combined with the tremendous progress in diagnostic imaging systems in this speciality make it especially possible to send images over telemedicine networks for the diagnosis or even prevention of eye diseases, thus making the demand for the use of these types of methods extremely important.

Romero-Aroca P, Sagarra-Alamo R, Pareja-Rios A, López M. Importance of telemedicine in diabetes care: Relationships between family physicians and ophthalmologists. *World J Diabetes* 2015; 6(8): 1005-1008 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i8/1005.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i8.1005>

INTRODUCTION

If telemedicine is especially suited for a particular medical specialization it would undoubtedly be ophthalmology. There is enormous pressure on healthcare systems by the general population's high demand for vision control and the prevalence of certain diseases which affect the eyes, such as diabetes mellitus (DM). The tremendous progress in diagnostic imaging systems make it possible to send images over telemedicine networks for the diagnosis or even prevention of eye diseases.

We should not forget that eye-care centers are often a considerable distance away from the population that require healthcare, so being able to send information over a telemedicine network is a great advantage. Good liaison among specialists, such as the ophthalmologist and the family doctor, means that it is possible to avoid patients traveling and enabling them to be diagnosed and controlled closer to home.

In spite of ophthalmology perhaps being the best example of the use of telemedicine, the reality is that its use is still far from extensive. There are many problems that ophthalmologists and other specialists who could use this communication system may face, including incompatibility among data-processing systems used by different specialists, not only among specialists and general practitioners, but even among hospital centers themselves. This is heightened by the existence of personal data-protection laws that cover the sending of images along with other personal details of patients. All this can make the regular use of telemedicine quite difficult^[1].

Even within the field of ophthalmology, not all eye diseases can benefit from the use of telemedicine, the most common being to send images of diseases that affect the retina, many of them highly prevalent.

TELEMEDICINE IN DIABETIC RETINOPATHY

DM is recognized by the World Health Organization as an genuine pandemic^[2] that affects more than 10% of the population over 14 years of age^[3], with type diabetes mellitus 2 (DM2) being the most common presentation and associated with lifestyle habits such as sedentarism or obesity. Considered a chronic disease, its morbidity is brought about by the complications it causes throughout a patient's lifetime, these being mainly derived from damage to large vessels, or macroangiopathy (complicated by cerebrovascular accidents and myocardial infarction),

or damage to small vessels, or microangiopathy, leading to nephropathy, neuropathy or retinopathy.

Eye diseases caused by microangiopathy or diabetic retinopathy (DR) are the main cause of blindness among young adults in the western world (aged between 45 and 60) and are closely related to poor metabolic control of the DM and aggravated by other comorbidities that are present in DM, such as high blood pressure, dyslipidemia or nephropathy. Early diagnosis of DR is very important because it has been shown that strict control over glycemia and high blood pressure slows the progress of the retinopathy and, if it is not present, extends the time until its appearance^[4]. Screening diabetics is therefore fundamental for detecting the existence of DR as soon as possible. This should be carried out by taking retinal photographs with non-mydratic cameras, an accepted cost-effective method that makes it feasible to cover a large number of patients with DM^[5,6].

In spite of the fact that a system such as the one presented here would enable the screening of a large number of patients, with the benefit for the diabetic population this represents, the truth is that, to date, the screening of diabetic patients does not take place on a general basis and many patients with DM do not undergo regular eye examinations. So much so that in developed countries such as those in the European Union area, there are considerable deficiencies in compliance with eye examinations for diabetic patients.

With this in mind, the European "Screening for DR in Europe" group revised the 1990 St Vincent Declaration^[7]. A large group of ophthalmologists and endocrinologists from 29 European countries attended a number of meetings between 2005 and 2011, which revealed a series of difficulties in applying screening recommendations. Such difficulties were identified as a paucity of information supplied to the public regarding screening visits, a shortage of teams and training programs, and insufficient collaboration among general practitioners, endocrinologists and ophthalmologists. In view of this data, it was decided to implement systematic screening programs designed to reach at least 80% of diabetics by using staff and professionals specially trained for this purpose.

When deciding to implement a system of DR screening, we need to consider what type of healthcare professional should be responsible for controlling patients with DM. In the majority of countries this is the family doctor, with control by endocrinologists being restricted to patients with very poor metabolic control of the DM.

The family doctor was, therefore, the professional who it was thought should be able to ensure collaboration with DR screening, even though there was some reticence among some sectors, especially ophthalmologists and optometrists. The insufficient number of ophthalmologists for such large populations such as diabetics, combined with different studies on the effectiveness of screening by general practitioners^[8-10], led the different working groups to decide that the family doctor needs to be involved in the DR screening programs provided they are

experts in the analysis of retinal photographs and have the support of an ophthalmologist who can supervise them, without this implying non-performance of complete eye examinations^[11]. In the United Kingdom, a country where screening has been more widely developed, general practitioners are included in the programs and different professionals are involved in assessment (general practitioners or optometrists)^[2,12]. Furthermore, in the authors' healthcare areas, the general practitioner plays an important role in DR screening^[13,14]. It is therefore essential to impart the necessary training to these professionals so that they can detect the presence of an incipient retinopathy and establish contact with reference ophthalmologists^[14,15] so that the latter can then provide the required support. If more advanced forms of retinopathy are detected, they would be able to refer the patient for treatment as quickly as possible.

Telemedicine is clearly of great use to this system of DR screening making it possible to send images and information for a correct diagnosis, and disturbing diabetic patients as little as possible.

In the authors' experience, including general practitioners and ensuring they are appropriately supervised by ophthalmologists who are experts in DR, has enabled the screening of a large number of diabetics^[16,17]. Since 2007, from an estimated population of 17792 diabetics, it has been possible to screen 15396 patients (86.53%), with 3.18 ± 1 visits during these 7 years. The scheme involves firstly training general practitioners, who would then be responsible for analyzing the retinal photographs of diabetics in their area. In the event of any suspicion of the presence of signs suggesting DR, the reference ophthalmologist would be consulted, and he/she then makes the final diagnosis and decides how to manage the patient. This procedure has led to the detection of an annual incidence of between 8.06% and 8.92% of patients with DR, with the incidence of patients with diabetic macular edema being between 2% and 2.8% per year. It is also important to note that between 9.2% and 10.3% of other pathologies have been detected each year, including macular degeneration associated with age, pathological myopia and the presence of pigmented lesions such as nevi. In spite of the efforts we have made in our area, only between 32.40% and 41.16% of diabetic patients undergo screening for DR every year. Part of the problem is that screening is opportunistic rather than systematic, and poor awareness among the population.

With the current world economic crisis and the explosion of the prevalence of DM we are witnessing, we should strive to ensure that screening programs are more sustainable. Measures that should be considered include: (1) Extending screening intervals for patients who do not apparently have DR and have good metabolic control (biannual is sufficient). This has been studied by some groups and proven to be feasible; (2) Developing a diagnosis aid system by implementing the design of clinical decisions support system software to enable risk

factors to be considered when scheduling successive screening tests; and (3) Automatic reading of retinal photographs. Considerable advances have been made in this field, although these systems are currently very sensitive but not specific.

CONCLUSION

Telemedicine is a field that is extremely useful in ophthalmology and which has enormous potential, even though it is currently under-used and often limited to merely transmitting images and information about very specific pathologies such as DR or retinopathy in premature babies but has potential for many more eye diseases. Problems in data-processing systems can be solved and do not need to be an obstacle, but the lack of government regulation in many countries makes it difficult to apply in the vast majority of cases. Guidelines for government regulation are essential if communication among professionals is to increase, which can only lead to improving public health.

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Respiratory failure in diabetic ketoacidosis

Nikifor K Konstantinov, Mark Rohrscheib, Emmanuel I Agaba, Richard I Dorin, Glen H Murata, Antonios H Tzamaloukas

Nikifor K Konstantinov, University of New Mexico School of Medicine, Albuquerque, NM 87122, United States

Mark Rohrscheib, Division of Nephrology, Department of Medicine, University of New Mexico School of Medicine, Albuquerque, NM 87122, United States

Emmanuel I Agaba, Division of Nephrology, Department of Medicine, University of Jos Medical School, Jos, Plateau State 930001, Nigeria

Richard I Dorin, Section of Endocrinology, Medicine Service, Raymond G. Murphy Veterans Affairs Medical Center, Albuquerque, NM 78108, United States

Glen H Murata, Section of Informatics, Medicine Service, Raymond G. Murphy Veterans Affairs Medical Center, Albuquerque, NM 78108, United States

Antonios H Tzamaloukas, Section of Nephrology, Medicine Service, Raymond G. Murphy Veterans Affairs Medical Center, Albuquerque, NM 78108, United States

Richard I Dorin, Glen H Murata, Antonios H Tzamaloukas, Department of Medicine, University of New Mexico School of Medicine, Albuquerque, NM 87108, United States

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Correspondence to: Antonios H Tzamaloukas, MD, MACP, Section of Nephrology, Medicine Service (111C), Raymond G. Murphy Veterans Affairs Medical Center, 1501 San Pedro, SE, Albuquerque, NM 87108 United States. antonios.tzamaloukas@va.gov
 Telephone: +1-505-2651711
 Fax: +1-505-2566443

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Abstract

Respiratory failure complicating the course of diabetic ketoacidosis (DKA) is a source of increased morbidity and mortality. Detection of respiratory failure in DKA requires focused clinical monitoring, careful interpretation of arterial blood gases, and investigation for conditions that can affect adversely the respiration. Conditions that compromise respiratory function caused by DKA can be detected at presentation but are usually more prevalent during treatment. These conditions include deficits of potassium, magnesium and phosphate and hydrostatic or non-hydrostatic pulmonary edema. Conditions not caused by DKA that can worsen respiratory function under the added stress of DKA include infections of the respiratory system, pre-existing respiratory or neuromuscular disease and miscellaneous other conditions. Prompt recognition and management of the conditions that can lead to respiratory failure in DKA may prevent respiratory failure and improve mortality from DKA.

Key words: Diabetic ketoacidosis; Respiratory failure; Hypokalemia; Hypomagnesemia; Hypophosphatemia; Pulmonary edema; Adult respiratory distress syndrome; Pneumonia; Neuromuscular disease

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Core tip: Despite progress in its management, diabetic ketoacidosis (DKA) continues to cause significant morbidity and mortality. One of the conditions aggravating the course of DKA and causing several deaths is respiratory failure, which can be detected at presentation or, more frequently during the course of treatment of DKA. Several risk factors for respiratory failure in DKA are preventable. Early recognition and management of these risk factors, as well as early recognition of respiratory failure have the potential to improve both morbidity and mortality resulting from DKA.

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INTRODUCTION

Ketoacidosis in subjects with type 1, or less frequently, type 2 diabetes mellitus remains a potentially life-threatening diabetic manifestation. The subject has justifiably attracted attention in the literature. Sequential reviews^[1-9] have documented important changes in the clinical concepts that are related to diabetic ketoacidosis (DKA) and its management. A large number of case series of DKA have addressed various aspects of its clinical presentation and management. For this review, we selected representative studies focused on management, outcome, age differences, gender differences, associated morbid conditions, ethnicity and prominent clinical and laboratory features^[10-35].

In recognition of the complexity of treatment, the recommendation to provide this care in intensive care units was made more than 50 years ago^[36]. Severe DKA is treated in intensive care units today^[31]. Evidence-based guidelines for the diagnosis and management of DKA have been published and frequently revised in North America^[37,38] and Europe^[39]. Losses of fluids and electrolytes, which are important causes of morbidity and mortality in DKA, vary greatly between patients. Quantitative methods estimating individual losses and guiding their replacement have also been reported^[40,41].

The outcomes of DKA have improved with new methods of insulin administration^[42] and adherence to guidelines^[43-46]. The aim of treatment is to minimize mortality and prevent sequelae. One study documented that the target of zero mortality is feasible^[42]. However, mortality from DKA, although reduced progressively in the early decades after the employment of insulin treatment^[1], remains high. Up to fifty plus years ago, mortality from DKA was between 3% and 10%^[1,16]. A recent review reported a death rate from hyperglycemic

crises of 7.5% in the United States, with greater mortality from hyperglycemic hyperosmolar state (HHS) than from DKA^[9]. Reported mortality from DKA varies among age groups and countries. In various academic medical centers, death rate from DKA was < 1%^[26,27] and < 2% among adult patients younger than 65 years^[24] in the United States, 0.4% in Japanese children without and 4.7% with coma^[23], 6.5% in adult Mexican patients^[25], 4.1% in adult Israeli patients^[34] without differences between men and women^[29], 5.8% in adult Thai patients^[30], 3.6% in adult Nigerian patients^[32], around 13% in Indian children^[33,47], and 22% in American patients older than 65 years^[24]. In an autopsy study, DKA was identified as a major cause of death in diabetic patients^[48].

One general observation impacting the outcome of DKA is that its management is not always optimal. Several reports documented varying degrees of non-adherence to guidelines^[49-51], despite their proven effectiveness. The last of these reports also documented an increasing prevalence of DKA^[51]. In addition to adherence to guidelines, efforts to reduce mortality from DKA should focus on individual causes of death. Causes of death in DKA were analyzed in several studies^[23,25,27,30-34,52-55]. Cerebral edema and sepsis were the two most common causes. In a study from Greece reporting a 12.9% death rate, multivariate analysis identified the following as predictors of mortality from DKA: co-morbidities, severe acidemia at presentation (arterial blood pH < 7.0), high dose of insulin and persistence of hyperglycemia, and the development of coma or fever during treatment^[54]. Another study from Indonesia reporting 40% death rate identified coma plus high serum lactate levels (> 4 mmol/L) as poor prognostic factors^[55].

In this review, we analyzed the causes, mechanisms, management and prevention of respiratory failure which is one of the causes of death in DKA. Respiratory or cardiorespiratory deaths were reported in several series of DKA^[25,27,31,34,47]. Respiratory failure may either be recognized at presentation or, more frequently, develop during the course of treatment of DKA. The main purpose of the review is to underline the diagnosis, pathogenesis, management and, in particular, prevention of respiratory failure in DKA through proper management.

DIAGNOSIS OF RESPIRATORY FAILURE IN DKA

The key features establishing the diagnosis of DKA are the presence of metabolic acidosis and large amounts of ketones (acetone, acetoacetic acid, beta-hydroxybutyric acid) in serum and urine. Hyperglycemia may be absent in some patients complicating the diagnosis of DKA^[56]. DKA should be differentiated from other conditions producing increased ketone formation including alcoholic ketoacidosis and starvation ketosis. Lactic acidosis from sepsis or hypovolemia is another type of metabolic acidosis which occurs frequently during the course of

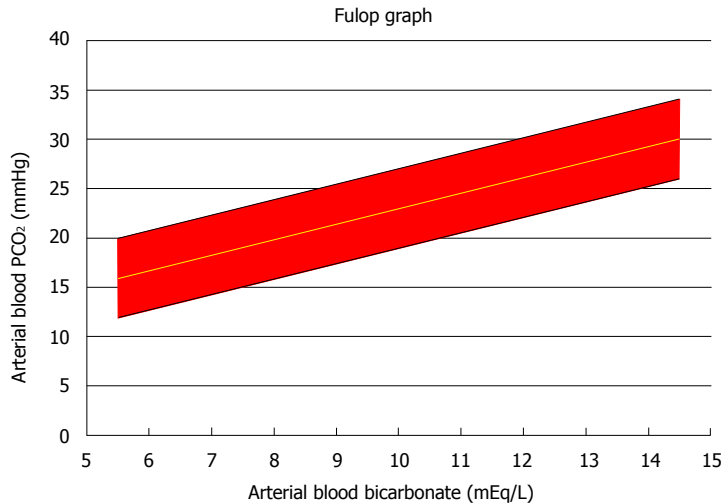


Figure 1 Arterial PCO₂ response to serum bicarbonate concentration in diabetic ketoacidosis. The draft, which was drawn using Fulop's regression equation^[60], shows the mean and 95%CI of this response.

both DKA and HHS that should be differentiated from DKA. Serum lactate level should be measured in all patients with hyperglycemia and metabolic acidosis.

Assessment of the severity of DKA is primarily based on the degree of the acid-base disturbance. The most recent version of the United States guidelines for hyperglycemic crises in adults^[38] set three levels of DKA severity: mild, moderate and severe. Criteria common to all three categories included plasma glucose > 250 mg/dL, positive serum and urine ketone test, and variable levels of effective serum osmolality, calculated as $[2 \times (\text{serum sodium}) + (\text{serum glucose.mg/dL})/18]$. The criteria that differed between the three categories were arterial blood pH (mild: 7.25-7.30, moderate: 7.00 to < 7.25, severe: < 7.00), plasma bicarbonate ($[\text{HCO}_3^-]_p$, mild: 15-18 mEq/L, moderate: 10 to < 15 mEq/L, severe: < 10 mEq/L), serum anion gap calculated as $[\text{serum sodium} - (\text{serum chloride} + \text{serum bicarbonate})]$ (mild: > 10 mEq/L, moderate: > 12 mEq/L, severe: > 12 mEq/L) and mental status (mild: alert, moderate: alert/drowsy, severe: stupor/coma). The European guidelines for DKA in children^[39] classify the severity of DKA only by the magnitude of metabolic acidosis (mild: venous pH < 7.30, $[\text{HCO}_3^-]_p$: < 15 mEq/L. Moderate: pH < 7.20, $[\text{HCO}_3^-]_p$: 10 mEq/L. Severe: pH < 7.10, $[\text{HCO}_3^-]_p$: < 5 mEq/L).

In addition to its use in the diagnosis of DKA and establishing its severity, blood gas analysis provides two objective criteria for assessing the presence and severity of respiratory failure complicating DKA. The universal feature of respiratory failure is hypoxemia. Arterial blood PO₂ (PaO₂) at room air should be evaluated in all patients presenting with DKA. The second parameter of arterial blood gases that allows detection of respiratory failure in DKA is the arterial PCO₂ (PaCO₂). The application of PaCO₂ in the detection of respiratory failure complicating DKA merits some discussion.

It has been known for a long time that there is a predictable alveolar ventilatory compensation, within

narrow limits, to a given degree of metabolic acidosis^[57-59]. In its simplest form, this compensation is expressed as PaCO₂ as a function of $[\text{HCO}_3^-]_p$. The ventilatory response to metabolic acidosis was analyzed by Fulop^[60] in a series of 27 episodes of DKA uncomplicated by lactic acidosis or other acid-base disturbances. Fulop derived the following regression equation, which is almost identical to the original Winters equation^[57] that was derived from several types of metabolic acidosis: $\text{PaCO}_2, \text{ mmHg} = 7.27 + 1.57 \times ([\text{HCO}_3^-]_p, \text{ mEq/L})$

Figure 1 shows the 95% confidence area defined by Fulop's study. Fulop's diagram should be used to evaluate every case of DKA. This diagram may assist in the detection of associated other primary acid-base disorders, for example metabolic alkalosis from vomiting^[61]. In addition, its use is critical for the detection of respiratory abnormalities accompanying the DKA. Fulop's diagram allows detection of an associated primary respiratory alkalosis. In this instance, the measured PaCO₂ is below the corresponding low 95% confidence limit in the Fulop diagram. Primary respiratory alkalosis is not rare in DKA^[62,63]. Detection of primary respiratory alkalosis in a patient with DKA has great importance because it often provides a clue for the presence of sepsis^[62] which is the underlying cause of DKA in many instances^[37-39], respiratory distress secondary to cerebral edema, and other causes of respiratory alkalosis.

The second critical use of Fulop's diagram is in the detection of respiratory failure complicating DKA. In this case the PaCO₂ value is higher than the corresponding upper limit for PaCO₂ in the Fulop diagram. Such a finding should lead to a systematic search for potential causes of respiratory failure and frequent monitoring of the respiratory status of the patient including arterial blood gases.

Detection of respiratory failure in DKA is not based only on PaO₂ and PaCO₂ values. A diligent search for coexisting conditions adversely affecting the respiratory function has also great importance. Respiratory failure is

Table 1 Risk factors for respiratory failure in diabetic ketoacidosis

Depletion of primarily intracellular ions
Potassium
Magnesium
Phosphate
Pulmonary edema
Hydrostatic (cardiogenic)
Non-hydrostatic (adult respiratory distress syndrome)
Respiratory tract infections
Pneumonia
Infections of the airways
Miscellaneous conditions
Neuromuscular disease
Non-infectious diseases of the respiratory tract
Other

more often encountered during treatment of DKA than at its presentation, as will be shown later. Consequently, vigilance for any clinical or laboratory clue suggesting development of respiratory failure should be maintained throughout the treatment of DKA.

One potential pitfall of Fulop's equation is that it may not compute the respiratory response to profound degrees of DKA with accuracy. Soubani *et al.*^[64] suggested that there are limits for hyperventilation resulting from metabolic acidosis or sepsis. Subsequently, Guh *et al.*^[65] derived different regression equations of PaCO_2 on $[\text{HCO}_3^-]_p$ in DKA patients with arterial pH > 7.10 ($\text{PaCO}_2 = 6.6 + 1.65 \times [\text{HCO}_3^-]_p$) and those with arterial pH \leq 7.10 ($\text{PaCO}_2 = 2.88 + 3.18 \times [\text{HCO}_3^-]_p$). The regression equation for DKA with moderate acidemia was similar to Fulop's equation. The Guh equation for severe acidemia appears to differ from Fulop's equation. Indeed, there are substantial differences between the two equations for $[\text{HCO}_3^-]_p$ values that are not extremely low. For example, for $[\text{HCO}_3^-]_p$ equal to 10 mEq/L, Fulop's equation calculates an appropriate PaCO_2 of 23 mmHg (95%CI: 23-27 mmHg). From the Henderson-Hasselbach equation, the resulting arterial pH value is 7.24 (95%CI: 7.19-7.34). The corresponding values obtained from the Guh equation for severe acidemia are PaCO_2 34.0 (95%CI: 30.6-37.4) mmHg, and arterial pH 7.09 (95%CI: 7.05-7.14). However, the differences between the two equations are trivial in cases of profound acidosis. For example, we used the same equations to calculate the ventilatory responses and arterial pH values for $[\text{HCO}_3^-]_p$ equal to 3 mEq/L: from Fulop's equation, the values were PaCO_2 12.0 (95%CI: 8-16) mmHg; arterial pH 7.02 (95%CI: 6.89-7.19); from Guh's equation, the values were PaCO_2 12.4 (96%CI: 9.0-15.8) mmHg; arterial pH 7.01 (95%CI: 6.90-7.14). We suggest that Fulop's diagram is appropriate for evaluating the alveolar ventilation in profound DKA. In addition, vigilance for other clues of potential respiratory failure (hypoxemia, history of pulmonary or neuromuscular disease, clinical monitoring of the respiratory system) should be enhanced in this instance.

Metabolic acid-base parameters (pH, plasma bicar-

bonate concentration) were found to be comparable between venous and arterial blood samples in uncomplicated cases of DKA^[66-68]. However, venous blood gas determination is not appropriate for the detection of respiratory failure in the course of DKA^[66] for two reasons: The first reason is that venous blood measurements cannot detect abnormalities in the partial pressure of oxygen caused by ventilatory failure, especially in states of hypotension and tissue hypoperfusion. The second reason is that the regression equations used for detection of inadequate ventilatory response to DKA were developed in arterial blood. Transcutaneous monitoring of carbon dioxide has been proposed as a means of monitoring the treatment of DKA^[69]. Determining the accuracy of this technique in identifying respiratory failure in DKA will require further research.

The diagnosis of respiratory failure during the course of DKA can be greatly facilitated by a systematic search for risk factors that have been identified to specifically complicate the course of DKA. Clinicians should also be alert for pre-existing respiratory insufficiency and for conditions that can cause respiratory failure independently of DKA (e.g., severe hypothyroidism). The next section addresses risk factors for respiratory insufficiency associated with DKA. Detailed discussion of all causes of respiratory failure is beyond the scope of this review.

RISK FACTORS FOR RESPIRATORY FAILURE IN DKA

Table 1 shows these factors. The first two categories listed in this table (depletion of primarily intracellular ions and development of pulmonary edema) are direct consequences of hyperglycemia and DKA. The last two categories (infections of the respiratory tract and miscellaneous risk factors) include conditions that may lead to DKA but are not caused by it.

Deficits of ions with primary intracellular distribution in DKA

Potassium, magnesium and phosphate are ions with primary intracellular distribution that are depleted as a consequence of DKA. Depletion of these ions has severe, but preventable, clinical consequences. Clinical manifestations relevant to this report are muscle weakness that can culminate in respiratory failure and cardiac dysrhythmias that may affect myocardial function. If appropriate replacement is not done, the depletion of these ions is more frequent and profound during treatment than at presentation with DKA^[70]. A major aim of the treatment of DKA is to address the deficits of these ions. The mechanisms of deficit and their clinical consequences are discussed below. The mechanisms of potassium deficiency and of changes in serum potassium concentration in DKA will be discussed in some detail. Similar mechanisms create the abnormalities in the other two ions.

Potassium: There are disturbances in internal and external potassium balance in DKA. During development of DKA, the internal imbalance is caused by movement of potassium from the intracellular into the extracellular compartment causing hyperkalemia. The external imbalance is attributed to the fact that DKA causes losses of body potassium causing hypokalemia. The losses can be profound and are usually accentuated during treatment. In Martin's report^[70], hyperkalemia was present in 39% of the DKA cases at presentation and in 4% of the cases after 12 h of treatment, while hypokalemia was present in 18% of the DKA cases at presentation and 63% of the cases after 12 h of treatment. The incidence of hypokalemia at presentation of DKA may be affected by factors independent of DKA, such as previous gastrointestinal loss of potassium or diuretic use^[71]. In published reports of DKA, the frequency of hypokalemia at presentation varied between 0^[17] and 36.7%^[13]. A recent study found hypokalemia at presentation in 5% of patients with DKA^[72].

Balance studies during development or treatment of DKA documented the abnormalities in external and internal potassium balance caused by DKA^[73-79]. Potassium is lost in the urine during development of DKA because of osmotic diuresis caused by glycosuria. Urinary excretion of ketoacids obviates the loss in the urine of equivalent amounts of cations, particularly sodium and potassium. The contribution of ketonuria to the urinary potassium loss has not been studied in DKA, to our knowledge. Nevertheless, the loss of potassium in DKA is often large. Patients with previously undiagnosed type 1 diabetes who present with DKA after protracted polyuria may have life threatening potassium deficits^[41].

Despite the urine losses, large numbers of patients exhibit hyperkalemia at presentation with DKA^[70,80,81], because of transfer of potassium from the intracellular into the extracellular compartment. The mechanisms of this transfer have been studied extensively. The most important underlying mechanism is absence of insulin action, which has direct and indirect effects on internal potassium balance. Directly, inhibition of basal insulin secretion causes loss of intracellular potassium^[82] and hyperkalemia. Insulin causes hyperpolarization of the cell membranes and potassium entry into the cytoplasm^[83] through an increase in the sites of the alpha-2 subunit of the sodium-potassium ATPase of the cell membranes^[84]. The effect of insulin on cellular potassium uptake is dissociated from that on cellular glucose uptake^[85].

Indirectly, absence of insulin action causes hypertonicity (elevated serum effective osmolality) through both extracellular accumulation of solute (glucose) and osmotic diuresis, which causes loss of water in excess of monovalent cations^[41]. Hypertonicity leads to transfer of water and intracellular solutes, particularly potassium, into the extracellular compartment^[86]. Hyperkalemia will result in this case even if the state of hypertonicity has no effect on the transport mechanisms of cell membranes for potassium or on the electrical potential difference across the cell membrane^[87]. Contraction of the

extracellular volume as a result of osmotic diuresis tends to concentrate extracellular solutes including potassium and constitutes another source of hyperkalemia in DKA^[41].

The hyperkalemic effects of the disrupted internal potassium balance described so far are encountered in DKA and all other hyperglycemic syndromes. The question whether metabolic acidosis has additional hyperkalemic effects in DKA has been a matter of controversy^[88-90]. Two lines of research provide support for an added hyperkalemic effect of acidosis in DKA: Multivariate analysis in clinical studies identified arterial pH as a predictor of serum potassium level, in addition to serum glucose^[91,92]. The other line of evidence is the recent discovery that acidosis affects potassium distribution across cell membranes though alterations in cellular membrane transporters^[93].

For patients on maintenance dialysis, the hyperglycemic effects on internal potassium balance are almost completely unopposed because of absent or minimal osmotic diuresis. Studies of hyperglycemic syndromes in this group have provided support for an additional hyperkalemic effect of DKA when serum glucose concentration and effective osmolality are comparable between DKA and HHS^[94-99]. Finally, we are unaware of studies showing that the catabolic state induced by acidosis causes the release of cellular potassium into the extracellular compartment and contributes to the hyperkalemia. Nevertheless, there is sufficient evidence to support the concept of an independent hyperkalemic effect of DKA that is added to the other hyperkalemic effects of hyperglycemia. This further complicates the evaluation of potassium deficits in DKA.

Treatment of DKA leads to substantial declines in serum potassium concentration, even when large amounts of potassium are infused^[100-107]. Multiple mechanisms contribute to the hypokalemic effect of treatment. These include a direct effect of insulin on cellular potassium uptake^[83], correction of the hyperglycemic hypertonicity^[96], dilution of extracellular potassium due to infusion of large volumes of fluids and continuing losses of potassium through the urine or the gastrointestinal tract.

Urinary potassium losses during treatment of DKA merit attention because they often do not constitute a treatment focus as they should. Urinary losses of potassium in DKA are accentuated by coexistent states of hyperaldosteronism^[108]. Insulin has an effect similar to aldosterone on renal transport mechanisms of sodium and potassium and its administration to patients with DKA causes excessive renal potassium losses^[109]. The other mechanism of excessive potassium losses during treatment of DKA is ongoing osmotic diuresis while serum glucose remains elevated^[41]. Improvement of the renal circulation as fluid deficits are corrected has the potential of worsening potassium losses through osmotic diuresis.

Clinical consequences of hypokalemia associated with DKA were reported first in 1946 in a seminal paper

by Holler^[110] who observed a patient who developed hypokalemia and respiratory failure during treatment of DKA and whose respiratory failure resolved after infusion of potassium salts. The significance of Holler's report was stressed in a more recent report^[111]. Subsequently, a series of articles reported severe clinical manifestations secondary to hypokalemia developing or worsening during treatment of DKA^[112-135]. The majority of the reported cases exhibited varying degree of respiratory failure. In several patients, respiratory failure was associated with severe cardiac manifestations and/or profound and generalized muscle weakness. Death occurred in some cases^[122,129].

The management of DKA should adhere to guidelines that recommend the administration of intravenous potassium salts to patients presenting with DKA and hypokalemia and initiation of insulin infusion only after serum potassium has reached values > 3.3 mEq/L^[38]. Serum potassium concentration should be monitored during treatment in all patients with DKA. In DKA patients presenting with hypotension, extreme hyperglycemia and hypokalemia, urine volume and urine potassium concentration should also be monitored during treatment in order to guide, along with serum potassium, changes in the rate of infusion of potassium salts^[41]. Measuring potassium levels with the apparatus used for blood gas determination is not appropriate because these levels may vary substantially from simultaneous serum potassium determinations^[136]. Finally, electrocardiographic changes may indicate changes in serum potassium during the course of DKA^[137,138]. Monitoring of electrocardiogram to prevent inappropriate administration of potassium salts to patients with DKA has been proposed^[137]. However, dissociation of plasma potassium concentration and electrocardiographic abnormalities in a patient on DKA has been reported^[139]. Monitoring of serum potassium during the course of treatment of DKA should be primarily based on frequent determinations of serum potassium concentration. Electrocardiographic monitoring should be used as a guide for management of life-threatening hyperkalemia and for timely detection of dysrhythmias complicating the treatment of DKA.

Magnesium: In DKA body magnesium deficits through urinary losses are routinely encountered and are the consequence of absence of insulin^[140]. However, magnesium exit from the cells may cause hypermagnesemia, which is frequent at presentation with DKA. The magnesium defect is unmasked during treatment. In Martin's study^[70], hypomagnesemia was recorded in 7% of the cases at presentation with DKA and 55% of the cases after 12 h of treatment, while hypermagnesemia was found in 68% of the cases at presentation and 21% of the cases after 12 h of treatment.

In one reported case, profound hypomagnesemia caused respiratory failure and asystole, and cardiac function recovered after cardiopulmonary resuscitation and infusion of a large bolus of magnesium salts^[141]. A small number of patients with DKA and severe hypoma-

gnesemia were subsequently reported^[142-144]. The development of hypomagnesemia during treatment of DKA was linked to infusion of potassium phosphate^[142,144]. Aldosterone promotes urinary magnesium losses^[145]. Magnesium loss in the urine during treatment of DKA may be increased because of the state of secondary hyperaldosteronism discussed in the subsection on potassium.

An important consequence of magnesium deficiency is that it causes excessive urinary losses of potassium and phosphate^[146]. It is difficult to replete potassium stores when there are large magnesium deficits^[147]. In addition to its direct and indirect effects on respiration, magnesium deficits have major effects on both cardiac contractility and rhythm. Insulin, along with its effects on cellular uptake of potassium and nutrients, increases intracellular free magnesium concentration in myocardial cells^[148].

Magnesium deficit should be anticipated in patients with DKA. Serum magnesium concentration should be measured at presentation with DKA and should be monitored during treatment. Magnesium replacement should be guided by serum magnesium levels in this state.

Phosphate: Changes induced by DKA on both external and internal phosphate balances are similar to those of the balances of potassium and magnesium. Hyperglycemic osmotic diuresis causes urinary losses of phosphate, while metabolic acidosis causes shifts of phosphate from the intracellular into the extracellular compartment^[149]. Insulin causes cellular phosphate uptake and a decrease in serum phosphate concentration^[150,151]. The insulin-mediated decrease in serum phosphate concentration may be accentuated by dilution through intravenous replacement fluids and by continuing urinary losses. In Martin's study^[70], hypophosphatemia was found in 11% of the cases at presentation with DKA and 71% of the cases after 12 h of treatment, while hyperphosphatemia was detected in 90% of the cases at presentation and was not detected after 12 h of treatment.

Severe hypophosphatemia has multiple adverse consequences^[149]. Oxygen delivery to peripheral tissues is impaired by the depletion of red cell 2, 3 diphosphoglycerate (2,3-DPG), which causes a shift of the oxygen dissociation curve to the left thus impeding oxygen release. Depletion of high-energy phosphate compounds in muscles secondary to phosphate deficits causes muscle weakness and rhabdomyolysis, dysrhythmias, myocardial dysfunction and seizures^[149,152,153].

A number of cases of development of severe hypophosphatemia with varying degrees of respiratory failure during the treatment of DKA have been reported^[154-161]. Rhabdomyolysis was present in one patient^[161]. A recent report found that the severity of metabolic acidosis at presentation affects the degree of hypophosphatemia during treatment of DKA^[162]. Monitoring of serum phosphate should guide the replacement of phosphate deficit. Phosphate replacement was shown to be

effective in preventing the development of severe hypophosphatemia in this instance^[163,164]. However, phosphate infusion has not been shown to improve the outcome of DKA in prospective studies^[38] and may have adverse consequences including hypocalcemia and hypomagnesemia^[142,143]. Phosphate should be replaced during treatment of DKA only if serum phosphate levels are low. The guidelines suggest rates of infusion of potassium phosphate and other ions^[38]. The critical measure during treatment of DKA consists of close monitoring of the patient's clinical status and all serum components that are replaced^[41].

Pulmonary edema secondary to DKA or its treatment

The second category of direct consequences of DKA is the development of pulmonary edema. Arterial blood gases are necessary for evaluation of its severity and to guide its treatment. Oxygen administration is guided by the degree of hypoxemia, which is universal in patients with pulmonary edema^[165]. Abnormalities of PaCO₂ accompany the hypoxemia in the majority of the cases^[166]. Respiratory alkalosis, triggered by the hypoxemia, is frequent in pulmonary edema. Eucapnia and respiratory acidosis are also present in substantial numbers of patients with acute pulmonary edema^[165]. "Normal" or elevated values of PaCO₂ in patients with pulmonary edema indicate inadequate respiratory response to hypoxemia and should be considered as indicators of the severity of this condition. Elevated PaCO₂ levels have been reported in a small number of patients with end-stage renal disease and extreme hyperglycemia without DKA^[63]. Two varieties of pulmonary edema in DKA have been recognized, a hydrostatic form attributed to elevated pulmonary venous pressure and a form that develops because of increased pulmonary capillary permeability.

Hydrostatic pulmonary edema in DKA: Hydrostatic pulmonary edema is usually diagnosed at presentation with DKA or severe hyperglycemia without DKA and is corrected during the treatment of these syndromes. The sequence of pulmonary edema at presentation with severe hyperglycemia with or without DKA and its correction with insulin administration has been reported in patients with advanced renal failure^[166-170]. Development of circulatory overload and hydrostatic pulmonary edema in these patients was initially attributed to the acute shift of a substantial volume of fluid from the intracellular into the extracellular compartment. This volume shift is an osmotic consequence of solute accumulation in the extracellular compartment during development of hyperglycemia. Correction of hyperglycemia with insulin administration shifts fluid back into cells^[166].

The magnitude of osmotic translocation of fluid between the two major body fluid compartments that is secondary to hyperglycemia should affect the severity of the ensuing circulatory overload. This magnitude is affected by two main factors: The first and most obvious factor is degree of hyperglycemia. The volume of the

osmotic fluid transfer increases as the serum glucose level increases in the same episode of hyperglycemia. The second factor affecting the volume of fluid transferred from the intracellular into the extracellular compartment during development of hyperglycemia is the baseline status of the extracellular volume. For the same degree of hyperglycemia, patients with preexisting peripheral edema develop larger osmotic fluid transfers than those without edema and the same baseline intracellular volume^[171,172]. Insulin administration without any other therapeutic measures has led to correction of the pulmonary edema in the reported cases^[166-170]. However, other measures, including mechanical ventilation and emergency ultrafiltration may be required in some patients.

The development of extracellular volume expansion may not be the only cause of hydrostatic pulmonary edema in DKA. This syndrome has been reported in DKA patients without advanced renal failure, who usually have volume deficits at presentation^[173,174]. This suggests that the development of DKA may be due to factors other than extracellular volume expansion in some cases. In one instance, DKA was diagnosed during treatment of high altitude pulmonary edema^[173]. Recovery required treatment of both conditions. It is not clear which condition appeared first.

In another case, hydrostatic pulmonary edema developed during treatment of DKA in a 9-year-old child^[175]. Serum troponin levels were elevated and echocardiography showed segmental myocardial dysfunction when pulmonary edema was diagnosed. Repeated cardiac echocardiography was normal 6 d later. This case report illustrates the potential of DKA to cause acutely myocardial dysfunction. This dysfunction could be secondary to excessive fluid replacement. Another cause of myocardial dysfunction in DKA is absence of insulin. Insulin has inotropic effects in subjects with type 1 diabetes^[175], subjects with type 2 diabetes^[176] and normal controls^[176]. It is unclear whether the resolution of pulmonary edema at presentation results from a correction of the extracellular volume excess or a direct action of insulin on myocardial contractility.

Non-hydrostatic pulmonary edema in DKA:

Diabetes mellitus may affect the structure and function of the lungs, in addition to other target organs. Histological changes in the lungs of diabetic patients involve the wall of the alveoli and the pulmonary capillaries, while the most consistent functional changes include reduced lung volumes, reduced pulmonary elastic recoil, and reduced capillary lung volume leading to impaired diffusion capacity^[177]. Respiratory function in these patients is apparently preserved under normal conditions, but their lung reserves are reduced and can cause clinical lung dysfunction under stressful conditions including volume overload^[178]. The development of non-hydrostatic pulmonary edema [adult respiratory distress syndrome (ARDS)] in DKA may be related to the effects of stress on diabetic lungs.

Characteristically, ARDS is not present initially, but develops during the course of treatment of DKA. ARDS appears to be a more frequent and severe complication of DKA than hydrostatic pulmonary edema. A number of publications reported patients who developed ARDS during treatment of DKA^[179-192]. ARDS developing during treatment of DKA may lead to death^[192]. The severity of ARDS complicating the treatment of DKA is underlined by its association with cerebral edema.

DKA is one of the major causes of cerebral edema^[193]. Cerebral edema usually develops during treatment of DKA^[194] and is a major cause of mortality and long-term neurological sequelae^[195]. Simultaneous development of cerebral edema and ARDS has been reported in several publications^[28,196-203]. Research efforts have addressed factors that affect fluid transfers across blood capillary membranes of the brain and lungs during treatment of DKA. Early studies focused on Starling forces controlling fluid exchanges across capillary membranes. Infusion of large volumes of crystalloid solutions leads to increase in the capillary hydrostatic pressure and to dilution of serum proteins and decrease in the colloid osmotic pressure of the serum. Decreased serum colloid osmotic pressure was identified as a risk factor for ARDS during treatment of DKA^[204-208].

Decreased serum colloid osmotic pressure may lead to increased fluid transfer from the intravascular into the interstitial space of various tissues including the lungs where it will cause respiratory distress. However, serum colloid osmotic pressure is not a key determinant of fluid transfers between interstitial fluid and intracellular compartment. Efforts to identify risk factors for the development of both ARDS and cerebral edema in DKA have been focused on altered capillary membrane permeability and changes in the serum effective osmolarity (tonicity). Increased pulmonary capillary permeability during treatment of ARDS was found in early reports^[209,210]. The potential explanations include activation of lymphocytes^[211] and release of cytokines, particularly interleukin-1 (IL-1)^[212-215], the serum levels of which are much higher during treatment of DKA than at its presentation. These findings have linked the development of cerebral edema and ARDS in patients with DKA.

A potent driver of fluid exchanges between the extracellular and intracellular compartments is the tonicity of the extracellular compartment, which changes during treatment of DKA. Current strategy for preventing cerebral edema and ARDS consists of careful infusion of crystalloid solutions. Care should be exercised when selecting their volume and tonicity^[41]. Replacement of volume deficits should be guided by the clinical picture: Deficits causing severe clinical manifestations should be replaced promptly with isotonic solutions. Monitoring of the clinical signs of hypovolemia (hypotension, tachycardia, low urine output) should guide the rate of infusion, which should be slowed down when these signs are corrected. Clinical monitoring is critical for adequate volume replacement and prevention of overshooting.

Hypertonicity is common in hyperglycemic syndromes and can be severe. Tonicity changes may play a role in the development of cerebral edema during treatment of DKA^[203]. Tonicity changes have, in general, a substantially larger effect on intracellular volume than extracellular volume changes. Unlike volume deficits hypertonicity should be corrected slowly during treatment of DKA. The guidelines for management of hyperglycemic crises recommend an hourly decline in serum glucose concentration between 50 and 65 mg/dL^[37], which corresponds to a decrease in serum effective osmolarity of between 1.2 and 2.8 mOsm/L^[41]. An hourly rate of decrease in effective osmolarity ≤ 3 mOsm/L during treatment of DKA is desirable. Whether measures addressing lymphocyte function and cytokine production can be effective in preventing cerebral edema and ARDS during treatment of DKA are topics for future research.

Respiratory tract infections in DKA

Infections are known to be a major category of conditions precipitating DKA. A systematic search for infections is warranted in all patients presenting with DKA. This search is complicated by the similarity between symptoms of infection and those of DKA (malaise, abdominal distress, dyspnea, etc.) and by finding elevated white blood cell counts in both conditions. In addition, infections in the respiratory tract have the potential of causing respiratory failure in patients with DKA. Examples of respiratory tract infections reported to cause respiratory failure include pneumonia secondary to *Streptococcus pneumoniae*^[134,216], *Legionella pneumoniae*^[217], *Klebsiella pneumoniae*^[218], community-acquired pneumonia^[219], influenza^[220], pulmonary zygomycosis^[221], mucormycosis^[222-225], candidiasis^[224] and coccidiomycosis^[226]. The list of respiratory tract infections that can cause respiratory failure during the course of DKA is, in all probability, much larger.

A complicating feature of DKA associated with lung infections is that severe volume deficits may mask the clinical and radiographic manifestations of pneumonia, which blossom after hydration^[134]. This scenario represents one condition in which evaluation of the respiratory compensation to DKA in the arterial blood gases can offer an early sign of the presence of a condition complicating the DKA with the potential of respiratory failure^[134].

Pneumonia associated with hyperglycemic symptoms is an independent predictor of short-term (28 d) mortality in both DKA and HHS^[227]. Early diagnosis and management of pulmonary infections associated with DKA offers the promise of reducing this mortality.

Miscellaneous conditions associated with DKA

DKA may develop in patients with other serious medical conditions. Regardless of whether these conditions had an etiologic relationship with DKA or not, DKA aggravates their course. For example, development of respiratory stress from DKA during the course of conditions potentially causing respiratory failure, such as pre-

existing neuromuscular or pulmonary disease, should intensify the monitoring of the respiratory function.

Preexisting neuromuscular disease: DKA with respiratory failure has been reported in patients with Guillain-Barré syndrome^[228,229] and one of the mitochondrial myopathies, the Kearns-Sayre syndrome^[230]. Mitochondrial myopathies are associated with a high incidence of diabetes mellitus. Kearns-Sayre syndrome is characterized by progressive external ophthalmoplegia and cardiac conduction defects as its primary clinical manifestations. Patients with this syndrome exhibit multiple endocrine disorders including diabetes mellitus requiring insulin in about 15% of the cases^[231]. This condition exemplifies the potential severity of DKA in the course of a neuromuscular disease. It is probable that patients with other types of mitochondrial syndromes causing myopathy develop DKA with respiratory failure. The reported case of DKA with respiratory failure in a patient with Kearns-Sayre syndrome had a fatal outcome^[230].

Preexisting conditions of the respiratory system:

We found reports of respiratory failure during the course of DKA in a patient with tracheal stenosis^[232] and another patient with central venous catheter thrombosis^[233]. DKA creates a severe stress on the respiratory function that has the potential to aggravate chronic lung disease. There is a paucity of studies addressing the effects of DKA on respiratory function in patients with chronic lung disease and on the outcomes of DKA in this setting.

Other associated conditions: The development of acute kidney injury during the course of DKA may place greater demands on respiratory function. Two reports addressed respiratory distress in patients with DKA and acute renal failure^[234,235]. As previously noted, there is a great need for studies of DKA in patients with conditions potentially affecting the respiratory function (*e.g.*, drugs, severe hypothyroidism, *etc.*).

CONCLUSION

Respiratory failure developing during the course of DKA worsens its prognosis and is preventable in most instances. Prevention of respiratory failure has the potential to reduce mortality from DKA. Prevention and early detection of respiratory failure, by close monitoring of the clinical status and timely use and appropriate interpretation of arterial blood gases, have the potential of both decreasing mortality from DKA and preventing the debilitating somatic and psychiatric sequelae of prolonged mechanical ventilation^[134].

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Gestational diabetes mellitus: Challenges for different ethnic groups

Lili Yuen, Vincent W Wong

Lili Yuen, Diabetes and Endocrine Service, Liverpool Hospital, Liverpool NSW 1871, Australia

Lili Yuen, Vincent W Wong, Liverpool Diabetes Collaborative Research Unit, Ingham Institute of Applied Science, Liverpool NSW 2170, Australia

Vincent W Wong, South Western Sydney Clinical School, University of New South Wales, Liverpool NSW 2170, Australia

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Correspondence to: Dr. Vincent W Wong, Liverpool Diabetes Collaborative Research Unit, Ingham Institute of Applied Science, 1 Campbell Street, Liverpool NSW 2170, Australia. vincent.wong@sswahs.nsw.gov.au
 Telephone: +61-2-87384577
 Fax: +61-2-87384539

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Abstract

Ethnicity is defined as "belonging to a social group

that has a common national or cultural tradition". Membership of certain ethnic groups has long been associated with increased risk of gestational diabetes mellitus (GDM). Studies that examined ethnic differences amongst women with GDM were often conducted in western countries where women from various ethnic backgrounds were represented. The prevalence of GDM appears to be particularly high among women from South Asia and South East Asia, compared to Caucasian, African-American and Hispanic communities. For some, but not all ethnic groups, the body mass index is a risk factor for the development of GDM. Even within a particular ethnic group, those who were born in their native countries have a different risk profile for GDM compared to those born in western countries. In terms of treatment, medical nutrition therapy (MNT) plays a key role in the management of GDM and the prescription of MNT should be culturally sensitive. Limited studies have shown that women who live in an English-speaking country but predominantly speak a language other than English, have lower rates of dietary understanding compared with their English speaking counterparts, and this may affect compliance to therapy. Insulin therapy also plays an important role and there appears to be variation as to the progression of women who progress to requiring insulin among different ethnicities. As for peri-natal outcomes, women from Pacific Islander countries have higher rates of macrosomia, while women from Chinese backgrounds had lower adverse pregnancy outcomes. From a maternal outcome point of view, pregnant women from Asia with GDM have a higher incidence of abnormal glucose tolerance test results post-partum and hence a higher risk of future development of type 2 diabetes mellitus. On the other hand, women from Hispanic or African-American backgrounds with GDM are more likely to develop hypertension post-partum. This review highlights the fact that management needs to be individualised and the clinician should be mindful of the impact that differences in ethnicity may have on the clinical characteristics and pregnancy outcomes in

women affected by GDM, particularly those living in Western countries. Understanding these differences is critical in the delivery of optimal antenatal care for women from diverse ethnic backgrounds.

Key words: Gestational diabetes mellitus; Ethnicity; Perinatal outcomes; Medical nutrition therapy; Prevalence

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Core tip: The prevalence of gestational diabetes mellitus (GDM) is increasing world-wide, and studies have shown that optimal management of GDM improves pregnancy outcomes. This review summarises the differences in prevalence, clinical profile, management and pregnancy outcomes among women from various ethnic backgrounds who have GDM. Ethnicity is an important consideration in women affected by GDM, particularly in an antenatal service based in a Western society. There are particular challenges in individualising and tailoring medical nutritional therapy and insulin therapy. Also women from certain ethnic groups are at a higher risk of increased foetal and maternal morbidity and mortality. Understanding these challenges is important in providing optimal antenatal care for women of diverse ethnic backgrounds.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first recognized during pregnancy^[1]. GDM has been reported to affect between 1.4% to 12.3% of pregnancies^[2], and its prevalence is increasing and parallels the rising incidence of type 2 diabetes mellitus worldwide^[3,4]. Risk factors for developing GDM in pregnancy include obesity, previously GDM, glycosuria, family history, ethnicity and hypertension^[5,6]. Arguably, one of the strongest non-modifiable risk factor for GDM relates to the woman's ethnicity.

The Oxford Dictionary defines ethnicity as "belonging to a social group that has a common national or cultural tradition"^[7]. In particular, ethnic groups that are considered high-risk include Hispanic, African-Americans, Native American, South or South East Asian, Pacific Islander or Indigenous Australian^[8]. It is also recognised that women with GDM from these and other ethnic groups may differ with regards to peri-natal and maternal outcomes^[9-12].

In this review we discuss the differences amongst women from various ethnic groups in terms of prev-

alence, diagnosis, treatment of GDM and pregnancy outcomes. Because of the variance in the diagnosis and management of GDM around the world, it is difficult to compare women with GDM between countries. In order to delineate ethnic differences in terms of GDM prevalence, metabolic profiles of the women and pregnancy and long-term outcomes, studies were often conducted in the same country (or under the same health care system) where the diagnostic criteria, screening process, treatment regimen and delivery of health care are uniform for all women^[13-16] (refer A Table 1).

DIAGNOSTIC CRITERIA

There are numerous diagnostic criteria for GDM currently being utilized in various parts of the world, as shown in Table 2. Many countries have based their GDM diagnostic criteria on the 1999 World Health Organisation (WHO) Criteria^[17], while in Australasia and the United States, they have adopted different glucose cut-offs to diagnose GDM based on the oral glucose tolerance test (OGTT)^[18,19]. Findings from the Hyperglycaemia and Adverse Pregnancy Outcomes study has put impetus on revising the diagnostic criteria for GDM, and the International Association of Diabetes in Pregnancy Study Group (IADPSG) had subsequently recommended new threshold glucose levels on the 75 g OGTT for diagnosing GDM^[20,21]. In 2013, the WHO adopted the IADPSG guidelines and revised the cut-offs for fasting plasma levels to ≥ 5.1 mmol/L (92 mg/dL), 1-h glucose level to ≥ 10.0 mmol/L (180 mg/dL) and 2-h glucose level to ≥ 8.5 mmol/L (153 mg/dL) following 75 g OGTT^[22]. It is expected that the 2013 WHO diagnostic criteria may standardise the diagnosis of GDM worldwide, but to date the implementation of this new criteria has been slow internationally.

There is preliminary data reflecting on the impact the new diagnostic criteria may have on the prevalence of GDM amongst different ethnic groups. A Singaporean study demonstrated that the proportion of women diagnosed with GDM in the Asian population using the 2013 WHO Criteria would be lower^[23]. The prevalence could drop from 30.9% to 18.9% in women of Chinese ethnicity, and from 33.5% to 28.1% among the South Asian population^[23]. On the other hand, in a predominantly Anglo-European population in Australia, the prevalence of GDM will increase from 9.6% to 13.0%^[24]. The reason for this divergence is that there are differences between ethnic groups in the glycaemic profiles on the OGTT from which GDM is diagnosed. In a cohort of over 850 women diagnosed with GDM from a multi-cultural community in south western Sydney, Australia, from the 75 g OGTT, those from South-East Asia had the lowest fasting glucose levels (4.95 ± 0.65 mmol/L) but the highest 2-h glucose level (8.75 ± 1.17 mmol/L). In contrast, Pacific Islanders had the highest fasting levels (5.71 ± 1.19 mmol/L) but the lowest 2-h levels (7.73 ± 1.27 mmol/L)^[25].

Table 1 Large studies highlighting the prevalence of gestational diabetes mellitus in women of different ethnicities living within a geographic region

Ref.	Year	City/region	Number of Women with GDM by ethnicity	Rate of GDM by ethnicity
Beischer <i>et al</i> ^[27]	1979-1998	Melbourne, Australia	66 Indian subcontinent	15% Indian subcontinent
			91 Chinese	13.9% Chinese
			60 Egypt and Arab countries	7.2% Egypt and Arab countries
			132 Other Asian	10.9% Other Asian
			95 Vietnamese	7.3% Vietnamese
			143 United Kingdom and Northern Europe	5.2% United Kingdom and Northern Europe
Solomon <i>et al</i> ^[63]	1990-1994	The Nurses Health Study II: 14 states in the United States	270 Mediterranean	7.3% Mediterranean
			1008 Australian and New Zealand	4.3% Australian and New Zealand
			655 White	4.7% White
			12 African-American	10.6% African-American
			17 Hispanic	7.6% Hispanic
			26 Asian	10.5% Asian
Sullivan <i>et al</i> ^[64]	1997	Sydney, Australia	730 Vietnamese	5.3% Vietnamese
			7226 Australian	1.6% Australian
Savitz <i>et al</i> ^[28]	1995-2003	New York City, United States	398 North African	7.2% North African
			1018 Sub-Saharan Africa	5.9% Sub-Saharan Africa
			3512 East Asia	6.2% East Asia
			1027 South-East Asia and Pacific Islands	8.6% South-East Asia and Pacific Islands
			4758 South Central Asia	14.3% South Central Asia
			5038 Non-Hispanic Caribbean	6.8% Non-Hispanic Caribbean
			8767 Hispanic Caribbean	4.9% Hispanic Caribbean
			2780 Mexico	6.3% Mexico
			1133 Central American	4.9% Central American
			4189 South American	6.6% South American
			6387 African-American	34.3% African-American
			9846 Non-Hispanic White	3.6% Non-Hispanic White
			5326 Japanese	3.45% Japanese
			32460 Asian Indian	8.03% Asian Indian
Chu <i>et al</i> ^[29]	2005-2006	Up to 19 states in the United States	25530 Chinese	6.44% Chinese
			25785 Filipino	6.9% Filipino
			11561 South Korean	3.9% South Korean
			21721 Vietnamese	6.14% Vietnamese
			20718 Other Asian	5.07% Other Asian
			5761 Pacific Islander	5.17% Pacific Islander
			1873925 White non-Hispanic	3.82% White non-Hispanic
			394091 Black non-Hispanic	3.54% Black non-Hispanic
			677392 Hispanic	3.63% Hispanic
			14617 American Indian	5.13% American Indian
			20129 Asian and Pacific Island	11.9% Asian and Pacific Island
			316 American Indian	7.6% American Indian
			3371 Black American	5.6% Black American
			52256 Hispanic	8.4% Hispanic
Kim <i>et al</i> ^[32]	2007-2009	California, United States	1483 Other	6.6% Other
			18806 Non-Hispanic White	5.4% Non-Hispanic White

GDM: Gestational diabetes mellitus.

ETHNICITY AND THE PREVALENCE OF GDM

Specific ethnicities of women have long been considered as a risk factor for developing GDM. At-risk ethnic groups identified in the literature, are Aboriginal women in Australia, Middle Eastern (Lebanese, Syrian, Iranian, Iraqi or Afghanistan) women and Pacific Islanders^[2,8,26,27]. Table 1 outlines some large population studies describing the prevalence of GDM among different ethnic groups who resided in western societies.

Among Asian women, the prevalence for GDM varies greatly. For instance, a study conducted in New York showed the prevalence of South-Asian (Indian, Sri Lankan, Pakistani, Fijian Indian) women having

GDM are generally higher than the risk of South-East Asian (Cambodian, Vietnamese, Laotian, Thai, Filipino, Malaysian) women and the East-Asian (Chinese, South Korean, Taiwanese and Japanese) women. The prevalence of GDM in women who were born in Asian countries varied from 3.0% to 21.2%^[28]. Many studies have shown Asian women had a much higher risk of GDM than women of United States Caucasian or Australian descent (Table 1). The highest risk appears to belong to women from South Asia and their adjusted relative risk is quoted by Savitz *et al*^[28] to be as high as 7.1 (95%CI: 6.8 to 7.3).

Interestingly, studies have demonstrated that women who migrated from their native countries to a western society had a higher rate of GDM compared

Table 2 Diagnostic criteria for gestational diabetes mellitus prior to recommendations by the International Association of Diabetes in Pregnancy Study Group in 2010

	ADA-NDDG ^[65]	ADIPS ^[19]	NZSSD ^[66]	WHO (1999) ^[17]	CDA ^[67]	EASD ^[62]
Glucose load (g)	100	75	75	75	75	75
FPG (mmol/L)	5.3	5.5	5.5	7	5.3	6
1-h Glc (mmol/L)	10	-	-	-	10.6	-
2-h Glc (mmol/L)	8.6	8	9	7.8	9	9
3-h Glc (mmol/L)	7.8	-	-	-	-	-
Abnormal results to diagnose GDM	2 or more	1 or more	1 or more	1 or more	1 or more	1 or more

ADA-NDDG: American Diabetes Association National Diabetes Diagnostic Group; ADIPS: Australian Diabetes in Pregnancy Society; NZSSD: New Zealand Society for the Study of Diabetes; WHO: World Health Organization; CDA: Canadian Diabetes Association; EASD: European Association for the Study of Diabetes; FPG: Fasting plasma glucose; Glc: Glucose; GDM: Gestational diabetes mellitus.

to women of a foreign ethnicity but who were born in western countries^[28]. However this trend did not apply to Japanese and South Korean women^[29]. Table 3 summarises two large studies showing the prevalence of GDM amongst women of various ethnic groups who were born in western countries compared with those born in their native countries. Again the data seems to suggest women born in South Asian and Pacific Islander countries who have migrated to the United States had the highest rate of GDM than United States born women from the same ethnicity^[29].

The demographic profiles of migrant mothers also varied among different ethnic groups. Studies had shown that Vietnam-born pregnant women with GDM who moved to Australia were more likely to be older, underweight and pregnant for the first time^[30]. Similarly, Shah *et al*^[31] found that United States Caucasian and Asian women with GDM were more likely to be over the age of 35 and have a higher education level. Compared with other Asian groups, Japanese and South Korean women have the lowest risk of GDM^[12,29,32].

BODY MASS INDEX

Body mass index (BMI) has long been considered as a risk factor associated with the development of GDM^[33]. Ethnic origin also appears to be a factor with a twofold higher rate in obese Hispanic women compared to African-American and Caucasian women^[34]. Women with GDM from Pacific Islands had the highest pre-pregnant BMI ($34.5 \pm 8.0 \text{ kg/m}^2$), while those from South East Asia had the lowest ($23.7 \pm 4.8 \text{ kg/m}^2$)^[25]. As BMI increases, the sensitivity of BMI to identify GDM in each racial/ethnic group decreases while the specificity increases. In a retrospective study of 24325 patients presenting at the University of San Francisco using a BMI of ≥ 25 as a screening tool classified 76.8% of African-Americans with GDM in this category but only 24.9% of Asian women. Using a BMI cut-off of > 21.0 identified 91.5% of African-American women with GDM, 90.1% of Hispanic, and 79.8% of United States Caucasian, but only 68.4% of Asian women. African-Americans were shown to have the highest increased risk (OR: 5.1) of GDM when BMI > 25.0 was used as a screening tool, compared with US

Caucasians (OR: 3.6), Hispanics (OR: 2.7) and Asians (OR: 2.3)^[31].

Women from Asia were shown to have GDM during pregnancy despite having a BMI that is within or below normal range^[30,32,35]. Therefore the role of BMI as a screening tool or risk factor for GDM in women from Asia is certainly questionable^[31]. Hunsberger *et al*^[15] found that Asian women had the greatest risk of having GDM compared to other ethnicities regardless of whether their BMI was greater or less than 26 kg/m^2 . This population tend to have more visceral or central fat, which is a known risk factor for insulin resistance and cardiovascular disease^[36]. Hence we would recommend screening pregnant Asian women for GDM regardless of their BMI.

A recent study on the interaction between maternal age and BMI showed the odds ratios for GDM development were significantly higher in women older than 30 years if they were Caucasian, older than 25 years if they were African and older than 20 years if they were South-Asians. This study also found that Africans and South-Indians were at higher risk of developing GDM irrespective of BMI^[37].

MANAGEMENT OF GDM

Medical nutritional therapy (MNT) is the cornerstone in the management of GDM. The goal of MNT is to provide adequate calories and nutrients to meet the needs of pregnancy and consistent with maintaining normoglycaemia^[5]. Yet there is very little consensus on a specific recommended dietary approach in the treatment of GDM^[6,38,39]. A recent review of 6 randomised controlled trials in 250 women with GDM suggested that a diet higher in complex carbohydrate and fibre, low in simple sugar and saturated fat may be effective in preventing postprandial hyperglycaemia and avoid worsening insulin resistance and excess foetal growth^[40]. Yet studies comparing low-glycaemic index (GI) with a high-GI or conventional high-fibre diet showed no difference in birth weight or adverse pregnancy outcomes^[41,42]. Similarly a 2013 Cochrane Review assessing 11 different types of dietary advice for women with GDM was unable to conclude on which was the most suitable dietary advice. The specific diets analysed were low-and high-

Table 3 Studies comparing the prevalence of gestational diabetes mellitus among different ethnicities in women born in Western countries with women born in foreign countries

Ref.	Year	City/Region	Rate of GDM in ethnic groups born in Western country	Rate of GDM in ethnic groups who migrated from their native country to a western country
Savitz, Janevic, Engel, Kaufman and Herring ^[28]	1995-2003	New York City, United States	1.7% North African	7.5% North African
			3.1% Sub-Saharan Africa	5.9% Sub-Saharan Africa
			5.6% East Asia	6.3% East Asia
			4.3% South-East Asia and Pacific Islands	8.9% South-East Asia and Pacific Islands
			6.8% South Central Asia	14.5% South Central Asia
			3.4% Non-Hispanic Caribbean	7.1% Non-Hispanic Caribbean
			4.4% Hispanic Caribbean	5.3% Hispanic Caribbean
			4.0% Mexico	6.4% Mexico
			3.4% Central American	5.1% Central American
			3.1% South American	7.0% South American
Chu, Abe, Hall, Kim, Njoroge and Qin ^[29]	2005-2006	Up to 19 states in the United States	4.91% Japanese	3.27% Japanese
			5.54% Asian Indian	8.81% Asian Indian
			4.64% Chinese	6.25% Chinese
			5.95% Filipino	7.31% Filipino
			5.31% South Korean	4.92% South Korean
			5.16% Vietnamese	6.2% Vietnamese
			4.39% Other Asian	6.21% Other Asian
			5.82% Pacific Islander	8.38% Pacific Islander

GDM: Gestational diabetes mellitus.

carbohydrate, high-monounsaturated fat, fibre-enriched diet, low-, moderate-, and high-GI, and energy-restricted and unrestricted. Overall there were no significant differences seen in the rates of macrosomia, large-for-gestational age deliveries or caesarean section^[39].

To achieve treatment goals, dietary plans should be prescribed by an accredited dietitian and should be culturally appropriate and tailored to the individual^[5]. The ability to adjust the amount and type of carbohydrate by training patients in "carbohydrate counting" is important to achieve target blood postprandial glucose levels^[38]. However, the amount of carbohydrate intake varies greatly between different ethnic and cultural groups. For instance, in South East Asia, rice is the staple food and this may pose major challenges for women from this background to curtail their rice intake. The diet for South-Asians is similarly heavily reliant on carbohydrate, and multiple sources of carbohydrate are often included at any one meal (e.g., lentil, dhal, rice in combination)^[43].

On the other hand, some women from the Middle East typically have a large meal in the afternoon with relatively smaller meals consumed at breakfast and dinner. They also have a tendency to delay breakfast till mid-morning and have dinner very late in the evening. Ramadan, an annual month of fasting observed by people of the Muslim faith, has significant impact on the timing of carbohydrate intake and meal portions. Ironically, it is the month where food consumption increases dramatically for Muslim communities as the daytime fasting is broken each evening with large banquets among family and friends which can last until dawn^[44]. Although pregnant women are exempted from observing Ramadan, many pregnant women with GDM still choose to observe the important religious ritual with their family.

For Pacific Islanders, they also tend to have large

servings of carbohydrate at main meals and multiple sources of carbohydrate at the one meal (taro, yam, cassava, green bananas, bread and rice)^[45]. All these factors should be taken into consideration when prescribing MNT. An overly regimental dietary recommendation will therefore result in poor compliance to therapy and suboptimal glycaemic control.

Health literacy among women from different ethnic groups may be highly variable, and this could have a significant impact on management of GDM. A study of women with GDM in the United Arab Emirates showed they had little understanding of carbohydrate knowledge, but not significantly different to women who did not have GDM. Moreover 22% of women with GDM were not reviewed by a dietitian for nutrition counselling and 65% attended a dietitian only once or twice^[46]. Furthermore, migrants in a Western society may also face huge challenges in managing their GDM. This could be related to language difficulty or their inability to adapt to an unfamiliar health system. A cross-sectional study conducted in Melbourne Australia showed that women coming from Vietnam had the poorest English skills and lowest education levels, with the greatest risk of misunderstanding GDM^[47]. Women with a history of GDM were shown to have poor diet quality as determined by the Australian Recommended Food Score, and in particular women who spoke a language other than English had significantly poorer knowledge than those who spoke English only^[48].

There were few studies that examined compliance to therapy for women with GDM. In an Australian study looking at failure-to-attend (FTA) rates of women with GDM, women who FTA more than once during their pregnancy had higher BMI, greater incidence of previous GDM and were more likely to be from non-Caucasian backgrounds^[49]. Apart from language barriers,

women from non-Caucasian backgrounds may need greater resources and time from clinicians to help them understand their condition better in order to improve their adherence to treatment recommendations.

INSULIN THERAPY

The glycaemic targets set for the management of GDM may also differ between countries, and hence it would be difficult to compare the proportion of women requiring insulin therapy across different regions. From a database in south western Sydney, women from South-East Asia had the lowest prevalence for insulin therapy (37.2%), compared with Anglo-Europeans (56.7%)^[25]. Despite having the highest 2-h glucose level on OGTT, women from South-East Asia also had the lowest need for rapid-acting insulin for the management of post-prandial hyperglycaemia. In that cohort, Pacific Islanders had the greatest need for insulin therapy, with 65% failing MNT. These women also had higher glycosylated haemoglobin ($5.9\% \pm 0.9\%$, 41 ± 10 mmol/mol) at the time of diagnosis of GDM compared to women from South East Asia ($5.4\% \pm 0.4\%$, 36 ± 4 mmol/mol)^[25]. In a similar study conducted in Hawaii, women from Pacific Islands had the highest rate of commencement of insulin therapy (27.5%), while women from Chinese heritage had the lowest (11.1%)^[11]. The higher percentage Pacific-Islanders requiring insulin before 20 wk of gestation in that study suggested that there could be a larger subset of Pacific-Islander women with previously undiagnosed type 2 diabetes.

FOETAL AND PERINATAL OUTCOMES

There is good evidence that treatment of women with GDM leads to better obstetrics and peri-natal outcomes^[50]. GDM increases risks of adverse perinatal outcomes including large for gestational age, shoulder dystocia, surgically assisted delivery and hypertensive disorders in pregnancy^[20]. In a 2013 systematic review commissioned by the United States Preventative Services Task Force and the National Institute of Health Office of Medical Applications of Research showed that treating GDM will result in a reduction in rates of preeclampsia, shoulder dystocia and macrosomia, but the benefits on preventing neonatal hypoglycaemia and averting long-term adverse metabolic outcomes of offspring are yet to be established^[51].

Among Asian subgroups, Cambodian and Laotian women with GDM had increased odds of macrosomia when compared with Japanese women with GDM. However, South East Asian women had lower rates of foetal macrosomia when compared with United States Caucasian women but preterm delivery with preeclampsia occurred more often when compared with Japanese and United States Caucasian women^[52]. Pacific Islanders have a higher rate of macrosomia than Asian or Caucasian women, but Asian neonates born with macrosomia had comparatively higher levels of NICU admission, need for

intravenous dextrose treatment for hypoglycaemia and respiratory distress, although the overall numbers were small^[10].

However another study from Ontario Canada showed that mothers of Chinese heritage had a significantly lower risk of adverse outcome at delivery compared to South Asian mothers. Chinese women also had a lower risk of adverse maternal outcomes compared with the general population^[53]. Several recent studies suggest infants born to mothers of non-Caucasian nationalities have lower adverse outcomes. A retrospective cohort study of 1865 adolescent women of different ethnicities born in a Californian University found that African-American, Hispanics and Asians had significantly lower rates of Caesarian delivery and low Apgar scores, while Asians and African-Americans had decreased rates of preterm delivery^[54]. There is no evidence to suggest any increase in peri-natal mortality for a particular ethnic group within a health care system. In a study of neonates admitted to intensive care unit, the mortality of 9813 infants of Australian-born mothers was not different to the 2166 infants born from migrant mothers^[55].

MATERNAL OUTCOMES

GDM represents relative beta-cell dysfunction which is caused by insulin resistance, revealed in response to the metabolic stress experienced during pregnancy^[56,57]. Women from particular ethnic groups who have GDM may be more susceptible to developing diabetes in the future^[2]. In particular, it appears that women with GDM from an Asian background who live in western societies are more likely than Anglo-European women to subsequently develop diabetes^[30,58]. Moreover a recent meta-analysis showed that women from ethnic groups other than "non-Hispanic white" had a higher rate of GDM recurrence of 56% compared with 39% in "non-Hispanic white" women who experienced GDM^[59].

A study of three ethnic groups of European, South Asian and Afro-Caribbean found that women who had a history of GDM had a range of metabolic abnormalities including beta-cell dysfunction with variable insulin resistance despite normal fasting blood glucose levels postpartum^[60]. Similarly a study in the United Kingdom of 221 women with GDM or impaired glucose tolerance (IGT) showed Asian women were shown to have significantly higher rates of persisting glucose intolerance compared with Afro-Caribbean or Anglo-European women post-partum. Use of insulin in Asian women during pregnancy was also associated with postpartum IGT^[14]. Development of type 2 diabetes mellitus in all ethnic groups was 3.5 times greater in women using insulin^[58].

An Australian study found that all ethnic groups living in a multicultural region with a high percentage of foreign-born residents all had a high rate of post-GDM diabetes or IGT^[2]. After a mean follow-up of 5.5 years, the study found that South Asians had the highest rate of either diabetes or IGT at 69%, more than the other

ethnic groups combined. South Asians and South-East Asians with either diabetes or IGT were also shown to have significantly lower BMI than Middle-Eastern or South European counterparts^[2].

A recent large retrospective analysis of women who delivered at Massachusetts General Hospital between 1998 and 2007 showed that women with GDM were 2.45 times more likely to develop hypertension compared to women without GDM. Furthermore, African-American and Hispanic women with GDM had a higher risk of developing hypertension and Asian women had a lower risk compared to United States Caucasian women subsequent to pregnancy^[61].

CONCLUSION

The increased risk of pregnant women developing GDM who belong to specific ethnic groups is widely acknowledged in the literature. This review highlights the major challenges in the provision of diabetes education and delivering MNT for GDM in an antenatal service where women may come from diverse ethnic and cultural backgrounds. Treatment involving MNT needs to be individually tailored and culturally sensitive, and insulin use may be more prevalent among some ethnic groups. Clinicians should appreciate that a "one size fits all" approach may not be appropriate in managing these women with GDM.

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Exercise guidelines for gestational diabetes mellitus

Cliantha Padayachee, Jeff S Coombes

Cliantha Padayachee, Jeff S Coombes, Physical Activity and Health, the School of Human Movement Studies and the Centre for Research on Exercise, the University of Queensland, St Lucia QLD 4072, Australia

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Correspondence to: Jeff S Coombes, PhD, Physical Activity and Health, the School of Human Movement Studies and the Centre for Research on Exercise, the University of Queensland, Blair Drive, St Lucia QLD 4072, Australia. jcoombes@hms.uq.edu.au
 Telephone: +61-7-33656767
 Fax: +61-7-33656877

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Abstract

The prevalence of gestational diabetes mellitus (GDM)

is increasing worldwide. This disease has many detrimental consequences for the woman, the unborn foetus and child. The management of GDM aims to mediate the effects of hyperglycaemia by controlling blood glucose levels. Along with pharmacology and dietary interventions, exercise has a powerful potential to assist with blood glucose control. Due to the uncertainty of risks and benefits of exercise during pregnancy, women tend to avoid exercise. However, under adequate supervision exercise is both safe and beneficial in the treatment of GDM. Therefore it is vital that exercise is incorporated into the continuum of care for women with GDM. Medical doctors should be able to refer to competently informed exercise professionals to aid in GDM treatment. It is important that exercise treatment is informed by research. Hence, the development of evidence-based guidelines is important to inform practice. Currently there are no guidelines for exercise in GDM. This review aims to assess the efficacy of exercise for the management of GDM in order to establish an exercise prescription guideline specific to the condition. It is recommended that women with GDM should do both aerobic and resistance exercise at a moderate intensity, a minimum of three times a week for 30-60 min each time.

Key words: Gestation; Pregnancy; Glucose; Physiology; Guidelines; Physical; Activity

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Core tip: Exercise has been proven to be beneficial in improving pregnancy outcomes in women with gestational diabetes mellitus (GDM). However, there is currently no exercise guidelines published for this population. A review into research outcomes of exercise in pregnant women with and without gestation diabetes as well as guidelines pertaining to type 2 diabetes mellitus has been conducted. This review has shaped the first guidelines pertaining to exercise for GDM management.

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INTRODUCTION

The use of exercise as part of the continuum of treatment in patients with diabetic related disorders is accepted and widely encouraged^[1]. One increasingly prevalent metabolic disorder is gestational diabetes mellitus (GDM). Although exercise prescription as treatment in this population group may be encouraged, a general exercise prescription guideline is lacking. Therefore the purpose of this review is to assess the efficacy of exercises currently being prescribed for the management of GDM in order to establish an exercise prescription guideline specific to GDM.

DEFINITION AND DIAGNOSIS OF GDM

GDM is defined as a "carbohydrate intolerance of varying degrees of severity with onset or first recognition during pregnancy"^[2]. Other variants of this definition further refer to the period of onset of hyperglycaemia, specifically within 24-28 wk of gestation^[3] and a natural dispelling of the hyperglycaemic condition after child birth^[4].

The earliest record of hyperglycaemia during pregnancy is from the 1960's, when a research group led by O' Sullivan (and endocrinologist/gynaecologist) noted that hyperglycaemia in pregnant women was associated with poorer pregnancy outcomes and a higher occurrence of type 2 diabetes in the years ensuing pregnancy^[4]. In 2013, the World Health Organisation (WHO) released a classification and diagnostic criteria for GDM (Table 1)^[5]. The new criteria was developed to take into account a quantifiable relationship between hyperglycaemia and adverse short-term pregnancy outcomes for both mother and newborn, in light of findings from the study of Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO)^[6]. This diagnostic guideline can be used throughout pregnancy and can distinguish GDM from diabetes mellitus in pregnancy (Table 1)^[5]. However, as the new criterion recommends lower fasting plasma glucose levels than what has been previously used, the WHO anticipated an increase in the number of pregnant women diagnosed with GDM^[5]. The effects of these criteria changes are yet to be evaluated, especially in the area of pregnancy outcomes.

RISK FACTORS FOR GDM

There is a range of risk factors that increase the chance of developing GDM. Ethnicity may play a role in GDM development as elevated incidences have been reported in certain ethnic subgroups. In the United States epidemiological studies have reported a higher incidence

of GDM in African Americans, Native Americans, Hispanics and Orientals than in non-white Hispanic women^[7-9]. In Australia, the prevalence of GDM was found to be higher in Aboriginal Australians as well as women who were born in Asia and India^[10-12].

In Europe, a large scale epidemiological study ($n = 11205$) in London found that women of ethnic minority groups had higher prevalence of GDM^[13]. When compared to White women, relative risks ranged from 3.1 for Black, 5.9 for miscellaneous, 7.6 for South Asian and 11.3 for Indian women^[13]. Asian women living in Asia have less proportional incidence than Asian born women living in other continents^[14]. Indian women living in urban areas have greater observed incidence than those living in rural areas of India^[15].

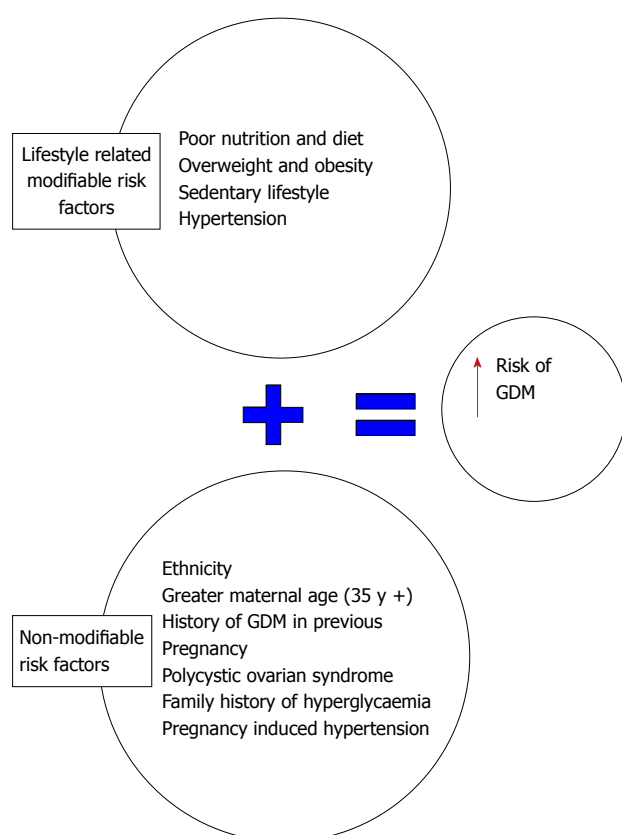
Furthermore, non-modifiable risk factors include greater maternal age (defined as 35 year of age plus)^[16,17], polycystic ovarian syndrome, family history of diabetes mellitus and pregnancy induced hypertension^[17]. Incidence of GDM has also been 30%-60% greater in women who have experienced the disease in a previous pregnancy^[18-22]. In a 16 year longitudinal retrospective study of 651 Canadian women who had GDM during their first pregnancy, 35% developed GDM in their second pregnancy^[23]. Greater pre-maternal weight was a strong predicting factor of GDM re-occurrence in ensuing pregnancies^[23].

This finding leads into a discussion of lifestyle related factors that are largely modifiable. Factors such as overweight and obesity can be modified. Weight gain during pregnancy was investigated using a nested-case control study of 1145 women^[24]. Findings suggested that subjects with greater weight gain during pregnancy (0.27-0.41 kg/wk or more) had an increased risk of developing GDM by 43%-74%. This effect was further exacerbated in overweight and obese women^[24]. Furthermore poor diet and nutrition are reported as mediators of increasing maternal weight and risk of GDM^[25-27]. Diets particularly high in refined sugars, with a high glycaemic index and fat content have been thought to increase the risk of GDM and hypertension during pregnancy^[25,28]. However, the majority of studies reporting these findings are small and have not been able to provide conclusive evidence on the role of diet in increasing the risk of GDM^[25,28]. Even so, one large prospective study ($n = 13475$), investigated the consumption of sugar sweetened beverages and incidence of GDM. After a 10 year follow up and 860 cases of GDM, excessive consumption (≥ 5 servings a week) of sugar sweetened cola was found to increase the risk of GDM by 22%^[29].

In a population based longitudinal study of 824 women, hypertension was found to increase relative risk of developing GDM up to twice as much (relative risk 2.03) as women without hypertension^[30]. The relationship between hypertension and GDM was confirmed in a Danish study of 215 women, in which higher rates of GDM was found in those with hypertension^[31]. Literature surrounding the pathophysiology of hypertension and gestational diabetes does not clearly underpin the

Table 1 Diagnostic criterion for gestational diabetes mellitus (WHO 2013) and diabetes mellitus in pregnancy (WHO 2006)^[5]

	Gestational diabetes mellitus	Diabetes mellitus in pregnancy
Fasting plasma glucose level	≥ 5.1-6.9 mmol/L	≥ 7.0 mmol/L
	OR	OR
75 g oral glucose tolerance test levels	1 h: ≥ 10.0 mmol/L 2 h: ≥ 8.5-11.0 mmol/L	1: Not required 2 h: ≥ 11.1 mmol/L
Random plasma glucose level	Not required	≥ 11.0 mmol/L

**Figure 1** Risk factors for gestational diabetes mellitus. GDM: Gestational diabetes mellitus.

mechanisms that cause such complications. However, it has been suggested that hypertension during pregnancy may heighten insulin resistance, alter immune responses and inflammatory pathways^[32]. These responses further encourage hypertension and hyperglycaemia, reminiscent of pathways in the metabolic syndrome^[32,33].

Sedentary behaviour and lifestyles characterised by low levels of physical activity have also been shown to increase the risk of GDM. In the largest reported study documenting physical activity and sedentary behaviour in relation to risk of GDM, Zhang *et al.*^[34] reviewed cases of 21765 women's pregnancies, 1428 of whom had GDM. They controlled for dietary factors and other covariates and found inverse relationships between vigorous activity, higher weekly physical activity levels and risk of GDM^[34].

Vigorous activity and brisk walking both had protective effects against developing GDM (RR = 0.77 and RR = 0.66 for each respectively). Women who were sedentary had a greater than two fold increase in developing GDM (RR = 2.30)^[34]. A more recent randomised control trial involving pregnant women had an exercise group ($n = 40$) which engaged in moderate land based resistance activity combined with aquatic aerobic activity^[35]. The control group ($n = 43$) was not given physical activity advice or exercise supervision. During 24-28 wk of gestation the women underwent a 50g maternal glucose screening test of which the exercise group had significantly better glucose levels (5.76 ± 1.13) than the control group (7.05 ± 1.73 mmol/L)^[35]. A case-control study by Dempsey *et al.*^[36] found a large reduction in GDM diagnosis in non-diabetic women who participated in recreational physical activity in the first 20 wk of pregnancy by 48%-78%. Further prospective studies of 909 non-diabetic American women, found that recreational exercise leading up to pregnancy, when performed for at least one year can induce a 56%-76% reduction in the risk of developing GDM^[37]. A large meta-analysis investigating seven pre-pregnancy and five early pregnancy studies found that pre-pregnancy exercise gave a pooled odds ratio of 0.45, a high protective effect against GDM^[38]. Furthermore, exercise in early pregnancy was also significantly protective of developing GDM with an odds ratio of 0.76^[38]. For a summary of risk factors see Figure 1.

EFFECTS OF GDM IN MOTHER AND CHILD

GDM affects the health of the women, the foetus and even after birth, the baby or child. Hyperglycaemic placental environments increase the risk of traumatic pregnancies influenced by macrosomia (larger than usual birth weight)^[39,40]. This in turn increases the risk of the baby having shoulder damage during birth^[40]. Macrosomia can be further exacerbated by excess levels of insulin circulating in the placenta^[3,41]. This is due to the increased growth effects of insulin on the foetus^[41]. Therefore care must be taken when prescribing anti-glycaemic maternal medication. The uses of different types of medications are still being investigated and there is a body of evidence yet to be filled regarding the direct impact of anti-glycaemic agents on foetal health. However, reviews have suggested that the use of insulin secretagogues is the safest form of pharmacological treatment in GDM, as they have little to no perfusion across the placenta^[41-43]. However, oral hypoglycaemic agents including metformin and glyburide have been used as alternative pharmacological treatment to insulin therapy^[44,45].

Further complications for the baby directly after birth include acute breathing difficulty, jaundice and nerve palsy^[46-48]. Babies born of women with poorly controlled GDM are likely to experience hypoglycaemia after birth due to an immediate impairment of environmental

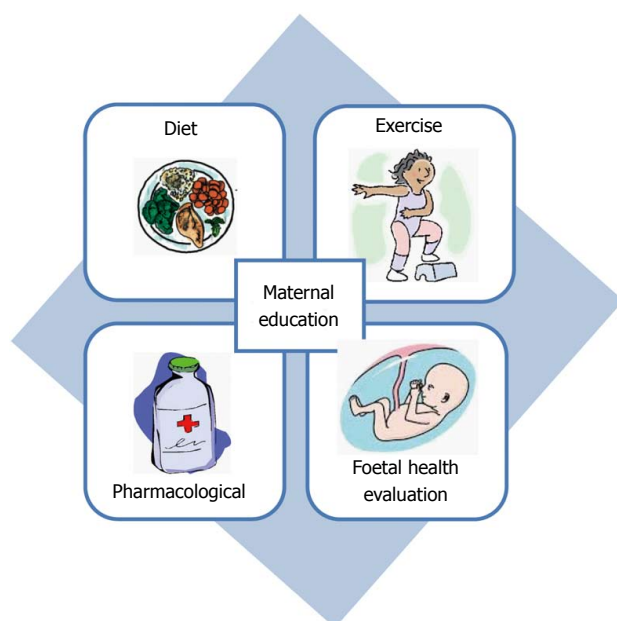


Figure 2 Gestational diabetes mellitus quintet management model.

glucose^[46,49]. Because of the complications presented, babies are more likely to be admitted to intensive care units and endure longer hospital stay times^[48,49]. As the child grows they are more likely to develop obesity as well as metabolic disorders such as type 2 diabetes^[50,51].

Traumatic births caused by the compounding effects of GDM also present complications for the women's immediate health^[47]. Women are more likely to undergo an emergency caesarean section, particularly with the presence of preeclampsia^[49]. Preeclampsia is a combination of high blood pressure and excessive protein in urine, also the second leading cause of maternal death^[49]. Due to the increased size of the baby, they are also more likely to have a longer active labour times, instrumental vaginal delivery as well as third degree perineal trauma and tearing^[49,51]. These traumatic events generally lead to post-partum haemorrhage and hence longer hospital stay times^[49]. Furthermore, women with GDM have greater weight retention post-partum leading to a greater risk of overweight and obesity^[51,52]. This has also been shown to correlate with increased risk of developing chronic hypertension, type 2 diabetes and GDM is ensuing pregnancies^[49,51,52].

Because of the vast effects of GDM on the health of both mother and child, the management of GDM is critical in minimising the effects of hyperglycaemia during pregnancy.

GENERAL MANAGEMENT OF GDM

The primary aim of GDM management is to optimise glucose control and improve pregnancy outcomes^[53]. Generally speaking the initial management of GDM involves diet modification and implementation of an exercise regime^[49,54]. If adequate glucose control has not been achieved, the woman will generally be prescribed

anti-diabetic medications to directly reduce blood glucose levels maternally and hence indirectly for the foetus^[42].

Although it is not a treatment method, constant evaluation of the foetus' health and development is recommended to continually assess for deformities and macrosomia^[54]. This includes foetal surveillance using ultrasound and Doppler of umbilical blood flow measurement^[49,54,55]. In a large randomly controlled trial, 1000 women diagnosed with GDM were randomly assigned gestational diabetes care ($n = 490$) or routine care ($n = 510$). The gestational diabetes care group received dietary advice, blood glucose monitoring and insulin therapy. The control group received no standard pregnancy care. Pregnancy outcomes were assessed and after controlling for various factors including ethnicity and age, the diabetes care group had lower rates of serious perinatal complications (1% vs 4%)^[49]. However, this intervention did not include maternal exercise or education. There were also no protective effects on admittance to the intensive care unit, induced labour or risk of caesarean delivery. A more recent and larger meta-analysis by Poolsup *et al.*^[56] investigated ten studies regarding the outcomes of GDM treatment methods including pharmaceutical and dietary care. It was found that such treatment significantly reduced the risk of macrosomia (RR = 0.47), shoulder damage during birth (RR = 0.42) and gestational hypertension (RR = 0.68). However alongside the absence of exercise from these treatments, there was no change in the risk of neonatal mortality, neonatal hypoglycaemia, birth trauma, premature births, preeclampsia, caesarean section and induced labour^[56].

The recommended quintet approach for GDM (Figure 2) includes maternal education, diet modification, exercise, pharmacology and foetal surveillance. This has been developed in light of the confounding evidence of increased positive pregnancy outcomes in studies of these areas independently. A study investigating the efficiency and practicality of this complete model of maternal and foetal care is yet to be investigated.

ROLE OF EXERCISE IN GDM MANAGEMENT

In regards to evidence specific to GDM treatment, exercise has been shown to be an effective tool in glucose control which may prevent, reduce or delay the need for insulin^[57,58]. Tight glucose control is considered especially important in the gestational patient, considering the increased risk of poor health outcomes for both mother and child in the presence of hyperglycaemia. For future ramifications, it has been found that any degree of abnormal glucose homeostasis during gestation can also independently predict diabetic re-occurrence in women with GDM^[57].

Although currently there are no GDM specific exercise prescription guidelines published, research has been conducted in general pregnancy and exercise.

Table 2 Absolute and relative contraindications for exercise during pregnancy^[75,82]

Absolute contraindications	Relative contraindications (aerobic exercise)
Restrictive lung disease	Heavy smoking
Ruptures membranes	History of extremely sedentary lifestyle
Preeclampsia	Orthopaedic limitations
Pregnancy-induced hypertension	Poorly controlled hypertension
Premature labour during current pregnancy	Extreme morbid obesity
Persistent bleeding (second or third trimester)	Extremely underweight (BMI < 12 kg/m ²)
Incomplete cervix or cerclage	Poorly controlled type 1 diabetes
Placenta previa (placental implanting into lower uterus) after 26 wk of gestation	Chronic bronchitis
Hemodynamically significant heart disease	Severe anaemia
High order multiple gestation (\geq triplets)	Unevaluated maternal cardiac arrhythmia
	Intrauterine growth restriction in current pregnancy
	Poorly controlled seizure disorder
	Poorly controlled hyperthyroidism
	Previous spontaneous abortion
	Anaemia (hb < 100 g/L)
	Twin pregnancy after 28 wk
	Malnutrition or eating disorder

BMI: Body mass index.

There has been no suggestion of the need to any extra precautions than the precautions taken when exercising pregnant women without GDM. However, considering the added hyperglycaemia, the same considerations and precautions concerning type 2 diabetes should also be considered when exercising women with GDM. Hence the FITT (frequency, intensity, time/duration and type) principles of exercise examined and presented in this review will take into consideration research including women with GDM, without GDM and type 2 diabetes. Given the lack of large cohort studies implementing exercise as management of GDM, recommendations have been drawn from exercise in pregnancy guidelines and exercise in type 2 diabetes guidelines.

For women who were previously sedentary before pregnancy, it is advised that they consult their medical practitioner who may assess their suitability to exercise. It is recommended that a suitability qualified exercise physiologist be actively involved in exercise prescription and delivery. This person would then be able to liaise with the medical practitioner to apply advice regarding suitability. As with any clinical population there are some contraindications to exercise in pregnancy. The absolute contraindications as recommended by the American Congress of Obstetricians and Gynaecologists (ACOG) are medical conditions which may be exacerbated by engaging in exercise. It is important that to educate patients that these conditions are not caused by exercise and until the condition is stabilised, they should not engage in exercise. Furthermore, ACOG has developed relative contraindications for engaging in aerobic exercise during pregnancy. Clinical knowledge and expertise must be applied when assessing each individual situation in regards to exercising with relative contraindications. It is recommended that advice from the medical practitioner is carefully interpreted by the exercise physiologist to determine if the benefits outweigh any risks of exercise.

Both absolute and relative contraindications for exercise in pregnancy are summarised in Table 2.

BENEFITS OF EXERCISE DURING PREGNANCY

Benefits to the mother

Exercise has been proved to be a beneficial therapeutic tool during pregnancy. Records as early as the 17th and 18th Centuries have shown encouragement of exercise during pregnancy as it was thought to ensure good health and prevent miscarriage^[59]. Further in the late 18th Century maternal physical activity was thought to help encourage an easier labour and reduce the baby size, also advantageous during delivery^[60]. In the early 20th Century particular the 1920's and 1930's, scientific studies began to investigate the impact of physical activity on pregnancy outcomes. These studies found inverse relationships between birth weight and household physical activity^[61]. In the 1920's studies began to inform prenatal exercise programs with benefits recorded as increased ease of labour, improved muscle tone, increased foetal oxygenation and facilitating post-partum weight loss^[62].

Key epidemiological studies came later in the 1990's. Clapp *et al.*^[63] found that women who exercised during pregnancy had babies with a significant lower birth weight than those who had decreased their physical activity during pregnancy. In 1991 Bung *et al.*^[64] investigated the use of exercise in women with GDM. This randomised control trial was one of the first of its kind and findings were imperative to influence future research into the efficacy of exercise in GDM management. Seventeen of the twenty-one women in the exercise group, of whom were all previously insulin dependent were able to maintain normal glucose levels without using insulin^[64]. Maternal complications did not differ between the

Table 3 Benefits of maternal exercise for the foetus and the child

Benefits to the foetus ^[100-104]	Benefits to the foetus ^[1,58,73,104,105]
Lower heart rate response to acute maternal exercise	Lower birth weights
Increased amniotic fluids	Increased gestational ages (lower risk of preterm birth)
Increased in placenta viability and volume	Improved neurodevelopment and lower body fat percentage
Increase in vascular function	Infants have higher behaviour regulatory ability and orientation
Faster placental growth and greater villous tissue	At the age of five children have less body fat, higher general language intelligence and oral language
Higher tolerance to labour	

Table 4 Modified heart rate target zone for aerobic exercise in pregnancy^[82,95]

Maternal age	Heart rate target zone (beats/min)	Heart rate target zone (beats/10 s)	Heart rate target zone (beats/min) (SOwt/SOb)
< 20	140-155	23-26	-
20-29	135-150	22-25	102-124
30-39	130-145	21-24	101-120
≥ 40	125-140	20-30	-

SOwt: Sedentary overweight; SOb: Sedentary obese.

exercising group and the control group. Exercise was now deemed to be safe and advantageous for glucose control for women with GDM^[64]. These findings were also confirmed by Jovanovic-Peterson *et al.*^[65] in a smaller study of women with GDM utilising exercise and diet ($n = 10$) and those using diet alone ($n = 10$). The findings of this study concluded that women engaged in diet plus exercise had lower fasting plasma glucose after 6 wk of training than women who underwent the diet only intervention (diet = 4.87 mmol/L, exercise and diet = 3.89 mmol/L)^[65]. This was further confirmed in a more recent study in which physical activity and diet interventions resulted in a lessened dependence on insulin for glucose control in women with GDM^[66]. Since there is little more exercise interventional trials conducted in women with GDM, this review is also informed by exercise in pregnancy.

Multiple studies have reported the positive effects of exercise on decreased lower back pain in pregnant women, two of which were recent randomised controlled trials^[67,68]. Other physiological studies have reported exercise in pregnant women to improve cardiovascular functions such as fitness, blood pressure, and peripheral oedema^[69]. Preeclampsia has also been show to decrease with an increase in physical activity^[70,71]. As previously demonstrated exercise may decrease the risk of developing GDM and type 2 diabetes^[36,72]. Furthermore, by increasing blood glucose control, exercise reduces the vast effects of hyperglycaemia on the women, foetus and child (see above section Benefits to the mother). As pregnancy is a period associated with physiological and psychological change, the benefits on mood and psychological wellbeing are also well documented. Due to a limitation in weight gain and fat retention, exercise has also been shown to improve self-image^[73]. The ACOG have also recently report an improvement in constipation

and bloating as well as fatigue and insomnia^[74].

Benefits to the foetus and child

Pregnancy outcomes are largely associated with foetal health and upon birth, the health of the child as well. Maternal exercise has also been shown to provide significant benefits to both the health of the foetus and the child. Because neonatology and paediatrics is beyond the scope of this review, these findings are summarised in the Table 3.

EXERCISE GUIDELINES

Type

Safety during pregnancy is paramount and studies have shown a variety of exercises ranging from low exerting forces such as Yoga to higher exerting forces such as aerobic classes and jogging can be safe for both mother and foetus^[75]. Considering the importance of safety, it is advised that some forms of exercise should not be practiced during pregnancy, including the following: recreational sports with increased risk of forceful contact or falling (*i.e.*, basketball, rugby, horseback riding and gymnastics), exercising in a supine position after the first trimester (may obstruct inferior vena cava flow), motionless standing and scuba diving (risk of foetal decompression sickness)^[75]. Recreational physical activity is encouraged and has been shown to improve general wellbeing, pregnancy outcomes^[76]. Furthermore maternal mood and mental health have been shown to benefit from recreational physical activity^[76] (Table 4).

Programmed exercise is also very important in pregnancy and is vital to aid in glucose control for women with GDM^[76].

Exercise guidelines for pregnancy stress the prescription of aerobic exercise and to a lesser extent the prescription of resistance strength training. Women who regularly exercise during pregnancy have more positive pregnancy outcomes and fewer negative adverse events^[77]. However, there is little evidence of what is the physiological role of aerobic exercise in these relationships^[77]. The only truly quantifiable relationship thus far is that of aerobic exercise and significantly improved maternal fitness during pregnancy^[77]. Positive foetal outcomes are yet to be quantified as the majority of this research area has focused on foetal safety during exercise^[75]. There is a general agreement that appropriate exercise does not induce any harm on the

foetus^[75]. However, in regards to elevate fasting glucose, aerobic exercise can indeed reduce blood glucose levels in individuals with hyperglycaemia, potentially reducing and delaying the need for insulin medication^[78]. These effects may last for more than 24 h but less than 72 h. Furthermore, following aerobic exercise insulin levels also drop, reducing the chance of hypoglycaemia^[78]. However, after an intense bout of exercise, a hyperglycaemic response may be observed for up to 2 h post exercise^[78-80]. This may be important to consider when measuring blood glucose levels after exercise.

Aerobic exercise can consist of any activity that uses large muscle groups in a continuous rhythmic manner, *i.e.*, walking, jogging, aerobic dance, swimming, hydrotherapy aerobics, rope skipping, hiking, rowing, *etc*^[75]. However, clinical judgement should be exercised when choosing the appropriate and practical mode of aerobic exercise. This is particularly important in the first stages of an exercise program if intensity is to be tightly controlled (see below in intensity).

In addition to aerobic exercise, resistance strength training (*i.e.*, weightlifting) and flexibility exercise are also beneficial and safe for gestational women and foetus^[75,81,82]. Although the Royal College of Obstetricians and Gynaecologists (RCOG), ACOG, the Society of Obstetricians and Gynaecologists of Canada (SOGC) and Canadian Society for Exercise Physiology (CSEP) all recommend the use of resistance training for pregnant women, they are yet to provide specific guidelines for practice. However, Hall *et al*^[83] investigated the effects of moderate intensity strength training in healthy pregnant women. They used a protocol of 12 reps and one set of 8-10 exercises and found pregnancy outcomes were improved with no adverse effects to foetal health^[75,83]. Women who used resistance band exercise training at a moderate intensity three days a week had improved glucose control. This was reflected in lower capillary glucose levels and significantly less users of insulin ($n = 18$ control vs $n = 7$ exercise group)^[84]. Pregnancy specific pelvic floor exercise training has also been shown to reduce incontinence and bladder weakness after pregnancy^[69]. However, in the treatment of elevated fasting glucose, the American College of Sports Medicine (ACSM) and Exercise and Sports Science Australia (ESSA) both recognise that resistance training lowers fasting blood glucose levels for 24 h after exercise^[78,85]. This response is further exaggerated with an increase in training volume and intensity^[78,85]. Mode of exercise used in resistance training may include but is not limited to, resistance machines, free weights and body weight exercises^[78,85].

The ACOG reports hydrotherapy exercise to be considered safe during pregnancy with the potential to improve positive outcomes and pregnancy management^[75]. It has been shown that aerobic water based exercise at a moderate intensity may improve fitness, strength and decrease peripheral oedema^[75]. With the added effects of increased buoyancy, hydrotherapy

may minimise the risk of musculoskeletal joint injuries and provide a pain relieving manner of exercise for suffers of pregnancy induced lower back pain^[1,75]. Thermoregulatory issues should also be considered. Although significant research has not yet been conducted on humans, animal studies have shown that an increase in core temperature by as little as 1.5 °C during embryogenesis (in early stages of pregnancy) may result in major congenital malformations^[86]. Although these findings have not yet been supported in human studies, it may highlight the importance of remaining adequately hydrated and exercise in environments that are cool, shaded and well ventilated. During pregnancy it is important to note that core temperature is already raised due to an increase in the basal metabolic rate^[87]. Furthermore during exercise increases in body temperature strongly correlate with work intensity^[87]. Therefore, prolonged intensity workouts that encourage temperature fluctuations and an accelerated loss of fluid through perspirations may need to be avoided^[75].

Both ACSM and ESSA recommend that combined aerobic and resistance exercise are more effective if blood glucose management, body composition improvement and fitness outcomes^[78,85]. However, training using both of these modes may be more time consuming and greatly dependent on the individual's comorbidities, complications, accessibility to equipment and preference^[88-90]. Even so, combination training shows improvements in blood glucose control utilising different physiological mechanisms that may be of greater use when activated together^[78]. Resistance training resulting in an increased muscle mass can increase blood glucose uptake independent of intrinsic insulin response as insulin does not have influence on musculature glucose uptake^[84,88]. Aerobic training increases insulin stimulatory action and thus increases blood glucose uptake *via* a different pathway^[88]. Activating both of these metabolic pathways may be more physiologically beneficial than utilising only one pathway or exercising using only resistance training or aerobic training^[78].

Performing a warm up before exercise is recommended for all clinical populations^[91]. Warm up's of between 5-10 min at a low to moderate intensity using aerobic activities can increase body temperature and reduce post-exercise muscle soreness and stiffness^[91]. Warm ups are an important stage in exercise, as physiological systems gradually adjust to meet the bioenergetics and biomechanical demands of the working component of the exercise session^[91]. Performing a post exercise cool down is recommended if vigorous exercise is performed to reduce the risk of a vasovagal response which may lead to syncope^[92]. Stretching and flexibility training is distinct from the warm up or cool down phase and can be performed after either^[91]. Although there are limited studies investigating the role of warm up and cool down phases in exercise during pregnancy, there is no evidence to suggest that this may cause any harm. Considering the general benefits implicated for most populations,

Table 5 Exercise guidelines for gestational diabetes mellitus

Type of exercise	Intensity	Duration	Frequency
Aerobic (large muscle activities in a rhythmic manner) <i>e.g.</i> , walking, running, swimming and cycling	Moderate 60%-90% of APHRM RPE 12-14 Previously sedentary Owt/Ob should begin training at 20%-30% of APVO ₂ R RPE 12-14 Vigorous RPE 14-16	≤ 30 min continuously (up to 45 min if self-paced)	No more than two consecutive days without exercising
Resistance (multi joint exercises, large muscle groups) <i>e.g.</i> , dumbbells, resistance band and pregnancy Pilates	Moderate 50% 1RM 5-10 exercises 8-15 repetitions 1-2 sets	60 min	At least 2 but ideally 3 times a week

APHRM: Age predicted heart rate maximum; RPE: Rate of perceived exertion; Owt: Overweight; Ob: Obese; APVO₂R: Age predicted VO₂ reserve; RM: Repetition maximum.

it can be safe to assume the same would apply for pregnant women.

Frequency and duration

When prescribing exercise it is important to take into consideration the woman's previous physical activity history, cardiorespiratory fitness and strength^[1]. For women who were previously sedentary it may be more convenient for them to start an exercise program in the second trimester, after which most of the initial discomforts of morning sickness, nausea and fatigue have settled down^[81]. This is recommended so that extra discomforts of initiating an exercise program may not in turn impair adherence or compliance. Yet, as previously discussed, exercise in early pregnancy can reduce the risk of GDM, therefore sooner the woman can comfortably exercise the better^[35-37]. Women with little physical activity history should begin with 15 min of continuous aerobic exercise three times a week with a graded increase to 30 min at least four times a week^[78]. This was also previously recommended in the 2002 ACOG and is also supported in by the Society of Obstetricians and Gynaecologists of Canada (SOGC) and Canadian Society of Exercise Physiologists (CSEP) guidelines^[75,82]. There is no recommendation of an upper limit of time spent performing aerobic exercise, but the ACOG advises against exercising for more than 45 min continually because of a risk of increased foetal temperature^[75]. However this temperature rise is seen to be negligible when the exercise is self-paced in an environment that has adequate temperature control^[75]. Exercise guidelines for Type 2 Diabetes from the American College of Sports Medicine (ACSM) and Exercise and Sports Science Australia (ESSA) are generally the same as the pregnancy guidelines. However ACSM and ESSA have an additional note that there exercise should be conducted with no more than 2 consecutive days between aerobic exercise sessions^[78,85]. This is due to the transient improvement of insulin action and passive glucose uptake after exercise for up to 48 h^[78,85]. In regards to resistance

training, ACSM and ESSA recommend a minimum of twice a week on non-consecutive days and ideally three times a week^[78,85]. Each training session should include 5-10 (ACSM) or 8-10 (ESSA) exercises involving the major muscle groups (upper body, lower body, and core) and 10-15 repetitions (ACSM) or 8-10 repetitions (ESSA) each set at a minimum of one (ACSM) or two (ESSA) sets for strength gains, but up to four sets for optimal glucose uptake and strength gains. Considering that it is recommended that women do not train for optimal gains during pregnancy, up to three sets at moderate intensity may be more appropriate.

Intensity

During pregnancy, the majority of guidelines indicate the use of moderate intensity, but even low intensity exercise such as Yoga and Tai-Chi has shown benefits on mood, balance, lower back pain and urinary incontinence^[93,94]. As cardiorespiratory fitness is vitally important in encouraging positive outcomes during pregnancy and post pregnancy, moderate aerobic exercise is highly recommended^[1,94]. Heart rate is a relatively simple way to prescribe aerobic exercise in a manner that corresponds with perceived exertion and thus intensity. However during pregnancy, heart rate is elevated by 10-15 beats and is blunted at maximal exercise levels^[82]. RCOG, SCOG and CSEP have all recommended the use of a modified heart rate target zone developed by the CSEP, when prescribing moderate intensity aerobic exercise (see Table 5)^[1,82]. This target zone aims for an exercising level of 60%-90% of age predicted maximal heart rate. Furthermore, Davenport *et al*^[95] have developed and validated an exercise target heart rate zone especially for sedentary overweight and obese pregnant women (Table 5). This model aims to exercise previously sedentary pregnant women at 20%-39% VO₂ reserve for as recommended by ACSM. Even so, it is interesting to know why this was only recommended for sedentary overweight and obese women and not previously sedentary normal weighted women. Furthermore, during

pregnancy heart rate variability increases^[75]. Therefore, ACOG recommends that prescribers exercise caution using heart rate to guide intensity and should consider using Borg's Modified Rate of Perceived Exertion Scale instead^[75]. Exercisers are recommended to aim for a working intensity of 12 to 14 (somewhat hard) on a 6-20 scale^[75]. McMurray also reported that women who self-paced exercise intensity would gradually reduce intensity as pregnancy progressed^[96].

High intensity short duration interval training has also been shown to be safe with showing no added complications during pregnancy but caution should be exercised for previously sedentary women^[75,82,97]. Furthermore, it may have protective effects from the foetus developing macrosomia and risk of preterm birth^[98].

ACSM's also recommends high intensity interval training for those exercising using moderate intensity and are in need of more glucose control^[78].

Resistance strength training has also been shown to positively influence the mother's general health and pregnancy outcomes^[81,99]. It is especially important to consider the physiological changes during pregnancy when prescribing resistance training and that high intensity resistance training should be avoided. Changes include increased urinary tract pressure (cause of urinary incontinence) and increased laxity in joints^[69]. Therefore care must be taken not to induce Valsalva manoeuvres that may increase the risk of injury or adverse event^[82]. There are few recommendations for resistance training guidelines during pregnancy, due to a lack of large quality studies. Therefore in accordance to other general pregnancy exercising guidelines, a recommended intensity of a moderate level is suggested. In regards to impaired fasting glucose, ACSM recommends strength training at a moderate (50% of 1-repetition maximum) or vigorous (75%-80% of 1-repetition maximum) intensity for optimal gains in strength and insulin action^[78]. ESSA generally recommends similar intensity of resistance exercise, yet with a greater inclination encourage vigorous intensity in light of dose related glucose control^[85]. Considering the added physiological changes in pregnant women, a moderate intensity training model is more appropriate. Furthermore, to avoid injury a slow progression of intensity, frequency and duration of strength training sessions occur^[78].

PRECAUTIONS AND RECOMMENDATIONS TO TERMINATE EXERCISE

Although there are vast benefits from exercising during pregnancy, some precautions need to be observed to encourage safety for both mother and child. As previously noted, the ACOG has advised against some forms of exercise including the following: recreational sports with increased risk of forceful contact or falling (*i.e.*, basketball, rugby, horseback riding and gymnastics), exercising in a supine position after the first trimester,

motionless standing and scuba diving^[69,75].

Furthermore, if any of the following warning signs occur, it is advised that exercise should be terminated: vaginal bleeding, dizziness, headache, chest pain, muscle weakness, preterm labour, decreased foetal movement, amniotic fluid leakage, calf pain or swelling and dyspnoea without exertion. It is important to regain stability of the mother's and foetus' condition as soon as possible. Treatment and advice from a medical practitioner should be incorporated in the exercise program^[69,75].

Exercising individuals with impaired fasting glucose presents its own sense of challenges and special considerations. Preventative measures should be in place to minimise the risk of an adverse event occurring and not prevent individuals from exercising. One especially important possible complication, however rare is hypoglycaemia. It is suggested that continual self-monitoring of blood glucose levels with physician consultation should be encouraged. Furthermore, if at pre-exercise the blood glucose level is ≤ 4.0 mmol/L this should be considered low and exercise should not begin till administration of some long and short acting glucose in food or drink^[85]. In order to take advantage of the hyperglycaemic effect of food, it may be advantageous to exercise an hour after a meal^[85]. It also may be important to consider taking insulin medication well before exercise to further reduce the risk of hypoglycaemia.

CONCLUSION

All pregnant women should engage in physical activity and may benefit from planned and programmed exercise. Women with GDM have extra physiological challenges that when left unattended to, have the potential to increase negative pregnancy outcomes for both mother and child. When used effectively, exercise can be used as a tool of treatment as part of the continuum of care for women with GDM. General guidelines encourage these women to engage in moderate intensity aerobic and strength training along with recreational physical activity. Exercise programs should be tailored by appropriately trained and qualified professionals (*e.g.*, Exercise Physiologists) who have knowledge, training and experience to understand the individual's physiological needs and associated risks.

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Indicators of glycemic control in patients with gestational diabetes mellitus and pregnant women with diabetes mellitus

Kunihiko Hashimoto, Masafumi Koga

Kunihiko Hashimoto, Department of Internal Medicine, NTT West Osaka Hospital, Osaka 543-8922, Japan

Masafumi Koga, Department of Internal Medicine, Kawanishi City Hospital, Kawanishi, Hyogo 654-8533, Japan

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Correspondence to: Masafumi Koga, MD, PhD, Department of Internal Medicine, Kawanishi City Hospital, Kawanishi, Hyogo 664-8533, Japan. m-koga@kawanishi-city-hospital.com
 Telephone: +81-72-7942321
 Fax: +81-72-7946321

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Abstract

Recently, it has become clear that mild abnormal glucose tolerance increases the incidence of perinatal maternal-infant complications, and so the definition and diagnostic

criteria of gestational diabetes mellitus (GDM) have been changed. Therefore, in patients with GDM and pregnant women with diabetes mellitus, even stricter glycemic control than before is required to reduce the incidence of perinatal maternal-infant complications. Strict glycemic control cannot be attained without an indicator of glycemic control; this review proposes a reliable indicator. The gold standard indicator of glycemic control in patients with diabetes mellitus is hemoglobin A1c (HbA1c); however, we have demonstrated that HbA1c does not reflect glycemic control accurately during pregnancy because of iron deficiency. It has also become clear that glycated albumin, another indicator of glycemic control, is not influenced by iron deficiency and therefore might be a better indicator of glycemic control in patients with GDM and pregnant women with diabetes mellitus. However, large-population epidemiological studies are necessary in order to confirm our proposal. Here, we outline the most recent findings about the indicators of glycemic control during pregnancy including fructosamine and 1,5-anhydroglucitol.

Key words: Glycemic control; Hemoglobin A1c; Glycated albumin; 1,5-anhydroglucitol; Fructosamine; Gestational diabetes; Diabetes mellitus; Pregnancy

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Core tip: In patients with gestational diabetes mellitus (GDM) and pregnant women with diabetes, stricter glycemic control is required to reduce the incidence of perinatal maternal-infant complications. We have demonstrated that hemoglobin A1c does not reflect glycemic control accurately during pregnancy because of iron deficiency. On the other hand, glycated albumin is not influenced by iron deficiency and therefore might be a better indicator of glycemic control in patients with GDM and pregnant women with diabetes. However,

large-population epidemiological studies are necessary. Here, we outline the most recent findings about the indicators of glycemic control during pregnancy including fructosamine and 1,5-anhydroglucitol.

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INTRODUCTION

The number of patients with diabetes mellitus has been steadily increasing worldwide^[1] and diabetes mellitus has become a global health problem. This tendency is also observed in women of child-bearing age partly due to the change in diagnostic criteria of gestational diabetes mellitus (GDM) as mentioned later. The incidence of GDM in Japan has increased by 4.1 fold from 2.9% to 12.1%^[2]. By detecting abnormal maternal glucose metabolism at an early stage of pregnancy and achieving excellent glycemic control during pregnancy, it is possible to prevent perinatal maternal-infant complications^[3,4]. According to a report on a meta-analysis of 20 studies, the relative risk for patients with GDM of developing type 2 diabetes mellitus after delivery is 7.43 times higher than that of women who had normal glucose tolerance during pregnancy^[5]. Therefore, it is important to follow up mothers after delivery. Moreover, the concept of developmental origins of health and diseases was proposed recently^[6] and the long-term effects of mothers with abnormal glucose tolerance on fetuses after birth have been actively discussed. Thus, it is important to manage glycemic control of mothers appropriately not only because it helps to maintain the health of mothers and infants in the short term, but also helps to maintain the long-term health of mothers and the next generation. In the following sections, we outline the management of pregnant women with abnormal glucose metabolism. Finally, we propose a reliable indicator of glycemic control.

CHANGES IN GLUCOSE METABOLISM DURING PREGNANCY

During the early stage of pregnancy, increased secretion of progesterone and 17 β -estrogen from the corpus luteum is observed; after the placenta is completed, it replaces the role of the corpus luteum. In humans, it is known that during pregnancy, the secretion of estrogen increases by about 30 fold and that of progesterone by about 10 fold compared with that during non-pregnancy. In addition, the secretion of prolactin and placental lactogen also increases gradually from week 12 of pregnancy. Placental lactogen is considered to be

one of the typical hormones involved in the change in insulin sensitivity during pregnancy. Moreover, it has been shown that tumor necrosis factor- α secreted from macrophages which infiltrate into fat cells and villous cells is deeply involved in decreased insulin sensitivity^[7]. It is known that high concentrations of estrogen and progesterone also induce decreased insulin sensitivity^[8,9]. Because of the involvement of hormones and cytokines as mentioned above, there is a substantial change in glucose metabolism during pregnancy. In clinical researches conducted by Catalano *et al.*^[10-12], increased gluconeogenesis in the liver during the end stage of pregnancy demonstrated decreased insulin sensitivity in the liver. In addition, it has been shown that systemic insulin sensitivity decreases by about 50% to 60% during the end stage of pregnancy^[13]. In pancreatic β -cells, increased β -cell volume and increased insulin secretion reaction take place to compensate for their insulin resistance. Regarding the mechanism of this phenomenon, it has been reported that serotonin is present downstream of the prolactin signal, which promotes pancreatic β -cell growth and greatly contributes to an increase in the cell volume^[14]. It is considered that abnormal glucose tolerance develops in pregnant women when this compensatory effect is insufficient. In fact, the presence of pancreatic β -cell dysfunction in GDM has been demonstrated^[15,16].

DEFINITION OF GDM

GDM, which was found or developed for the first time during pregnancy, is milder abnormal glucose metabolism than diabetes mellitus. It should be noted that GDM does not include overt diabetes in pregnancy^[17].

It is not appropriate to apply the diagnostic criteria of diabetes mellitus during non-pregnancy as the diagnostic criteria of abnormal glucose tolerance during pregnancy for the following reasons. Firstly, the altered hormonal environment and the presence of the fetus during pregnancy cease at the time of delivery. Secondly, the diagnostic criteria of diabetes mellitus during non-pregnancy are established based on the incidence of diabetic complications (especially, diabetic retinopathy); on the other hand, the diagnostic criteria of GDM are established for the prevention of diabetes mellitus of mothers and the prevention of perinatal complications of mothers and infants. The diagnostic criteria of GDM of each country that were used in the past were established to prevent future onset of diabetes mellitus, and differed among countries. These differences in the definition of GDM caused various problems in international discussions. Accordingly, the International Association of Diabetes and Pregnancy Study Groups announced worldwide uniform diagnostic criteria of GDM^[17] based on the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study^[18] reported in 2008 (Table 1). The HAPO study was conducted in 25505 pregnant women at 15 facilities in nine countries worldwide to evaluate outcomes of mothers and infants; the data of the partici-

Table 1 To diagnose gestational diabetes mellitus and cumulative proportion of Hyperglycemia and Adverse Pregnancy Outcome cohort equaling or exceeding those thresholds

Glucose measure	Glucose concentration threshold ¹		Above threshold (%)
	mmol/L	mg/dL	cumulative
FPG	5.1	92	8.3
1-h plasma glucose	10.0	180	14.0
2-h plasma glucose	8.5	153	16.1 ²

¹One or more of these values from a 75-g OGTT must be equaled or exceeded for the diagnosis of GDM; ²In addition, 1.7% of participants in the initial cohort were unblinded because of FPG > 5.8 mmol/L (105 mg/dL) or 2-h OGTT values > 11.1 mmol/L (200 mg/dL), bringing the total to 17.8% (modified from Ref.^[17]). FPG: Fasting plasma glucose; OGTT: Oral glucose tolerance test.

pants were opened to the primary physician only when fasting plasma glucose was not less than 105 mg/dL or plasma glucose at 2 h after meal was not less than 200 mg/dL; otherwise, the data were blinded to the primary physician. Primary outcomes included birth weight of not less than the 90 percentile, percentage of cesarean section, neonatal hypoglycemia, and cord serum C-peptide of not less than the 90 percentile; secondary outcomes included premature labor, shoulder dystocia/dystocia, hyperbilirubinemia, neonatal intensive care unit management, and pregnancy-induced hypertension syndrome. As a result, there was no threshold at which the incidence of primary outcomes showed a clear increase; plasma glucose level, whose odds ratio is 1.75 times higher than that in the lowest category, was adopted as the diagnostic cut-off value^[17]. Regarding glycemic control indicators, there were no clear diagnostic threshold. Thus, the shift from the diagnostic criteria of GDM based on the future incidence of diabetes mellitus of mothers to diagnostic criteria of GDM for improving perinatal outcomes of mothers and infants during pregnancy was a major event.

ADVERSE EVENTS DURING PREGNANCY OF GDM AND OVERT DIABETES MELLITUS

Because the definition of GDM is based on the incidence of perinatal complications of mothers and infants, we give an outline of perinatal complications once again. Maternal complications include pregnancy-induced hypertension syndrome, polyhydramnios, shoulder dystocia, and cesarean section. In pregnant women with diabetes mellitus, careful attention should also be paid to diabetic ketoacidosis, worsening of diabetic retinopathy and diabetic nephropathy, and hypoglycemia. For pregnancy-induced hypertension syndrome, 2% to 8% of all pregnant women are complicated with preeclampsia, which worsens perinatal outcomes. The late-onset form, which accounts for 80% of all cases of pregnancy-induced hypertension syndrome, is of maternal origin and is often accompanied by old age, obesity, diabetes mellitus, and chronic hypertension. That is to say, it is considered that abnormal glucose metabolism of mothers influences the

onset of pregnancy-induced hypertension syndrome. Next, it has been reported that 0.5% to 0.7% of normal pregnant women and 2.0% to 2.1% of patients with GDM are complicated with polyhydramnios^[19,20]. Polyhydramnios induces complications leading to perinatal death including premature labor, premature rupture of membranes, fetal malpresentation, weak labor, umbilical cord prolapse, premature separation of normally implanted placenta, and atonic hemorrhage after delivery. For pregnant women with diabetes mellitus, it has been reported that glucose concentration in amniotic fluid is related to maternal plasma glucose level^[21] and that there is a positive correlation between amniotic fluid volume and glucose concentration in amniotic fluid^[22]. Shoulder dystocia is a condition in which after the head of the infant is delivered in cephalic vaginal delivery, the shoulder of the infant is not delivered. It is a disease which may cause dystocia in both the mother and the infant. It is known that macrosomia is a risk factor for shoulder dystocia; on the other hand, it has been reported that pregnant women with abnormal glucose tolerance tend to experience shoulder dystocia regardless of the presence or absence of macrosomia^[23]. For these reasons and also because of complications of fetuses as mentioned later, the percentage of cesarean section is obviously higher in pregnant women with abnormal glucose tolerance; the percentage is 10.7%-18.9% in normal pregnant women, compared with 19.3%-30.9% in pregnant women with GDM and 45.2% in pregnant women with diabetes mellitus^[20,24-27].

Congenital anomaly is one of the complications of fetuses born from mothers with diabetes mellitus. According to a report in Japan, the incidence of congenital anomaly does not increase obviously when hemoglobin A1c (HbA1c) during the early stage of pregnancy is less than 7.4%; however, the incidence increases when HbA1c is 7.4% or more; the incidence is as high as 24.1% when HbA1c is 8.4% or more^[28]. Macrosomia as a developmental anomaly is a fetal developmental anomaly unique to pregnancy in women with diabetes mellitus. The hyperglycemia-hyperinsulinemia hypothesis proposed by Pedersen^[29] is that hyperglycemia of mothers induces hyperglycemia of fetuses, and hyperplasia of pancreatic β -cells of fetuses results in hypersecretion of insulin, leading to excessive growth of fetuses. Infants

born from mothers with diabetes mellitus are called infants of diabetic mothers and are known as high-risk infants in whom multiple complications develop at a high incidence^[30]. Such complications include hypoglycemia, polycythemia, hyperbilirubinemia, hypocalcemia, neonatal respiratory distress syndrome, and myocardial hypertrophy.

TREATMENT OF PREGNANT WOMEN WITH GDM AND OVERT DIABETIC MELLITUS

The basis of plasma glucose management during pregnancy is dietary therapy similar to that during non-pregnancy. As nutrition for fetuses during pregnancy, glucose, amino acids, and free fatty acids are supplied through the maternal placenta; the main energy source for fetuses is glucose. The main points of dietary therapy for pregnant women with abnormal glucose tolerance are to prevent ketosis of mothers resulting from insufficient carbohydrate intake and to perform strict glycemic control. In other words, energy intake at which the body weight of mothers before pregnancy does not decrease is determined as the basic food intake, and energy for fetuses according to the stage of pregnancy is added to it.

If the goal of glycemic control is not achieved in spite of dietary therapy, insulin therapy is selected. Currently, the types of insulin that can be used safely during pregnancy are human insulin as well as insulin aspart, insulin lispro, and insulin detemir; they are classified into the United States Food and Drug Administration (FDA) Pregnancy Category B. Insulin glargine and insulin glulisine are currently classified into the FDA Pregnancy Category C, and their potential risk cannot be ruled out. The principle of insulin therapy during pregnancy, which consists of supplementation of basal insulin secretion and supplementation of additional insulin secretion at the time of dietary intake, is the same as that during non-pregnancy. It should be noted that during the early stage of pregnancy, the insulin requirement decreases because of hyperemesis gravidarum, *etc.*; during and after the middle stage of pregnancy, insulin resistance increases; during the end stage of pregnancy, the insulin requirement increases to about two times that before pregnancy.

MEASUREMENT OF BLOOD GLUCOSE

In order to prevent perinatal complications of mothers and infants mentioned above, the goal of glycemic control during pregnancy should be to bring plasma glucose level as close to normal as possible without development of hypoglycemia. Strict glycemic control should be performed by self-monitoring of plasma glucose (SMBG) or continuous glucose monitoring (CGM).

SMBG

SMBG enables strict glycemic control. To achieve this, it is important to make patients understand why blood glucose should be measured, and to remind patients of the relationship between activity, events, meals, snacks, *etc.* and blood glucose levels. Because pregnant women tend to become anemic easily, this tendency should be taken into consideration. When hematocrit is low, plasma volume increases and plasma glucose level increases; conversely, when hematocrit is high, plasma volume decreases and plasma glucose level decreases. If insulin therapy is being performed, the insulin dose for glycemic control is adjusted based on the result of SMBG. The basic principle is to adjust basal insulin dose according to fasting blood glucose level and to adjust additional insulin dose according to postprandial blood glucose level. Langer *et al.*^[31] have reported that when fasting blood glucose is not less than 95 mg/dL in patients with GDM, the incidence of macrosomia significantly increases, and the incidence is decreased by insulin therapy. In addition, they have demonstrated that it is possible to decrease the incidence of both small-for-gestational-age and large-for-gestational-age by bringing mean blood glucose level to 87-104 mg/dL^[32]. On the other hand, it has been reported that adjusting insulin therapy according to postprandial blood glucose level results in an improvement of glycemic control, a decrease in neonatal hypoglycemia, a decrease in macrosomia, and a decrease in cesarean section^[33].

CGM

The CGM device can monitor blood glucose level every 5 min for up to 7 d. The device has demonstrated that there is a difference in circadian rhythm of blood glucose among non-diabetic pregnant women between normal-weight pregnant women and obese pregnant women^[34]. That is, compared with normal-weight pregnant women, obese pregnant women have comparable fasting blood glucose and comparable mean blood glucose but higher postprandial blood glucose and lower nighttime blood glucose. Compared with SMBG, the CGM device can monitor glycemic excursion in greater detail, but continuous wearing of the device is neither economically viable nor suitable for continuous evaluation. However, the CGM device is a useful education tool for pregnant women with diabetes mellitus, and it has been reported that by performing CGM every 4 to 6 wk during pregnancy, glycemic control during the third trimester of pregnancy improved, and the risk of macrosomia decreased^[35]. In addition, the ability to identify hypoglycemia is another noteworthy benefit of CGM. Rosenn *et al.*^[36] have demonstrated by CGM that 30% of pregnant women with type 1 diabetes mellitus experience at least three episodes of hypoglycemia during 2 wk. In such patients, it is possible to perform safer and more appropriate insulin therapy by performing CGM.

INDICATORS OF GLYCEMIC CONTROL

Plasma glucose measurement is important as part of glycemic control during pregnancy; however, it is actually difficult to measure blood glucose in all patients. Therefore, it is necessary to evaluate glycemic control condition using indicators of glycemic control such as HbA1c, glycated albumin (GA), fructosamine, and 1,5-AG. Each of these indicators of glycemic control has different characteristics as well as advantages and disadvantages^[37,38]. In addition, there are both appropriate and inappropriate indicators of glycemic control during pregnancy. The following sections give an outline of these indicators of glycemic control.

HbA1c

HbA1c is a ketoamine formed from nonenzymatic reaction and binding between the aldehyde group of glucose and valine at the N-terminus of the hemoglobin β -chain. Because the life span of red blood cells is 120 d, HbA1c reflects glycemic control status during the past 1 to 2 mo. Specifically, the following findings have been reported: 50% reflect plasma glucose level during the past 1 mo; 25% reflect plasma glucose level during the past 1 to 2 mo; 25% reflect plasma glucose level during the past 2 to 4 mo^[39]. Since the Diabetes Control and Complications Trial study^[40], extensive evidence on the development and progress of complications has been gathered, and HbA1c has certainly become a gold standard indicator of glycemic control. Therefore, it is recommended to maintain excellent glycemic control in pregnant women with diabetes mellitus or patients with GDM using SMBG and HbA1c as indicators^[41]. However, pregnant women are usually excluded from clinical studies of complications, and therefore little evidence has been obtained from pregnant women. Because chronic diabetic complications usually do not develop within a period as short as several months, there is no problem in using HbA1c as an indicator; however, there is little benefit in discussing glycemic control status during the past 1 to 2 mo of pregnancy. In addition, it is well known that HbA1c is influenced by the life span of red blood cells. Moreover, we have reported that for premenopausal women, HbA1c is significantly higher not only in women with iron deficiency anemia but also in women with iron deficiency compared with HbA1c in women without iron deficiency^[42,43]. It is well known that the demand for iron increases during the end stage of pregnancy and that most mothers experience iron deficiency anemia; therefore, HbA1c may be higher relative to plasma glucose level during the end stage of pregnancy. The time course of HbA1c during pregnancy will be explained in detail later.

Fructosamine

Protein undergoes glycation reaction in accordance with plasma glucose concentration, and ketoamine, an early Maillard reaction product, is produced *via* aldime. Because the side chain binding of ketoamine has the fructose structure, ketoamine is generically

named fructosamine. Fructose-lysine (fructosamine), in which glucose is bound to lysine residue of protein, has a reducing ability under alkaline conditions; glycemic control is measured using this reducing ability. A large part of such measurement is made by the fructosamine method; glycemic control is measured by colorimetric determination by producing reduction color reaction using nitroblue tetrazolium as a substrate. Because 60% to 70% of serum protein is albumin, the main component of fructosamine is GA, but fructosamine contains other components such as glycated lipoprotein and glycation globulin. Fructosamine is not influenced by anemia or abnormal hemoglobin. In addition, albumin, which accounts for the majority of serum protein, has a faster turnover than hemoglobin; therefore, short-term glycemic control can be evaluated by measuring fructosamine^[44]. In hyperthyroidism^[45,46] and nephrotic syndrome^[47] in which protein (albumin) metabolism is increased, fructosamine measured by this method is low; in hypothyroidism^[45] in which protein (albumin) metabolism is delayed, fructosamine measured by this method is high.

HbA1c is a glycation product of hemoglobin (single protein) and GA is a glycation product of albumin (single protein); on the other hand, fructosamine is the generic name of all glycated proteins and lacks specificity. Because 60% to 70% of serum protein is albumin, the characteristics of fructosamine are similar to those of GA. However, this method measures other glycated proteins as well; therefore, there is a problem that in myeloma, fructosamine measured by this method is high^[48]. In addition, it has been reported that fructosamine is associated with a larger intra-individual variability compared with HbA1c, and fructosamine is disadvantageous for detecting a significant change^[49]. HbA1c is expressed as the ratio of hemoglobin and GA is expressed as the ratio of albumin; therefore, HbA1c and GA are not influenced by dilution of serum. On the other hand, fructosamine is expressed as reducing ability per 1 mL of serum; therefore, fructosamine is influenced by serum protein concentration, and in dilutional anemia, fructosamine measured by this method is apparently low. In this respect, fructosamine measured by this method is likely to be influenced by dilutional anemia which may develop during pregnancy. Because fructosamine is measured by colorimetric determination produced by reduction color reaction, it is influenced by substances with reducing ability such as bilirubin. It is considered that the effects of ascorbic acid and vitamin E are small; however, if they are consumed in large amounts, measurement of fructosamine may be influenced.

GA

GA is a ketoamine formed from nonenzymatic reaction and binding between four lysine residues of albumin and glucose. In other words, GA is an amadori compound similar to HbA1c, but it has been reported that the binding rate between albumin and glucose is 4.5 times higher than that between hemoglobin and glucose^[50]. Because the half-life of albumin is about 14 d, GA is

an indicator of glycemic control during a shorter period (during the past 2 to 3 wk) compared with HbA1c. In addition, it is known that GA reflects postprandial plasma glucose more accurately than HbA1c^[37]. In the management of abnormal glucose metabolism during pregnancy, evaluation of mean plasma glucose level at a time point closer to the time of consultation with a doctor and evaluation of postprandial plasma glucose level are important, and GA is useful in this respect. Moreover, we have already reported that GA is not influenced by iron deficiency anemia or iron deficiency state^[43]. It should be noted that because GA is influenced by albumin metabolism, evaluation of measured GA levels requires attention in conditions such as nephrotic syndrome^[51] and abnormal thyroid function^[52]. Unlike fructosamine, GA is not influenced by dilutional anemia during pregnancy. The time course of GA during pregnancy will be explained in detail later.

1,5-AG

1,5-AG is a polyol with a structure in which hydroxyl at the first position of glucose is reduced; it is contained in a wide variety of food, but is hardly metabolized in the body^[53]. When plasma glucose is within the normal range, 1,5-AG is filtered in the kidney and then reabsorbed completely; therefore, serum 1,5-AG remains unchanged.

Usually, about 180 g of glucose is excreted from glomeruli daily, but almost 100% of the excreted glucose is reabsorbed by sodium glucose cotransporter 2 (SGLT2) which is a glucose-specific transporter and located in proximal tubules and SGLT1 which is a transporter for glucose and galactose and located downstream of SGLT2^[54]. When diabetes mellitus develops, excretion of glucose increases; if excretion of glucose exceeds the reabsorptive capacity of SGLT2 and SGLT1, reabsorption of glucose by 1,5-AG/mannose/fructose cotransporter (sodium glucose cotransporter 4: SGLT4) which is present downstream from SGLT2 and SGLT1 takes place. Usually, there is no glucose in locations where SGLT4 is present; therefore, 99.9% of 1,5-AG is reabsorbed by SGLT4; however, because SGLT4 reabsorbs glucose as well, if the inflow of glucose into tubules increases, reabsorption of 1,5-AG is inhibited^[55-57]. Specifically, if plasma glucose level exceeds 180 mg/dL, glucose is excreted in urine; therefore, 1,5-AG is also excreted in urine and serum 1,5-AG decreases.

Because of this mechanism, serum 1,5-AG reflects glycemic status during the past 24 h and is used as an indicator of very short-term glycemic control^[58,59]. In addition, serum 1,5-AG is an indicator which reflects postprandial hyperglycemia more accurately than HbA1c^[60,61]. It should be noted that in patients with marked hyperglycemia and a large urinary glucose excretion, even if glycemic control improves, serum 1,5-AG does not increase in a short period because the 1,5-AG pool in the body has decreased.

Because serum 1,5-AG is influenced by the threshold for urinary glucose excretion as well, serum 1,5-AG is low in renal glycosuria in which the threshold decreases. In

chronic renal failure^[62-64] in which reabsorption of 1,5-AG decreases, 1,5-AG is low because of transient glucosuria. In other conditions such as oxyhyperglycemia^[65], patients receiving long-term hyperalimentation^[66], and liver cirrhosis^[67,68], serum 1,5-AG is abnormally low. On the other hand, one of the causes of abnormally high 1,5-AG levels is oral administration of Ninjin-yoei-to and Kami-kihi-to^[69] which contain a large amount of 1,5-AG.

It has been reported that because the threshold for glucose in the kidney decreases during pregnancy, glucose tolerance may not change, and glucosuria may appear^[70]. It has also been reported that serum 1,5-AG during pregnancy is low because of this mechanism^[71]. Therefore, serum 1,5-AG during pregnancy does not reflect glycemic control accurately and is not an appropriate indicator of glycemic control.

CHANGE IN INDICATORS OF GLYCEMIC CONTROL DURING PREGNANCY

Change in indicators of glycemic control during normal pregnancy

In the past, Phelps *et al.*^[72] reported the time course of HbA1c during pregnancy in 377 non-diabetic pregnant women and the time course of plasma glucose level at 1 h after 50 g oral glucose loading in 1756 normal pregnant women. It was demonstrated that HbA1c shows a biphasic change with the trough level occurring at week 24 of pregnancy and that 1 h plasma glucose level also shows a biphasic change with the trough level occurring at week 20 of pregnancy. In a report of the Japanese Society of Diabetes and Pregnancy^[73], similar tendencies were shown; according to an analysis of 574 normal pregnant women, HbA1c tends to decrease during the middle stage of pregnancy and increase during the end stage of pregnancy, and GA tends to decrease gradually toward the end stage of pregnancy (Figure 1). Judging from this report, the reference range in Japanese normal pregnant women was considered to be 4.4% to 5.7% for HbA1c and 11.5% to 15.7% for GA. In any case, there is undoubtedly a difference between the time course of HbA1c and GA during pregnancy, so which indicator of glycemic control is reliable? We investigated the effect of iron deficiency on HbA1c in 17 normal pregnant women^[74]. HbA1c increased significantly from the middle stage of pregnancy (wk: 20-23) to the end stage of pregnancy (wk: 32-33) ($4.7\% \pm 0.2\%$ vs $5.1\% \pm 0.2\%$; $P < 0.0001$). On the other hand, GA showed no significant change. Mean corpuscular hemoglobin (MCH), transferrin saturation, and serum ferritin, which are indicators of iron deficiency, showed a decrease toward the end stage of pregnancy; there was a significant negative correlation between HbA1c and MCH, transferrin saturation, and serum ferritin (Figure 2). On the other hand, there was no significant correlation between GA and MCH, transferrin saturation, and serum ferritin. Based on the above findings, it is considered that in normal pregnant women, iron deficiency progresses during the end stage of pregnancy, and therefore HbA1c

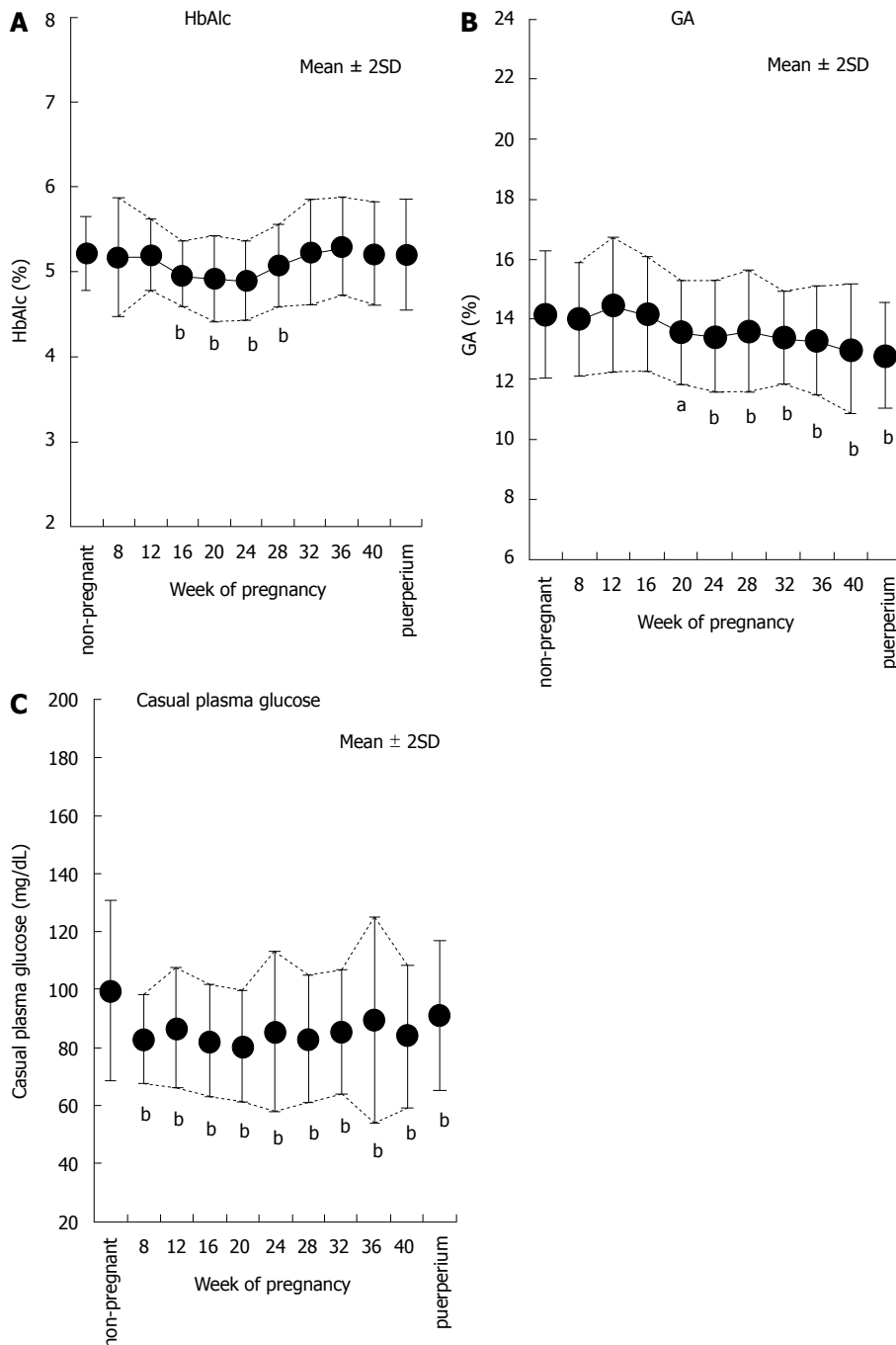


Figure 1 Time courses of indicators of glycemic control in normal pregnant women. The time courses of HbA1c (A), GA (B), and casual plasma glucose (C) in normal pregnant women are shown (modified from Ref.[73]). ^a $P < 0.05$, ^b $P < 0.01$ vs non-pregnant women. HbA1c: Hemoglobin A1c; GA: Glycated albumin.

level increases. That is, HbA1c during pregnancy may not be a reliable indicator of glycemic control, especially during the end stage of pregnancy. However, if pregnant women take in a sufficient amount of iron during pregnancy, increase of HbA1c may not occur from the middle stage to the end stage of pregnancy as shown in Figure 1.

Change in indicators of glycemic control of women with GDM and overt diabetes mellitus

Glycemic control status is important in pregnant women with diabetes mellitus and patients with GDM. For the time courses of HbA1c and GA in pregnant women with

diabetes mellitus and patients with GDM as well, the GA Study Group of the Japanese Society of Diabetes and Pregnancy has issued a detailed report^[75]. According to this report, in 193 pregnant women with diabetes mellitus and patients with GDM, HbA1c decreased during the middle stage of pregnancy and then increased during the end stage of pregnancy, as in the case of normal pregnant women. On the other hand, GA decreased as the gestational age advanced (Figure 3). However, gestational diabetes was not distinguished from pregnancy complicated with preexisting diabetes in this report. The time courses of HbA1c and GA were similar to

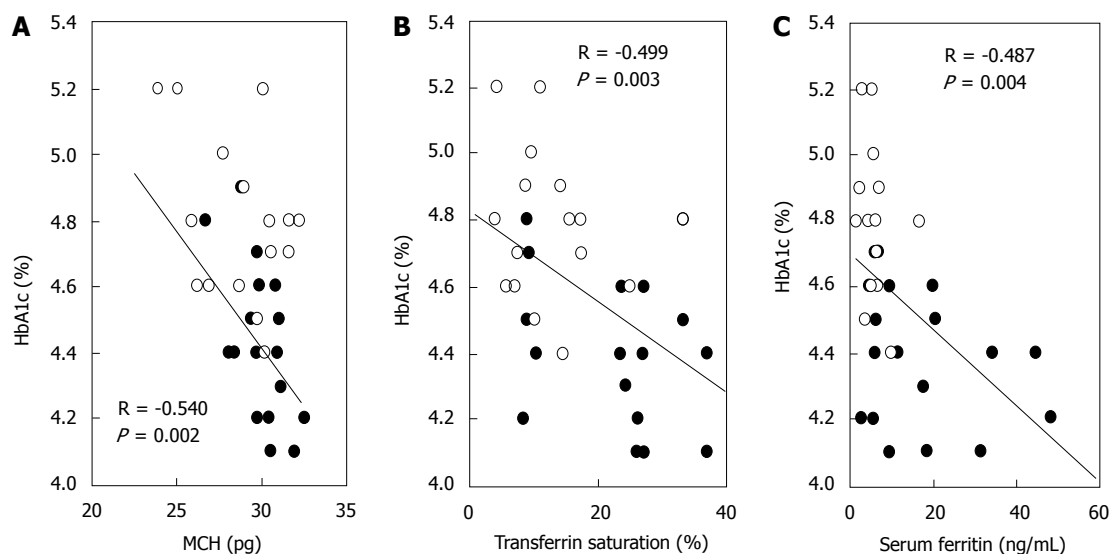


Figure 2 Correlations between hemoglobin A1c and indicators of iron deficiency in normal pregnant women. The correlations between HbA1c and mean corpuscular hemoglobin (MCH) (A), transferrin saturation (B), and serum ferritin (C) in normal pregnant women are shown (modified from Reference^[74]). ●: Middle stage of pregnancy (wk: 20-23); ○: End stage of pregnancy (wk: 32-33); HbA1c: Hemoglobin A1c.

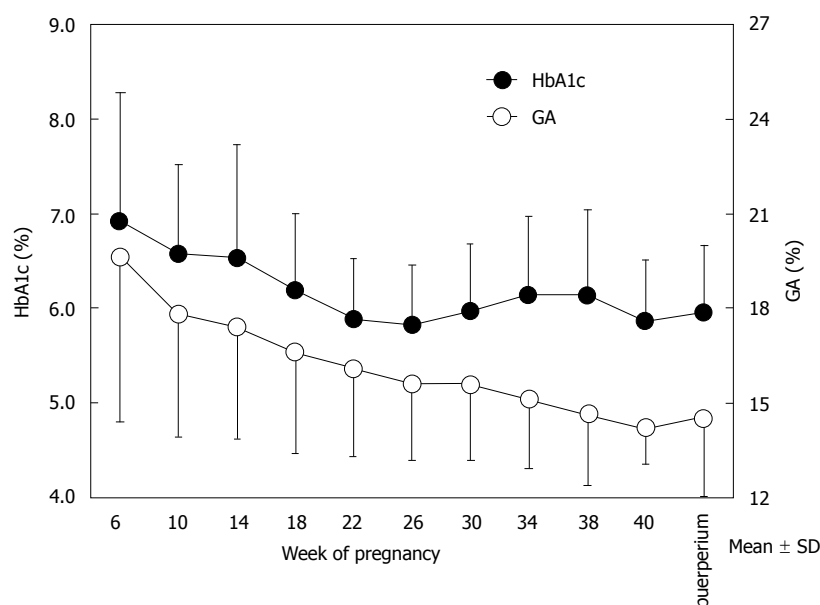


Figure 3 Time courses of hemoglobin A1c and glycated albumin in pregnant women with diabetes mellitus and patients with gestational diabetes mellitus. The time courses of HbA1c (closed circles) and GA (open circles) in pregnant women with diabetes mellitus and patients with gestational diabetes mellitus are shown (modified from Hiramatsu *et al.*^[73]). HbA1c: Hemoglobin A1c. GA: Glycated albumin.

those observed in normal pregnant women; as expected, there was a difference between time courses of different indicators of glycemic control. Therefore, we made a similar investigation in 11 pregnant women with diabetes mellitus (7 patients with type 1 diabetes mellitus and 4 patients with type 2 diabetes mellitus) and 6 patients with GDM^[76]. As in the case of normal pregnant women, HbA1c increased significantly from the middle stage of pregnancy (wk: 20-23) to the end stage of pregnancy (wk: 32-35) ($5.8\% \pm 0.7\%$ vs $6.1\% \pm 0.6\%$; $P < 0.05$), whereas GA showed no significant change. During the end stage of pregnancy, MCH, transferrin saturation, and

serum ferritin level decreased, and there was a significant positive correlation between transferrin saturation and the GA/HbA1c ratio. These results show that in pregnant women with diabetes mellitus and patients with GDM, iron deficiency progresses, and HbA1c increases during the end stage of pregnancy.

We introduce a typical pregnant woman with diabetes mellitus (36-year-old woman) as one of our patients. She was not obese before pregnancy (BMI before pregnancy was 22.7 kg/m^2) and treatment with insulin therapy was started before pregnancy. As the gestational age advanced, HbA1c increased from 6.2% (wk 18) to

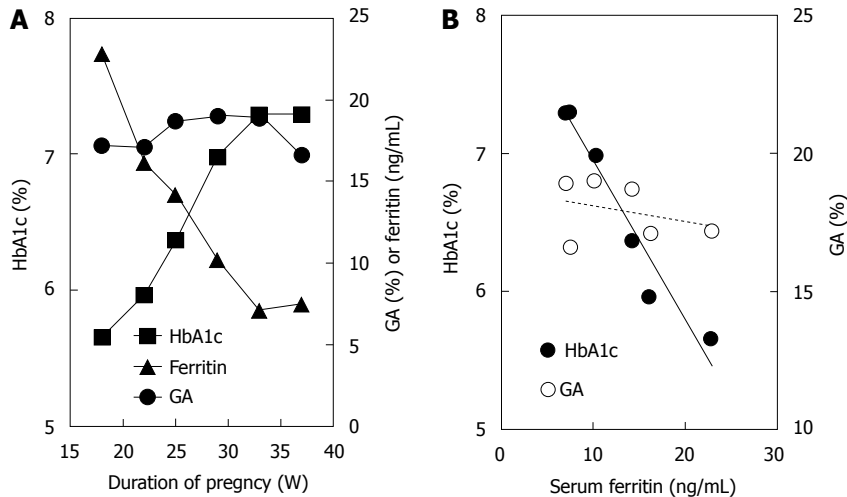


Figure 4 Time courses of hemoglobin A1c and glycated albumin and correlations between serum ferritin and hemoglobin A1c or glycated albumin in a pregnant woman with diabetes mellitus. A: The time courses of HbA1c (closed squares), GA (closed circles), and serum ferritin (closed triangles) in a pregnant woman with diabetes mellitus (a 36-year-old woman with type 2 diabetes mellitus receiving insulin therapy), are shown; B: Correlations between serum ferritin and HbA1c ($R = -0.975$, $P < 0.001$) or GA ($R = 0.322$, $P = 0.534$) in a pregnant woman with diabetes mellitus are shown. HbA1c: Hemoglobin A1c; GA: Glycated albumin.

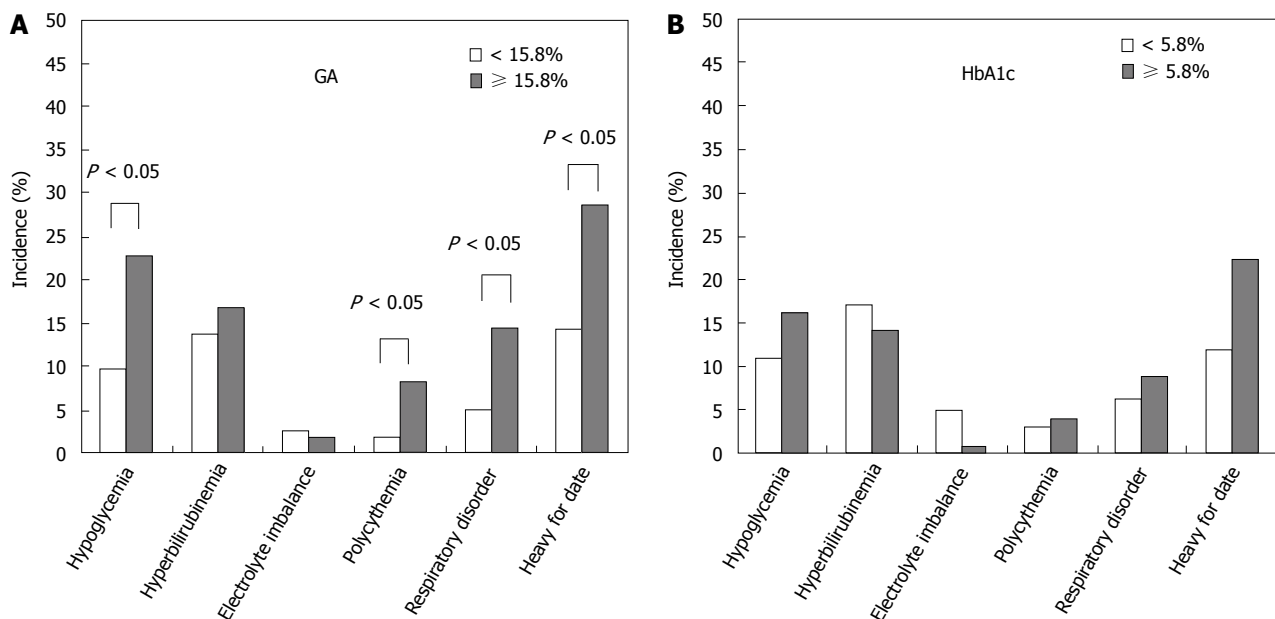


Figure 5 Comparison between glycated albumin and hemoglobin A1c during pregnancy and the incidence of neonatal complications. For GA (A) and HbA1c (B) measured during the end stage of pregnancy, the incidence of neonatal complications was compared between the group of women whose GA or HbA1c was within the reference range (GA < 15.8%; HbA1c < 5.8%) and the group of women whose GA or HbA1c exceeded the reference range (GA ≥ 15.8%; HbA1c ≥ 5.8%) (modified from Shimizu *et al.*^[75]). HbA1c: Hemoglobin A1c; GA: Glycated albumin.

7.3% (wk 37), which suggested worsening of glycemic control status. However, GA was stable between 17.2% (wk 18) and 16.6% (wk 37). In contrast, serum ferritin decreased from 22.8 ng/mL (wk 18) to 7.5 ng/mL (wk 37) during this period (Figure 4A). Because the time course of HbA1c and serum ferritin formed a mirror image, it was considered that the increase in HbA1c during the end stage of pregnancy was not due to poor glycemic control status but due to iron deficiency anemia (iron deficiency state). We emphasize that HbA1c ($R = -0.935$, $P < 0.001$), but not GA ($R = 0.322$, $P = 0.534$), was negatively correlated with serum ferritin (Figure 4B).

In conclusion, GA is not influenced by iron deficiency and so is a reliable indicator of glycemic control.

ASSOCIATION BETWEEN INDICATORS OF GLYCEMIC CONTROL AND COMPLICATIONS IN THE PERINATAL PERIOD

The GA Study Group of the Japanese Society of Diabetes and Pregnancy has analyzed the association between outcomes (neonatal complications and birth weight)

and indicators of glycemic control (HbA1c and GA)^[75]. The analysis was made considering the upper limits in normal pregnant women (HbA1c: 5.7%; GA: 15.7%); for neonatal complications, the incidences of neonatal hypoglycemia, polycythemia, and respiratory disorder were found to be significantly higher in the group of women with GA of more than 15.7% (Figure 5). In addition, it was reported that the incidence of large-for-gestational age was also significantly higher in the group of women with GA of more than 15.7% compared with the group of women with GA of 15.7% or less. On the other hand, it was reported that there was no significant increase in the incidence in the group of women with HbA1c of more than 5.7% compared with the group of women with HbA1c of 5.7% or less. Although a more accurate judgment should be made by ROC analysis for different cut-offs, GA is superior to HbA1c for prediction of perinatal complications. Furthermore, appropriate regression analysis is necessary to see if the indicator remains significant after eliminating the iron factors. As we demonstrated in our patients, if HbA1c is apparently high during the end stage of pregnancy, it may be misinterpreted that glycemic control has worsened and excessive insulin therapy may be performed, leading to hypoglycemia and increased incidence of perinatal complications of mothers and infants. Hence, management based on GA is essential during pregnancy also from the viewpoint of perinatal complications.

CONCLUSION

We outlined indicators of glycemic control in abnormal glucose metabolism during pregnancy. As explained, it is insufficient during pregnancy to use HbA1c as an indicator of glycemic control; glycemic control using GA is recommended. It is necessary to measure HbA1c to enable comparison with the large amount of data accumulated so far; the goal of management of abnormal glucose metabolism during pregnancy might be to maintain GA within the normal range (15.7% or less). However, because little data from clinical studies is available, large-population epidemiological studies would be necessary in order to confirm our proposal.

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Is there a relationship between vitamin D with insulin resistance and diabetes mellitus?

Kamal AS Al-Shoumer, Thamer M Al-Essa

Kamal AS Al-Shoumer, Division of Endocrinology and Metabolic Medicine, Department of Medicine, Faculty of Medicine, Kuwait University, 13110 Safat, Kuwait

Kamal AS Al-Shoumer, Thamer M Al-Essa, Division of Endocrinology and Metabolic Medicine, Department of Medicine, Mubarak Al Kabeer Hospital, 46304 Jabriya, Kuwait

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Correspondence to: Kamal AS Al-Shoumer, MD, FRCP, PhD, FACE, Professor and Consultant, Head, Division of Endocrinology and Metabolic Medicine, Department of Medicine, Faculty of Medicine, Kuwait University, PO Box 24923, 13110 Safat, Kuwait. kshoumer@gmail.com
 Telephone: +965-25-319596
 Fax: +965-25-313511

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Abstract

Available data suggest a possible link between abnormal

vitamin D level and abnormal glucose homeostasis, two of the most common chronic medical conditions. Both conditions are associated with inflammation, and the exact mechanism for role of either on the other is not well clear. Literature investigating the link between vitamin D and either pre-diabetic states or diabetes is reviewed. Vitamin D deficiency is detrimental to insulin synthesis and secretion in animal and human studies. In humans, it has been shown by majority of observational studies, that vitamin D is positively correlated with insulin sensitivity and its role is mediated both by direct mechanism through the availability of vitamin D receptors in several tissues and indirectly through the changes in calcium levels. Large number of, but not all, variable samples cross sectional human trials have demonstrated an inverse relation between vitamin D status and impaired glucose tolerance, insulin resistance or diabetes. To compliment this conclusively, evidence from intervention studies is critically warranted before we can frankly state that vitamin D plays a role in diabetes prevention or treatment. Absence of both sizable prospective observational trials utilizing 25(OH)D as the main variable and the non-availability of randomized studies specifically designed to assess the effects of vitamin D on pre-diabetes and diabetes states, are the main obstacles to draw solid and conclusive relationships.

Key words: Vitamin D; Insulin resistance; Type 2 diabetes

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Core tip: A potential role for abnormal vitamin D level in changes of glucose homeostasis has been described. It has been demonstrated that deficient vitamin D status is detrimental to the synthesis and secretion of insulin in animal and human studies. In several, but not all, human observational trials, an inverse correlation was seen between vitamin D with insulin insensitivity, pre-diabetic states and dysglycemia. However, evidence from randomized interventional studies assessing the

effects of changes in vitamin D status on markers of dysglycemia and diabetes prevention is not available. Therefore, firm and true protective influence of vitamin D on glucose homeostasis remains to be defined.

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INTRODUCTION

The widely common medical conditions, low vitamin D level and diabetes with its proceeding pre-diabetic state of insulin resistance, have become two of the most common chronic medical conditions diagnosed in modern years, both in developing and developed countries. There are around 387 million diabetic patients worldwide in year of 2014 and that number is projected to increase by 55% in 2035, as Africa is the highest projected region to have increased prevalence followed by the Middle East. The prevalence of impaired glucose tolerance, or insulin resistance, is even higher. Diabetes is leader in causing cardiovascular diseases and was responsible for 5.1 million deaths last year only^[1].

Vitamin D deficiency is now recognized as a pandemic. Its prevalence varies according to geographic location, season, ethnicity and the standard laboratory value of what is considered normal, deficient and insufficient vitamin D. It was estimated that there were about 1 billion individuals with low vitamin D in 2008 and the number most likely is higher now^[2]. Well-studied adverse outcomes of vitamin D deficiency are low bone density^[3], non-vertebral fractures^[4], increased risk of hip fracture^[5] and slowed walking speed^[6].

The link of vitamin D with insulin insensitivity or abnormal glucose metabolism gained much more scientific attention in the last decade. Several observations or associations were cited exploring the possible role for either altered vitamin D status and its metabolites or altered insulin sensitivity in the pathogenesis of the each disease. To gain more insight on the role of these variables, understanding of the metabolism of vitamin D and its relation to the pancreas is crucial.

VITAMIN D METABOLISM

In humans, vitamin D₃ is mostly obtained from endogenous vitamin D resources as a result of skin exposure to ultraviolet B light and only a minor portion is extracted from meals containing fortified milk and dairy food resources, eggs, and wild oily sea fish^[7] (Figure 1). It is crucial to note that vitamin D₂ is the non-animal plant derived form of vitamin D and is called ergosterol. Vitamin D₃ is a lipophilic precedent of the major circulating 25(OH)

D₃ metabolite which is hydroxylated, predominantly in the kidney, by a single enzyme 1 α -hydroxylase [1 α (OH)ase; CYP27B1] into the most active vitamin D₃ known as 1,25-dihydroxyvitamin D [1,25(OH)₂D₃], which may potentiate mineralization of bone *via* its role in the stimulation of calcium absorption in the intestine. Many immune cells also contain the machinery for the two-step conversion of vitamin D to 1,25(OH)₂D₃^[8]. Moreover, 1,25(OH)₂D₃ can be produced locally in the pancreas from the main circulating form, 25(OH)D₃, because 1 α -hydroxylase is present in islets^[9]. 25(OH)D₃ itself also has some biological activity, but the affinity of 1,25(OH)₂D₃ is about 1000-fold higher than 25(OH)D₃ for the vitamin D receptor (VDR). All metabolites of vitamin D are circulating in the bloodstream bound to the vitamin D-binding protein that has a different affinity for the individual metabolites^[10]. Seasonal factors, geographical variations, differences in skin color, age, and changes in lifestyle may make certain subjects more susceptible to develop vitamin D insufficiency [defined as 25(OH)D₃ concentrations 20-30 ng/mL or 50-75 nmol/L], or vitamin D deficiency [25(OH)D₃ concentration < 20 ng/mL or < 50 nmol/L]^[11] (Table 1).

POSSIBLE MECHANISMS BY WHICH VITAMIN D MAY INFLUENCE GLUCOSE INTOLERANCE AND TYPE 2 DIABETES MELLITUS

The development of abnormal glucose tolerance and type 2 diabetes mellitus is always preceded by alterations in the function of pancreatic β -cells, insulin sensitivity, and systemic inflammation. Available data suggest that these mechanisms are influenced by vitamin D.

β -cell function of the pancreas

Responses of insulin to glucose load appears to be exclusively influenced by vitamin D. Vitamin D does not appear to affect basal insulin^[12,13]. A positive role for vitamin D in the modification of the function of β -cells of the pancreas has been reported^[14]. This role is mediated through several pathways, including direct stimulation of insulin secretion by vitamin D through the presence of vitamin D receptors (VDRs) in β -cells of the pancreas^[14] and their expression of 1- α -hydroxylase enzyme^[9]. Also, 1,25-(OH)₂D is able to activate transcription of the gene of human insulin and thus play an essential role in insulin secretion^[15]. In mice, it has been shown that insulin secretory response may be impaired if the functional VDRs were absent^[13]. Several animal studies have also shown that when those were supplemented with vitamin D, they became able to restore their insulin secretion^[12,16-19]. In human studies, introduction of vitamin D was associated with improvement in release of insulin in some^[20-23], not all^[21,22,24], limited-scale short-term studies.

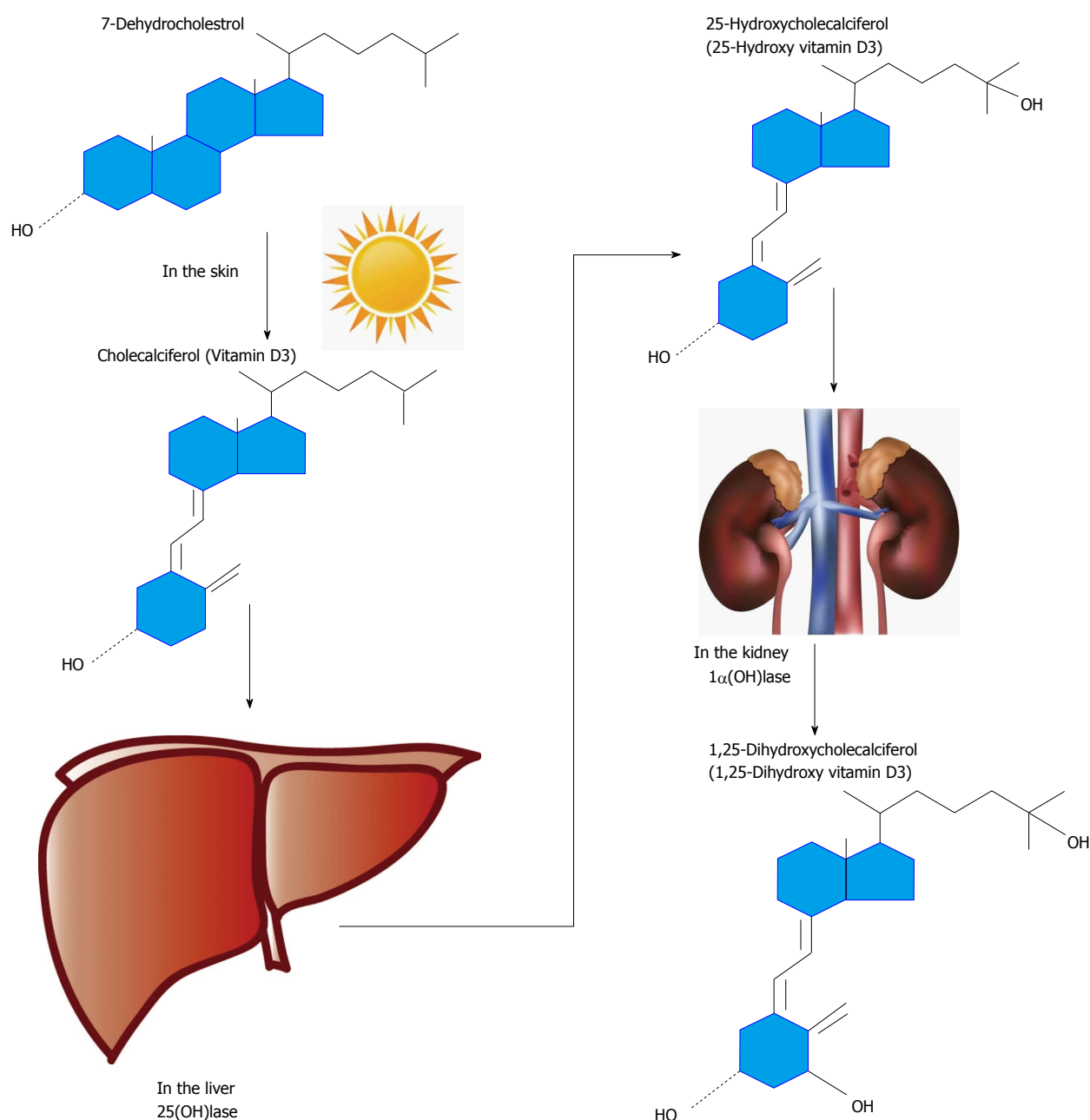


Figure 1 Schematic overview of the metabolism of vitamin D. Synthesis of vitamin D3 starts in the skin where 7-dehydrocholesterol is converted into vitamin D3 in response to UVB exposure. Vitamin D3 is hydroxylated by 25-hydroxylases in the liver. The resulting 25-hydroxyvitamin D3 is then hydroxylated in the kidney by 1 α hydroxylase, to produce the final activated product, 1,25-dihydroxyvitamin D3.

Through its regulatory role of the calcium pool of β -cell intracellularly and extracellularly, vitamin D insufficiency appears to affect normal release of insulin^[25] particularly in reaction to a glucose intake since the secretion of insulin is mediated by a calcium dependent mechanism. Some^[20,21,26-28], compared with other^[22,29], studies of variable cohorts including diverse baseline status of vitamin D have reported a link between deficiency of vitamin D and impairment of release of glucose-induced insulin. Moreover, a role of calbindin-D28k (a calcium-buffering protein in pancreatic beta cell) in calcium regulation and modulation of insulin release has been described^[25].

Insulin insensitivity

Improvement in action of insulin may be mediated by vitamin D directly through the presence of VDRs in skeletal muscles^[30], stimulation of expression of insulin receptors in bone marrow cells^[31] and through vitamin D activation of peroxisome proliferator activator receptor- δ ^[32], a transcription factor involved in the control of metabolism of fatty acids in adipose tissue and skeletal muscle^[33]. The indirect role of vitamin D is *via* the regulation of pools of intracellular and extracellular calcium and control of normal influx of calcium through the membranes of cells. Some^[27,34] studies have demonstrated a negative association of vitamin D with

Table 1 Accepted cut-off values of 25 hydroxyvitamin D3 (25OHD3) that describe vitamin D status

Vitamin D status	25OHD3 (nmol/L) ¹
Sufficient (optimal)	> 75
Insufficient	50-75
Deficient	< 50

¹Conversion factor, 1 nmol/L = 2.5 ng/mL.

insulin insensitivity, but this was not shown by others^[22].

Inflammation

In the state of systemic inflammation that T2DM can create based on wide range of clinical studies^[35-37], altered function of β -cells triggered by apoptosis of β -cell can develop due to the presence of elevated cytokines that can also induce insulin resistance directly. Vitamin D can act to lower systemic inflammation in general by interacting with components in the region of promotion of cytokine genes interfering with generation and action of cytokines through impeding the role of factors involved in nuclear transcription^[38-40].

Specifically to insulin insensitivity, vitamin D was demonstrated to under-regulate the activation of nuclear factor- κ B^[38,40,41], which plays a regulatory role for genes of cytokines of pro-inflammation implied in resistance of insulin^[42]. On the other hand, data from human research with inconsistent outcome that have directly assessed the association of vitamin D or calcium status and systemic inflammation in relation to type 2 diabetes mellitus were reported^[43-46].

EVIDENCE FOR VITAMIN D LINK WITH INSULIN RESISTANCE

Several trials have demonstrated an association between deficiency of vitamin D with increasing body mass index. One of those was a population trial from Norway with data from 10229 subjects, revealing an inverse association of 25(OH)D concentrations with BMI which was not only seen in summer, but also in winter months^[47]. So levels of 25(OH)D may change in seasons, but not body mass index. Furthermore, the same trial reported results from 2656 studied subjects in a longitudinal study between 1994 until 2008, which showed a negative predictor role for the changes of BMI in levels of 25(OH)D in that a reduction of more than 1 kg/m² in BMI, would result in an estimated increase of 2.8 ± 19.9 nmol/L in levels of 25(OH)D.

In a study of adults from North America by Devaraj *et al.*^[48], prediabetic state (state of a fasting plasma glucose concentration of 6.1-6.9 mmol/L, a 2 h glucose concentration of 7.8-11 mmol/L, or glycosylated hemoglobin of 5.7%-6.4%), a form of insulin resistance presentation, was noted to be associated with serum 25(OH)D in the first quartile in comparison with the fourth quartile in association with an adjusted odds ratio

of 1.47^[48]. The same study showed that in patients with metabolic syndrome, concentration of 25(OH)D was negatively associated with fasting glucose and homeostasis insulin resistance model of assessment.

Improvement in 25(OH)D status in T2DM patients was shown to be linked to some improvements in insulin sensitivity^[27], but still, other parameters of insulin resistance like obesity did not change significantly with vitamin D supplementations in other studies. The data from the Norwegian study (The Tromsø study), also included an intervention arm where 93 subjects of varying BMI values received vitamin D at 40000 IU weekly for a year^[47]. At the end of trial, increased vitamin D₃ doses were not associated with significant decrease in weight. The intervention showed that individuals with obesity needed bigger vitamin D doses than lean ones to achieve similar concentration of 25(OH)D, and a similar outcome to this was demonstrated in another study by Lee *et al.*^[49]. At the end of the trial with non-significantly different 25(OH)D values at baselines and vitamin D treatment doses, subjects with higher BMIs had lower concentrations of 25(OH)D compared with those of lower BMIs indicating that possibly body composition and insulin resistance in higher BMI subjects have a regulatory influence on vitamin D absorption, metabolism and/or storage.

The negative association between body weight, together with evidence of increased adiposity and low adiponectin levels, and low 25(OH)D concentrations also were shown in different age groups including both children and adolescents. Deficiency of vitamin D was prevalent in young Norwegian subjects^[50], African-American adolescents^[51], in both black and Caucasian youth^[52] and tropical locations like Malaysia and Colombia^[53,54].

Those prevalence studies remain with low scientific evidence since they are only observational and it is difficult to draw a causality relationship from them due to multiple confounders. Despite attempts to control those confounders, still full causality cannot be achieved.

DATA ON VITAMIN D LINK WITH T2DM

Large number of human trials, mainly cross-sectional and some longitudinal, have demonstrated a negative correlation of vitamin D status with predominant hyperglycemia. This correlation was shown both in children and adults, in each gender, and in diverse backgrounds of ethnicity^[48,55-61]. Also have been reported that seasonal variation in diabetes control being worse during winter months when vitamin D levels in their lowest^[62]. Beside prevalence, incidence of T2DM with decreased level of vitamin D has also been shown in majority of longitudinal but observational trials. It is therefore worth to go through the available observational or intervention trials.

Observational studies for T2DM incidence in association with altered vitamin D

In the recent review of systematic analysis of obser-

Table 2 Data of selected epidemiological studies on the relation between vitamin D level and markers of insulin resistance (A), insulin resistance (B) and type 2 diabetes (C)

Ref.	No. of subjects	Age, mean or range	Trial outcome
(A)			
Jorde <i>et al</i> ^[47]	10229	58	25OHD negatively associated with BMI
Lee <i>et al</i> ^[49]	95	68, 47-91	25OHD negatively related to BMI
Lagunova <i>et al</i> ^[50]	102	8-19	↑ prevalence of Vit D Def. (19%) and insuff. (> 50%) in obese
Suijder <i>et al</i> ^[60]	453	> 65	↑ BMI is associated with ↓ 25OHD
(B)			
Chiu <i>et al</i> ^[27]	125	26	+ve relation between 25OHD and insulin sensitivity
Nunlee-Bland <i>et al</i> ^[51]	34	10-20	↓ 25OHD is associated with insulin resistance
Shankar <i>et al</i> ^[61]	12719	> 20	↓ 25OHD is associated with pre-diabetes state
(C)			
Song <i>et al</i> ^[63]	76220	meta-analysis	inverse relation between 25OHD and risk for T2DM
Afzal <i>et al</i> ^[64]	9841	48-65	↓ 25OHD is associated with ↑ risk for T2DM
Pittas <i>et al</i> ^[65]	95243	meta-analysis	↓ incidence of T2DM in highest vs lowest 25OHD
Buijsse <i>et al</i> ^[69]	53088	50.9	HR of T2DM is ↓ with ↑ in 25OHD

T2DM: Type 2 diabetes mellitus; BMI: Body mass index.

vational studies, Song *et al*^[63] described a reduction of 38% in relative risk in diabetes incidence for subjects with the highest compared with the lowest group of serum of 25(OH)D₃ level. 21 prospective studies, largely population-based studies with white subjects, were included in the analysis involving about 70000 participants. The relations of 25(OH)D concentration and risk of diabetes were weakened but remained significant following corrections for hypertension and BMI. The association was not influenced by gender, sample size of study, period of follow up time, diagnostic criteria for diabetes, or method of 25(OH)D assay. About 4% reduction in T2DM risk was seen for each increment of 10 nmol/L (4 ng/mL) in serum 25(OH)D₃ level. In similar review studies, but with smaller numbers, a meta-analysis found a higher relative risk of 50% for the development of T2DM in low vs high 25(OH)D concentrations^[64]. In that last study, analyses stratified according to study design did not alter the appreciated association substantially.

Most of data analysis for risk of developing T2DM in relation to vitamin D status has used cutoffs categorizing vitamin D deficiency or insufficiency that is less clinically practiced in the last few years. In majority of trials, serum value of 25(OH)D above 50 nmol/L is considered sufficient while a large body of evidence supported by scientific agreement is considering a level above 75 nmol/L to be sufficient to execute its biological effect^[11].

Even though serum level above 75 nmol/L of 25(OH)D could be beneficial to multiple physiological effects, its protective effect against developing T2DM compared to levels in the insufficient range (75-50 nmol/L) is doubtful or at least needs further investigation. In data from large number of participants of 9841 from The Copenhagen City Heart Study, a long-term prospective study with a median of 20 years follow up of the general population of Denmark, increased hazard ratios (HR) for T2DM with decreased concentrations of 25(OH)D by clinical severities and season quartiles were noted. For 25(OH)D less than 12.5 nmol/L compared with levels

more than 50 nmol/L, the HR was 1.22, and was 1.35 for lowest compared with highest quartile^[64]. This was not clinically significant when the values of more than 75 nmol/L is used as a sufficient level compared with values of 25(OH)D between 75-50 nmol/L (HR 0.91).

Serum vitamin D levels are influenced significantly by dietary habits, mainly consumption of dairy products. After a prospective follow up for 20 years, in a cohort of the Nurses Health Study, an inverse association between T2DM risk with total 25(OH)D and calcium intake was described^[65]. The analysis showed that consumption of 3 or more vs only one daily dairy serving was associated with decreased risk of development of diabetes.

Several genetic studies have identified a relationship of circulating 25(OH)D with presence of polymorphisms of single nucleotide in six genetic regions^[66-68]. In an observational study of Buijsse *et al*^[69] where eight of those polymorphisms of single nucleotides, most strongly associated with 25(OH)D, were tested in relation to levels of 25(OH)D and T2DM incidence in an observational prospective case-control manner. In that study, it was found that in a population with relatively low serum values of 25(OH)D, 25(OH)D was inversely associated with T2DM risk, for concentrations of 25(OH)D below 45 nmol/L only (compared with higher levels), after controlling for measures of general and abdominal adiposity. After being adjusted for age, gender, center, and month of the year blood drawn, HR of T2DM per 5 nmol/L higher 25(OH)D was 0.92. But this study also found that genetically determined 25(OH)D was not related to T2DM across the entire 25(OH)D range or below 45 nmol/L. This latter finding argues against a strong causal relationship of 25(OH)D with T2DM but requires further investigation in larger research groups.

Caution has to be applied when making conclusions from observational studies due to possible multiple confounding factors affecting vitamin D status like age, race, dietary habits and level of activity, which are also known to play a role in increased risk for development of diabetes. Table 2 shows summary of selected epidemio-

logical studies on the association of vitamin D with markers of insulin insensitivity, pre-diabetic resistance to insulin and T2DM is displayed in Table 2.

Randomized intervention trials for the relation of vitamin D and T2DM

Direct evidence of a role for vitamin D in diabetes prevention and treatment is critically needed from interventional randomized studies before any conclusion can be made. Those needed intervention trials, ideally large-scale randomized trials, are lacking today. What is available are either data of scattered small-scale trials or some of post hoc data from analyses of somewhat larger studies on the influence of supplementation of vitamin D on parameters related to diabetes and those were either inconsistent or inconclusive, though vitamin D is known to have certain advantageous effects in subjects with increased diabetes risk^[43,64,70,71]. There are several limitations that can make it difficult to draw conclusions of solid nature from the limited available-to-date small interventional trials. Some of those trials were intended mainly to assess outcomes on glycemia and the majority of them were underpowered. Also, there was a variation in the dosing of vitamin D for replacement, some of these studies used supraphysiological vitamin D doses at infrequent manner, while others used daily doses and this would potentially cause different pharmacokinetic effect on concentration of 25(OH)D and pharmacotherapeutic effects on target cells.

In description of some of those trials, data analysis of the well known randomized trial of Women's Health Initiative^[72], in which about 50% of the women had 25(OH)D concentration less than 45 nmol/L, revealed no effect of administration of vitamin D3 at 400 IU and calcium at 1000 mg daily for 7 years on risk of diabetes (HR 1.01). Two smaller randomized trials tested the glycemic effect of applying vitamin D3 in subjects with impaired fasting glucose; the first one found that people using vitamin D3 daily at 700 IU and calcium at 500 mg for 3 years had a less rapid worsening of glycemia than those on placebo^[43]. The second trial supplemented with vitamin D3 to get 25(OH)D levels between 150 and 225 nmol/L^[73]. After 1 year, no effect was seen on incident diabetes. Together with the mixed findings of short-term trials on the influences of vitamin D on release of insulin and its sensitivity^[73,74], the experimental evidence today is inconsistent.

CONCLUSION

In conclusion, data from non-interventional observational trials have shown a negative relationship between the status of vitamin D and parameters of insulin insensitivity and incidence of T2DM. A biological active position for vitamin D in both insulin secretion and action, and in the function of β -cells has been considered. However, definitive conclusion for a causative link for vitamin D with T2DM can not be drawn due to the missing of

large-sized prospective observational investigations that use 25(OH)D as the target variable and the absence of randomized trials particularly designed to assess the influence of vitamin D on diabetes. Similar randomized prospective trials are needed to correctly explain the outcome of vitamin D administration as an interventional agent for preventing and managing diabetes. We anticipate that these future well designed randomized prospective trials to answer several important questions. Firstly, whether the daily interventional utilization of vitamin D in the pre-diabetic states works as a strong defensive tool against progression to type 2 diabetes? Secondly, whether the daily intake of vitamin D will be accompanied with significant glycemic improvement? And finally, whether supplementation of vitamin D to diabetics will delay or prevent some of the adverse diabetic complications or have positive effects on cardiometabolic outcomes in long term.

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Gestational diabetes: A clinical update

Ulla Kampmann, Lene Ring Madsen, Gitte Oeskov Skajaa, Ditte Smed Iversen, Niels Moeller, Per Ovesen

Ulla Kampmann, Lene Ring Madsen, Niels Moeller, Department of Endocrinology and Internal Medicine, Aarhus University Hospital, 8000 Aarhus, Denmark

Gitte Oeskov Skajaa, Ditte Smed Iversen, Per Ovesen, Department of Obstetrics and Gynecology, Aarhus University Hospital, 8000 Aarhus, Denmark

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Correspondence to: Ulla Kampmann, MD, PhD, Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Nørrebrogade 44, 8000 Aarhus, Denmark. ulla@opstrup.dk
Telephone: +45-2-2370857
Fax: +45-8-9492072

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Abstract

Gestational diabetes mellitus (GDM) is increasing in prevalence in tandem with the dramatic increase in

the prevalence of overweight and obesity in women of childbearing age. Much controversy surrounds the diagnosis and management of gestational diabetes, emphasizing the importance and relevance of clarity and consensus. If newly proposed criteria are adopted universally a significantly growing number of women will be diagnosed as having GDM, implying new therapeutic challenges to avoid foetal and maternal complications related to the hyperglycemia of gestational diabetes. This review provides an overview of clinical issues related to GDM, including the challenges of screening and diagnosis, the pathophysiology behind GDM, the treatment and prevention of GDM and the long and short term consequences of gestational diabetes for both mother and offspring.

Key words: Gestational diabetes; Diagnostic criteria; Treatment; Complications

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Core tip: Gestational diabetes is increasing in prevalence coincidentally with the dramatic increase in the prevalence of overweight and obesity in women of childbearing age. Much controversy surrounds the diagnosis and management of gestational diabetes, making it an important subject to discuss as the risk of foetal and maternal complications are increased in gestational diabetes. This review provides an overview of issues related to gestational diabetes, including the challenges of screening and diagnosis, the pathophysiology behind gestational diabetes, the treatment and prevention of gestational diabetes and the long and short term consequences of gestational diabetes for both mother and offspring.

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INTRODUCTION

Gestational diabetes mellitus (GDM) occurs in about 5% of pregnancies but figures vary considerably depending upon the criteria used and demographic characteristics of the population. The prevalence is expected to increase as the epidemic of obesity continues^[1]. Pregnancies affected by GDM impose a risk for both mother and child as the risk of cesarean and operative vaginal delivery, macrosomia, shoulder dystocia, neonatal hypoglycemia and hyperbilirubinemia is increased^[2]. Women with a history of GDM are also at an increased risk of developing type 2 diabetes mellitus (T2DM) in the years following their pregnancy and their children have a higher risk of developing obesity and T2DM early in life^[3].

For those reasons it is important to pay rigorous attention to GDM and the purpose of this review is therefore to cover a wide range of clinical issues related to GDM, including the challenges of epidemiology, diagnostic criteria and screening, the pathophysiology of GDM, the treatment and prevention of GDM and the long and short term consequences of GDM for both mother and child.

EPIDEMIOLOGY

It is problematic to determine the true prevalence of GDM. The prevalence varies worldwide and even within a country's population, depending on the racial and ethnic composition of the residents. Accordingly, in the United States the prevalence is higher amongst African American, Hispanic American, Native American, Pacific Islander, and South or East Asian women than in Caucasian women^[4]. Furthermore the prevalence of GDM differs depending on the variety of screening strategies (universal or selective), diagnostic criteria and the prevalence of T2DM in any specific country. While data from western countries are frequently reported, data from developing countries are sparse. Recently Jiwani *et al.*^[5] and Macaulay *et al.*^[6] tried to determine the prevalence of GDM worldwide, including developing countries. The prevalence was found to be ranging from < 5% in countries such as Pakistan, Belgium, Denmark, Estonia, Ireland, South Korea, South Africa and United Kingdom, to < 10% in Italy, Turkey, Brazil, United States, Morocco and Australia, to a prevalence as high as 20% in Bermuda and Nepal. A recent report from the International Diabetes Federation estimated that worldwide 16% of live births in 2013 were complicated by hyperglycemia during pregnancy^[7] and it is most likely that the prevalence of GDM will increase due to the increase in risk factors like obesity and physical inactivity.

SCREENING AND DIAGNOSIS

Recently the American Diabetes Association (ADA) defined GDM as "diabetes diagnosed during pregnancy that is not clearly overt diabetes"^[8]. Screening and diagnostic testing for GDM is however important in order

to identify the women at risk for developing GDM and thereby reduce or prevent the risk of adverse events for both mother and child associated with GDM.

In most countries a selective screening is carried out, using parameters such as previous GDM, previous large for gestational age babies, diabetes (of any kind) in first degree relatives, pre-pregnancy adipositas, belonging to a particular ethnic group associated with a high prevalence of GDM, glucosuria, and high maternal age. By using selective screening there is a risk of missing GDM cases. On the other hand, selective screening could help to concentrate medical resources on subjects with the highest risk of complications.

Also, screening for preexisting diabetes in the very early weeks of pregnancy by the measurement of a fasting glucose is warranted. This is important because of the rising prevalence of T2DM at younger ages. Accordingly there is an increasing number of young women in their twenties and thirties presenting with undiagnosed preexisting T2DM.

Pregnant women have a higher physiological turnover of erythrocytes, rendering glycosylated hemoglobin (HbA1c) inadequate as a diagnostic tool, because of underestimation of the average glucose level. In fact a reduction of HbA1c is seen in normal pregnancy^[9]. Instead, a variety of oral glucose tolerance tests (OGTT) have been applied, but a consensus regarding screening for and classification of GDM is yet to be achieved globally^[10]. However, a 2-h 75 g OGTT at 24-28 wk of gestation is now being recommended both by the European Association for the Study of Diabetes, International Association of Diabetes and Pregnancy Study Group (IADPSG), ADA and World Health Organization (WHO)^[6].

The HAPO study recently demonstrated that no specific threshold for the risk of adverse events for both mother and child associated with GDM can be set as the risk increase is continuous^[11]. Other studies^[12-14] have supported the idea of lowering the diagnostic threshold in the diagnostic criteria for GDM, taking the maternal and foetal risks of hyperglycemia into consideration. In 2010 the IADPSG outlined new diagnostic criteria for GDM^[15] based on the knowledge achieved in the HAPO study. This new guideline from IADPSG was adopted by the WHO in 2013^[16] and ADA in 2014^[8] and is based on the risk of adverse pregnancy outcomes As shown in Table 1 the threshold for a positive test is exceedance of one of the following three plasma glucoses; fasting plasma glucose ≥ 5.1 mmol/L (≥ 92 mg/dL), 1 h ≥ 10.0 mmol/L (180 mg/dL), or 2 h ≥ 8.5 mmol/L (153 mg/dL)^[15]. In comparison the WHO recommended threshold in 1999 was fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL) and in 1985 fasting plasma glucose ≥ 7.8 mmol/L (140 mg/dL)^[6].

It has been estimated that with this new diagnostic criteria the prevalence of GDM will increase to nearly 18%^[11], which will have a major impact on the costs, the capacity of the health care systems, and the pathologization of pregnancies that were earlier categorized as normal. The vast majority of the women diagnosed with

Table 1 New (2013) World Health Organization recommendations for the diagnosis of gestational diabetes based on the general principles behind how the IADPSG criteria were derived

Gestational diabetes mellitus should be diagnosed at any time in pregnancy if one or more of the following criteria are met	
Fasting plasma glucose	5.1-6.9 mmol/L (92-125 mg/dL)
1-h plasma glucose following a 75 g oral glucose load	≥ 10.0 mmol/L (180 mg/dL)
2-h plasma glucose following a 75 g oral glucose load	8.5-11.0 mmol/L (153-199 mg/dL)

If fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL), and/or 2-h plasma glucose ≥ 11.1 mmol/L (200 mg/dL) and/or random plasma glucose ≥ 11.1 mmol/L (200 mg/dL) in the presence of diabetes symptoms overt diabetes is diagnosed.

GDM will however have mild hyperglycemia, requiring non-pharmaceutical treatment, including lifestyle modifications.

PATHOPHYSIOLOGY

In normal pregnancy, maternal tissues become progressively insensitive to insulin. This is believed to be caused partly by hormones from the placenta and partly by other obesity and pregnancy related factors that are not fully understood.

Skeletal muscle and adipose tissue are the main whole-body glucose disposable sites. In normal pregnancy, insulin-mediated whole-body glucose disposal decreases by 50% and in order to maintain a euglycemic state, the woman must increase her insulin secretion by 200%-250%^[17].

GDM develops when the pregnant woman is not able to produce an adequate insulin response to compensate for this normal insulin resistance.

GDM is observed in obese as well as in lean women. However, the pathophysiology behind the disease is believed to differ between these groups. In obese women, the pathophysiology is primarily characterized by the pregnancy-induced insulin resistance being amplified by the already elevated pre-pregnant insulin resistance level. The elevated insulin resistance level is a known factor in the metabolic syndrome. In lean women, the same factors seem to play a role but a defect in the first-phase insulin response contributes to a larger extent^[18].

These defects culminate in a disruption of the action of insulin in maintaining glucose levels, resulting in maternal hyperglycaemia. Glucose is transferred *via* the placenta to the fetus. Maternal hyperglycaemia therefore stimulates a foetal hyperinsulinaemia to counter the excess placental glucose transfer. The high insulin level in the fetus stimulates growth which results in foetal macrosomia (birth weight over 4000 g)^[19].

RISK FACTORS FOR DEVELOPING GDM

There is a range of established risk factors for GDM, chief amongst which are the following. The Hyperglycemia

and Adverse Pregnancy Outcome (HAPO) study reported that a higher pre-pregnant BMI and the BMI at 28 wk are strongly correlated to increased insulin resistance at 28 wk^[11]. Adipose tissue is, like the placenta, believed to produce a large amount of diabetogenic adipokines. Especially the adipokine TNF- α , which the placenta likewise produces, is suspected to play an important role in insulin resistance pathways. This could be one explanation to the elevated pre-pregnant insulin resistance level seen in obese women^[20].

As mentioned previously, ethnicity seems to play an important role as well. Berkowitz *et al.*^[21] reported that the United States Native Americans, Asians, Hispanics, and African-American women have a higher risk of GDM compared to non-Hispanic white women. In addition studies have shown that women from Asia are at very high risk of developing GDM and the increased insulin resistance is observed at much lower BMI levels when compared to European women. Retnakaran *et al.*^[22] reported that Asian women's pre-pregnancy BMI has a greater influence on the pregnancy related insulin resistance than that of Caucasian women.

Cypryk *et al.*^[23] reported that maternal age over 25 years and previous GDM are strongly correlated to development of GDM. These findings are in agreement with other authors^[23-25]. In addition Polycystic Ovary Syndrome, multiparity, twin pregnancy and a family history of diabetes are well known risk factors^[26].

COMPLICATIONS DURING PREGNANCY AND BIRTH

Women with GDM are at higher risk of hypertensive disorders including gestational hypertension, preeclampsia, and eclampsia. In the HAPO study, 5.9% had gestational hypertension and 4.8% had preeclampsia. The study showed that the glucose level at the first glucose tolerance test was positively correlated with the risk of preeclampsia^[27]. Likewise, Rowan *et al.*^[28] reported that 5% had gestational hypertension and 6.3% had preeclampsia.

The HAPO study, found a direct correlation between Cesarean section rate and maternal glycemia with an overall frequency of 23.7%^[27]. Gorgal *et al.*^[28] reported a non-elective cesarean section rate for women with GDM of 19.5% compared to 13.5% for non-diabetic women.

Macrosomia in newborns of diabetic mothers is characterized by increased body fat^[16]. The IADPSG study found that percentage of body fat in newborns, maternal glycemia and foetal insulin levels estimated by cord C-peptide level were strongly positively correlated^[15]. Thus maternal glycemia is directly related to neonatal adiposity. Although rare, shoulder dystocia is a serious complication of childbirth. A clear association between increased foetal size and the risk of shoulder dystocia has been shown once the birth weight exceeds 4 kg^[29].

In older studies, the risk of stillbirth was increased fourfold^[30]. In more recent studies, this risk is found to

be lower; probably due to the initiation of monitoring and treatment of GDM. In the HAPO study, there was no increased risk of prenatal death with increased maternal glucose levels^[11]. In comparison, Crowther *et al.*^[31] observed five deaths in the Routine Care Group and none in the Treatment Group.

MATERNAL LONG-TERM CONSEQUENCES OF GDM

GDM is not only associated with adverse pregnancy outcomes, such as macrosomia, increased caesarian section rates, hypertensive disorders and foetal hyperinsulinaemia^[32,33], but also significantly increases the risk for long-term problems for both mothers and their offspring.

T2DM

Women who have had GDM have a substantially increased risk for development of T2DM, even though most women return to a euglycaemic state shortly after delivery^[34-36]. The evidence of this association is massive, but the magnitude of the risk varies among studies, primarily explained by differences in length of follow-up, number of women participating in follow-up, diagnostic criteria and in the selection of the population^[37]. A Danish study found that 40% of women with diet-treated GDM had developed diabetes 10 years after the index pregnancy. Compared to the 30-60-year-old females in the background population, the incidence of diabetes was increased 10 fold^[36]. A systematic review of 20 studies found an at least 7 fold increase in the risk of developing T2DM, when comparing women with a pregnancy complicated by GDM to women with a normoglycaemic pregnancy^[34]. In conclusion, GDM is one of the most predictive factors for the development of T2DM later in life. These women should be followed up with an OGTT 2-3 mo after delivery and then a yearly follow-up, ideally with an OGTT. Furthermore, a yearly fasting glucose test will allow detection of the development of T2DM early in these women.

The specific biological link between GDM and T2DM remains unclear. Both disorders are characterized by insulin resistance and/or abnormal insulin secretion. In addition studies provide evidence that several of the known T2DM risk genes are more frequent in women with previous GDM^[38], and many of the risk factors are the same, such as a raised body-mass index, high age, family history of diabetes and Asian and black ethnicity^[37,39]. It thus appears plausible that the pathogenesis is overlapping, and GDM may serve to identify women at high risk of future T2DM^[34,36].

Metabolic syndrome and cardiovascular disease

GDM may also increase a woman's risk of the metabolic syndrome and cardiovascular disease (CVD) postpartum. The metabolic syndrome is characterized by several risk factors, including central obesity, hypertension, insulin

resistance and dyslipidemia. These risk factors are also associated with the development of CVD and T2DM, and the metabolic syndrome has been demonstrated to increase the risk of both outcomes^[40]. The abnormalities of the metabolic syndrome and a high risk health profile are more frequent among women with previous GDM. The prevalence of the metabolic syndrome is found to be 3 times as frequent in Danish women with previous diet-treated GDM compared to population-based and age-matched control women^[41]. Another study has demonstrated that the 3 mo postpartum prevalence of the metabolic syndrome increases progressively from 10% in women with normoglycaemic pregnancies to 17.6% in women with gestational impaired glucose tolerance and to 20% in women with previous GDM^[42]. These results suggest that dysglycemia in pregnancy may provide an opportunity to detect otherwise unrecognized risk conditions, such as the metabolic syndrome and consequently allow targeted intervention to prevent diabetes and CVD.

The risk of CVD is found to be approximately 70% higher in women with previous GDM compared with women having normoglycaemic pregnancies when followed for 11.5 years after the index pregnancy^[43]. The increased risk may also extend to women with only mild glucose intolerance during pregnancy^[44]. When adjusting for the incidence of T2DM, the association was attenuated in both studies.

The increased risk of CVD in women with prior GDM is attributable to several interacting factors, primarily including the development of overt T2DM and the increased risk of the metabolic syndrome and vascular dysfunction^[44]. Therapeutic interventions to prevent the development of T2DM may therefore reduce the risk of CVD, and a potential modification of cardiovascular risk factors may also help to prevent development of CVD in women with a history of GDM.

LONG TERM EFFECTS IN OFFSPRING OF WOMEN WITH GDM

Offspring of women with a history of GDM are also at increased long-term risk of developing metabolic diseases such as obesity, T2DM and the metabolic syndrome. This long-term risk depends on genetic susceptibility and is further modulated by the postnatal environment. In recent years focus has been on the phenomenon of epigenetic transmission of acquired characteristics from mother to child due to perinatal programming of the fetus^[45]. Maternal glucose easily crosses the placenta and as a consequence maternal hyperglycemia leads to intrauterine hyperglycemia, which induces foetal hyperinsulinemia and possible modification of growth and future metabolism of the fetus (fuel-mediated teratogenesis)^[46,47]. Also worth noticing, is the finding that the relation between birth weight and risk of T2DM is U-shaped and therefore both infants with decreased and those with increased birth weight are at increased

risk of developing T2DM as compared to persons being born with a normal birth weight^[48].

Animal studies have convincingly shown that intrauterine exposure to maternal diabetes is associated with an increased risk of abnormal glucose tolerance, diabetes and obesity in offspring^[49]. Although it is difficult to study the effect of intrauterine hyperglycemia separately from a genetic effect in humans observational studies among the Pima Indians have added evidence for an epigenetic mode of diabetes transmission. Thus children of diabetic mothers had a 6 fold increased risk of developing T2DM compared to children born to non-diabetic mothers^[50]. Another study conducted in the Pima Indian population strengthened this association by showing a higher incidence of diabetes in siblings born after a maternal diagnosis of diabetes compared to a sibling born before the maternal diagnosis of diabetes (OR: 3.0, $P < 0.01$), which partly eliminates the genetic disposition. A greater frequency of diabetes is also seen in offspring of mothers with T2DM than offspring of T2DM fathers^[51]. These results are not directly applicable to other populations, as Pima Indians have a remarkably high incidence of T2DM, but they clarify the importance of intrauterine exposure to hyperglycemia, even within a population with a strong genetic inheritance of T2DM^[51].

A Danish long-term follow-up study based primarily on a Caucasian population found a high prevalence of T2DM and pre-diabetes in adult offspring of mothers with diet-treated GDM and in offspring of mothers with type 1 diabetes compared with the background population [Adjusted OR: 7.76 (95%CI: 2.58-23.39) vs 4.02 (95%CI: 1.31-12.33)]. These findings support the hypothesis that a hyperglycemic intrauterine environment plays a role in the pathogenesis of T2DM^[52] and are in accordance with earlier studies on children with a mixed ethnic composition, finding a similar prevalence of impaired glucose tolerance in offspring born to mothers with GDM^[53,54]. T2DM is characterized by both reduced insulin sensitivity and impaired B-cell function, but little is known about how these precursors are changed in the offspring after an exposure to maternal hyperglycemia in pregnancy. A recent study found that offspring exposed to intrauterine hyperglycemia due to GDM, primarily have reduced insulin sensitivity, but also a significantly lower relative insulin release taking insulin sensitivity into account (disposition index) when compared with the background population. The absolute insulin release did not differ significantly between the groups^[55].

Two other possible long-term consequences of pregnancies complicated by GDM is the development of the metabolic syndrome and obesity in the offspring. Development of obesity in offspring exposed to maternal diabetes in utero is found in the Pima Indian population, where the mean BMI was 2.6 kg/m² higher in offspring born to diabetic mothers compared to offspring born to non-diabetic mothers^[51]. This association is also seen in the multi-ethnic EPOCH study, where children of mothers with primarily GDM had a higher increase in BMI growth velocity than unexposed controls, with the increase

starting at the age of 10 to 13^[56]. According to a recent study, offspring of Caucasian women with GDM had a 2-fold increased risk of developing obesity and a 4-fold increased risk of the metabolic syndrome compared to the background population. This study also concludes that genetics play a major role in the development of the metabolic syndrome and obesity together with an effect of intrauterine hyperglycemia^[57]. The prevalence of obesity increases worldwide among all age groups and some of the predisposition to obesity in children may be due to epigenetic foetal programming. Randomized trials are needed to clarify the possible causal relationship between maternal hyperglycemia in pregnancy and the mentioned cardiovascular risk factors in human offspring.

TREATMENT OF GDM

Recently two large randomized controlled trials have been carried out to prove that identification and treatment of GDM and even mild carbohydrate intolerance during pregnancy confer a benefit. Thus the Australian Carbohydrate Intolerance Study in Pregnant Women, a large, randomized trial of treatment for gestational diabetes mellitus, concluded that treatment reduces serious perinatal complications and may also improve health-related quality of life using treatment of gestational diabetes in the form of dietary advice, blood glucose monitoring, and insulin therapy as required for glycemic control^[31]. The American Maternal-Fetal Medicine Units Network study provided further compelling evidence that among women who have GDM and normal fasting glucose levels, treatment that includes dietary intervention and insulin therapy, as necessary, reduces rates of foetal overgrowth, cesarean delivery, and preeclampsia^[58].

Accordingly, the primary intervention recommended to women diagnosed with GDM is dietary counseling in combination with physical activity and self-monitoring of blood glucose^[59,60]. If these measures are insufficient in terms of achieving optimal glycemic control subcutaneous insulin therapy is the therapy of choice as insulin does not cross the placenta and is therefore considered harmless to the foetus. However insulin is relatively expensive and difficult to administer. It requires education to ensure a safe administration and it is associated with an increased risk of hypoglycemia and weight gain. The use of safe and effective oral agents may therefore offer advantages over insulin but has not yet been formally approved for GDM therapy in all countries^[61]. A large randomized controlled trial was performed by Rowan *et al*^[62] in which 751 women with GDM at 20 to 33 wk of gestation were assigned to open treatment with metformin or insulin if lifestyle intervention had failed to achieve glycemic control. Three hundred and sixty-three women were assigned to metformin. 92.6% continued to receive Metformin until delivery and 46.3% in the Metformin group received supplemental insulin. The authors concluded that metformin, alone or with supplemental insulin, was not associated with increased

perinatal complications as compared with insulin. Thus the treatment with Metformin was considered safe and effective and moreover, the women preferred metformin to insulin treatment. Further follow-up data are however necessary to establish long-term safety.

Another randomized controlled trial included 404 women between 11 and 33 wk of gestation with singleton pregnancies and GDM that required treatment and assigned them to either glyburide or insulin. All the women received dietary advice and eight women in the glyburide group required additional insulin therapy. There were no significant differences between the glyburide and insulin groups regarding macrosomia, neonatal hypoglycemia, lung complications or foetal abnormalities and it was concluded that glyburide is a clinically effective alternative to insulin therapy^[63].

Other studies show that both metformin and sulfonylurea have been increasingly and safely used in the treatment of GDM^[64]. However, both glyburide and metformin cross the placenta and given the growing evidence of epigenetic foetal programming in utero, administration of drugs potentially affecting foetal metabolism is of major concern and as long term follow-up data on both mother and offspring are lacking oral antihyperglycemic agents should be used with caution.

Vitamin D and GDM

A growing body of epidemiological evidence suggests a possible association between vitamin D deficiency/insufficiency and GDM, maternal obesity and adverse maternal, neonatal and infant outcome^[65]. The molecular and cellular mechanisms with respect to the interaction between vitamin D and GDM are only partly understood. However, it appears that vitamin D acts directly on pancreatic beta cells through expression of the vitamin D receptors as well as through the enzyme 25(OH)D-1- α -hydroxylase by regulating intracellular calcium to increase insulin secretion and by attenuating systemic inflammation associated with insulin resistance^[66,67]. The association between vitamin D and glucose metabolism in GDM has been investigated in several observational studies^[65] but large randomized controlled trials are lacking and it remains to be determined whether vitamin D supplementation can reduce the risk of developing GDM and/or improve glycemic control in diabetic pregnant women with vitamin D deficiency/insufficiency.

As stated above lifestyle counseling concerning diet and exercise is one of the cornerstones in the treatment of GDM, but recently it was also reported that a healthful diet was associated with a lower risk of T2DM among women with a history of GDM^[68]. Additionally, newly published results from a large prospective study indicate that increasing physical activity may help lower the risk of progression from GDM to T2DM^[69].

CONCLUSION

Worldwide there has been a dramatic increase in the prevalence of overweight and obesity in women of

childbearing age. Overweight and obese women have an increased risk of developing GDM leading to complications during pregnancy, birth and neonatally. The clinical management of obese pregnant women and women with GDM is a challenge and puts additional stress on the healthcare system. In addition it seems more and more clear that maternal metabolic characteristics are crucial determinants of insulin resistance during pregnancy and in offspring and interventions, especially in the form of exercise, weight loss and a healthy diet before, during and after pregnancy might be a key to prevent the vicious circle that contributes to the epidemic of obesity, insulin resistance and T2DM.

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Steroid hyperglycemia: Prevalence, early detection and therapeutic recommendations: A narrative review

Héctor Eloy Tamez-Pérez, Dania Lizet Quintanilla-Flores, René Rodríguez-Gutiérrez, José Gerardo González-González, Alejandra Lorena Tamez-Peña

Héctor Eloy Tamez-Pérez, Dania Lizet Quintanilla-Flores, Alejandra Lorena Tamez-Peña, Internal Medicine Service, "Dr. José Eleuterio González" University Hospital and School of Medicine, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León 64460, México

Héctor Eloy Tamez-Pérez, José Gerardo González-González, Research Division, School of Medicine, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León 64460, México

René Rodríguez-Gutiérrez, José Gerardo González-González, Endocrinology Service, "Dr. José Eleuterio González" University Hospital and School of Medicine, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León 64460, México

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Correspondence to: Héctor Eloy Tamez-Pérez, MD, Research Division, School of Medicine, Universidad Autónoma de Nuevo León, Ave. Madero y Gonzalitos s/n, Colonia Mitras Centro, Monterrey, Nuevo León 64460, México. hectoreloytp@gmail.com
 Telephone: +52-81-83294050
 Fax: +52-81-83294050

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Abstract

Steroids are drugs that have been used extensively in a variety of conditions. Although widely prescribed for their anti-inflammatory and immunosuppressive properties, glucocorticoids have several side effects, being hyperglycemia one of the most common and representative. In the present review, we discuss the main epidemiologic characteristics associated with steroid use, with emphasis on the identification of high risk populations. Additionally we present the pathophysiology of corticosteroid induced hyperglycemia as well as the pharmacokinetics and pharmacodynamics associated with steroid use. We propose a treatment strategy based on previous reports and the understanding of the mechanism of action of both, the different types of glucocorticoids and the treatment options, in both the ambulatory and the hospital setting. Finally, we present some of the recent scientific advances as well as some options for future use of glucocorticoids.

Key words: Steroid; Hyperglycemia; Diabetes mellitus; Treatment; Insulin

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Core tip: Steroids are drugs that have been used extensively in a variety of conditions. Although widely prescribed for their anti-inflammatory and immunosuppressive properties, glucocorticoids have several side effects, being hyperglycemia one of the most common and

representative. We present the pathophysiology of corticosteroid induced hyperglycemia as well as the pharmacokinetics and pharmacodynamics associated with steroid use.

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INTRODUCTION

Steroids are drugs that have been used extensively in a variety of conditions, both acute and chronic^[1]. At supraphysiological doses, they reduce the synthesis of pro-inflammatory cytokines, T-cell function, and antibody Fc receptor expression, which activate anti-inflammatory and immunosuppressive processes, making them the cornerstone in treatment of numerous inflammatory diseases^[2,3].

Despite their efficacy, their use is limited by the wide variety of side effects, which can be divided into three categories: immediate, gradual and idiosyncratic. Immediate effects include fluid retention, blurred vision, mood changes, insomnia, weight gain, and modulation of the immune response. The more gradual effects are those related to endocrine metabolism, especially hyperglycemia, osteopenia with subsequent osteoporosis, dyslipidemia, central obesity, and adrenal suppression. Additionally, acne, skin thinning, and dyspepsia are considered of gradual onset. Some of the idiosyncratic effects are avascular necrosis, cataracts, open-angle glaucoma and psychosis^[3-5].

Steroids are the main cause of drug-induced hyperglycemia^[4]. They not only exacerbate hyperglycemia in patients with known diabetes mellitus (DM), but also cause DM in patients without documented hyperglycemia before the initiation of glucocorticoids (GC) therapy^[1,6], with an incidence that can reach up to 46% of patients, and increases in glucose levels up to 68% compared to baseline^[7-9]. Furthermore, in some populations they can precipitate acute complications such as nonketotic hyperosmolar state, and diabetic ketoacidosis^[10] and in a few instances death, especially in patients with pre-existing DM.

EPIDEMIOLOGY AND RISK FACTORS

Exacerbated and uncontrolled hyperglycemia is a common complication in patients with DM and carbohydrate intolerance as previously documented^[11]. Moreover, DM incidence in patients without a prior history of hyperglycemia to steroid use varies from 34.3% to 56%^[12,13], with a relative risk ranging from 1.36 to 2.31, and a number needed to harm ranging from 16-41 for

1-3 years of use, according to several authors^[14-16]. In terms of the steroid presentation, only oral GCs have demonstrated to increase the risk of diabetes in up to 2% of incident cases in a primary care population; there is either minimal or no association of incident diabetes with prescribing of GC-containing in-halers, topical preparations, eye drops, or infrequent GC injections^[17].

The main risk factors that have been identified as predictors of developing diabetes are: the dose and type of steroid, odds ratio (OR) (OR: 1.01, 95%CI: 0.996-1.018)^[18,19], duration of treatment^[9], a continuous GC scheme (OR: 2.0, 95%CI: 1.29-3.1)^[12], older age (OR: 1.05, 95%CI: 1.02-1.09)^[20], HbA1c, and body mass index (OR: 2.15, 95%CI: 1.12-4.13)^[11,14,21]. In addition, there are population groups with a greater risk of developing hyperglycemia during treatment with steroids, among these are patients with a history of gestational DM, a family history of diabetes (OR: 10.29, 95%CI: 2.33-45.54), concomitant use of mycophenolate mofetil (OR: 4.80, 95%CI: 1.32-17.45) and calcineurin inhibitors, abnormal fasting glucose, and impaired glucose tolerance^[3,8,19,22].

In the hospital setting, there is evidence that more than half of the patients receiving high-dose steroids develop hyperglycemia, with an incidence of 86% of at least one episode of hyperglycemia and 48% of patients presenting a mean blood glucose ≥ 140 mg/dL^[23]. The main associated factors related to inpatient hyperglycemia are previous history of DM, a higher prevalence of comorbidities, prolonged treatment with steroids and older age^[9,23].

PATHOPHYSIOLOGY

GC's provide a substrate for oxidative stress metabolism increasing lipolysis, proteolysis, and hepatic glucose production^[4]. The mechanism responsible for glucose intolerance after GC administration is similar to that of type 2 DM since steroids increase insulin resistance, which can be up to 60%-80% depending on the dose and type used^[14,15].

Among the notable factors that modify the biological effects of steroids, there is the enzymatic activity of 11 β -hydroxysteroid dehydrogenase, which is classified into two types: type 1, expressed in liver and adipose tissue and amplifies the local action of steroids to convert cortisone to cortisol, and type 2, which predominates in renal tissue and reduces the effect of converting cortisol to cortisone^[4].

Skeletal muscle is responsible for 80% of postprandial glucose storage and represents the largest reserve of glycogen in the body. Its storage is totally dependent on the presence of insulin and the availability of the glucose transporter type 4 (GLUT4) glucose transporter in the cell membrane. Steroids induce insulin resistance by directly interfering with signaling cascades, mainly the GLUT4 transporter, within muscle cells, with the subsequent 30%-50% reduction in insulin-stimulated glucose uptake and a 70% reduction in insulin-stimulated

Table 1 Pathophysiology of corticosteroid-induced hyperglycemia**Increase in insulin resistance with increased glucose production and inhibition of the production and secretion of insulin by pancreatic β -cells**

Corticosteroids increase endogenous glucose production, increment in gluconeogenesis and antagonizing the metabolic actions of insulin
 Enhance the effects of other counterregulatory hormones, such as glucagon and epinephrine, which increase the endogenous synthesis of glucose
 Also been shown that the expression of the nuclear receptor peroxisome proliferator-activated receptor α is necessary for the increment in endogenous glucose production induced by corticosteroids
 Corticosteroids reduce peripheral glucose uptake at the level of the muscle and adipose tissue
 Corticosteroids also inhibit the production and secretion of insulin from pancreatic β -cells and induce β -cell failure indirectly by lipotoxicity

glycogen synthesis^[24,25]. On the other hand, steroids are responsible for the catabolism of proteins with the subsequent increase in serum amino acids, which also interfere with insulin signaling in the muscle cell. Finally, they increase lipolysis, resulting in an increase in serum free fatty acids and triglycerides. These promote the accumulation of intramyocellular lipids (acetyl coenzyme A, diacylglycerol and ceramide), reducing the entry and storage of intramuscular glucose^[4].

In the fasting state, the liver maintains euglycemia *via* gluconeogenesis and glycogenolysis, effects that are counteracted by insulin after food intake. GCs antagonize the metabolic effects of insulin, particularly in the postprandial state through the induction of enzymes that promote gluconeogenesis, increased lipolysis and proteolysis, increased mitochondrial activity, the enhancement of the effects of counterregulatory hormones, such as glucagon and epinephrine, and the induction of insulin resistance *via* the nuclear peroxisome proliferator-activated receptor (PPAR) α ^[4,21,25].

At the level of adipose tissue they promote the deposition of fat in viscera, while reducing peripheral reserves. Steroids have direct effects on various adipokines: (1) promoting the expression of resistin and adipokines, which influence glucose tolerance; (2) decreasing the expression of adiponectins, which promote insulin sensitivity; and (3) stimulating expression and secretion of leptin. Finally, they are responsible for increasing triglyceride hydrolysis in adipocytes^[4]. These effects have the final result of increased plasma levels of non-sterified fatty acids, which accumulate within muscle cells and reduce glucose uptake by interfering with insulin signaling^[24,25].

It has been shown that GCs alter the function of pancreatic beta cells through the reduction of GLUT2 and glucokinase receptor expression at the same time increasing the activity of glucose-6-phosphate dehydrogenase, with the consequent alteration in β -oxidation. Additionally, they reduce insulin synthesis and it is thought that they reduce cell mass through the induction of beta cell apoptosis. Likewise, in response to the decrease in insulin sensitivity, the pancreatic beta cell normally increases insulin secretion to maintain glucose homeostasis, but at times this increase is not sufficient to compensate for the insulin resistance resulting in hyperglycemia^[4,15].

Based on the aforementioned, GCs increase insulin

resistance with the subsequent state of hyperinsulinism. In healthy subjects, this mechanism is compensated by an increase in pancreatic insulin secretion, causing serum glucose levels to remain within normal range^[14]. However, in susceptible populations, such as normoglycemic individuals with reduced insulin sensitivity and a low rate of production of the same prior to steroid use, this offsetting effect is lost, resulting in hyperglycemia^[4] (Table 1).

PHARMACOKINETICS AND PHARMACODYNAMICS

Steroids of adrenal origin are synthesized from cholesterol, and their secretion follows a circadian pattern and a pulsatile ultradian rhythm. Normal secretion ranges from 8 to 15 mg/d, of which 10% circulates in free form, the rest is bound to carrier proteins, mainly albumin and cortisol binding globulin. The plasma half-life ranges from 80-270 min depending on the type of GCs used, with an action in tissues that lasts for 8-12 h. They are metabolized in the liver and their conjugated metabolites are excreted mainly by the kidneys^[5,25,26].

The development of insulin resistance is mainly postprandial and varies depending on the type of steroid used: intermediate-acting and long-acting GCs. Prednisone and methylprednisolone are classified as intermediate-acting GCs, with a peak of action 4-6 h following administration. Their effect on glucose levels is mainly during the afternoon and night without effect in fasting glucose when they are administered in a single dose. On the other hand, they cause persistent hyperglycemia when administered in divided doses. Dexametasone fits in the long-acting GCs, with a steroid hyperglycemia that lasts for more than 24 h, with a slight decline during an overnight fast^[5,25,26].

The effect of steroids is usually transient and reversible. As steroid doses are reduced, their effect on endocrine metabolism returns to baseline and drug-induced diabetes is expected to resolve; however, this is not true in all cases^[1,6]. There are few studies that describe the effect of long-term use of GCs on pancreatic function and the development of DM. According to recently published data, GCs are likely to cause the greatest impact when it is administered acutely, especially during the second and fourth week, with a spontaneous remission in the majority of patients when a phenomenon of adaptation reduces the extent to which

glucose levels increase^[12,27].

EFFECTS OF STEROID HYPERGLYCEMIA

Despite its frequency, little is known about the impact of hyperglycemia associated with steroid use on clinical comorbidities and mortality. It is known that rheumatic diseases *per se* represent an important cardiovascular risk factor, which makes them the leading cause of premature mortality in these patients. Therefore, it is thought that the coexistence of inflammatory diseases and steroid-induced hyperglycemia may lead to worse cardiovascular consequences^[3,10]. Similarly the diabetic patient possesses a traditional cardiovascular risk factor for microvascular and macrovascular complications.

Fluctuations in serum glucose levels have been associated with increased cardiovascular mortality associated with increased LDL cholesterol, endothelial dysfunction, activation of the coagulation cascade, increased pro-inflammatory cytokine production, and oxidative stress resulting in macrovascular disease progression^[2]. Several studies have reported that transient increases in serum glucose are associated with acute inflammatory processes and endothelial dysfunction in both diabetic and non-diabetic patients^[14].

In the hospitalized patient, acute hyperglycemia is associated with increased hospital stay, repeated emergency room visits, risk of admission to intensive care, higher risk of infection rates, poor wound healing and higher hospital mortality rates^[9,23,28]. In susceptible populations such as the elderly, persistent hyperglycemia associated with GC use can precipitate hyperglycemic hyperosmolar states, which would require frequent hospital admissions for aggressive hydration and insulin therapy, as well as increased complications related to inpatient hyperglycemia^[19]. Additionally, steroid hyperglycemia represents a strong predictor of graft failure in the transplant population with a 2-3 fold increased risk of fatal and non-fatal cardiovascular events as compared with non-diabetic patients^[29,30].

DIAGNOSIS

All patients who are started on steroid treatment should have a baseline glucose, as well as education on daily self-monitoring of glucose^[6,8]. Daily monitoring should be started when hyperglycemia above 180 mg/dL is identified in more than one occasion in the presence or absence of symptoms associated with hyperglycemia^[1]. The diagnosis of steroid hyperglycemia is similar to the current criteria established by the American Association of Diabetes: blood glucose level of ≥ 126 mg/dL, glycemia at any time ≥ 200 mg/dL, HbA1c $> 6.5\%$ or blood glucose > 200 mg/dL 2 h after an oral glucose load^[31].

Based on the pathophysiology and pattern of GC-induced hyperglycemia it seems that some of the current criteria for diagnosis of DM underestimate the diagnosis itself. Since steroid-induced diabetes is detected mainly

in the postprandial state, we do not recommend the use of fasting glucose as well as the glucose tolerance curve as reliable diagnostic methods, because there is a high possibility of losing some of the hyperglycemic patients. According to observations in previous studies, postprandial glucose determinations and/or HbA1c determinations are suggested as a screening examination with long-term steroid use^[21,32,33]. The postprandial glycemia after lunch offers the greatest diagnostic sensitivity, especially when intermediate-acting GCs are administered in a single morning dose.

In hospitalized patients, monitoring should start with capillary glucose determination from the start of steroid treatment. Since almost 94% of cases of hyperglycemia develop within 1-2 d of initiation of steroid therapy in the hospital setting, in nondiabetic patients who maintain glucose levels < 140 mg/dL without insulin requirements for 24-48 h, glycemic monitoring can be discontinued^[23]. On the other hand, in patients with glucose levels > 140 mg/dL with persistent insulin requirements, a basal/bolus subcutaneous insulin scheme must be established. Additionally, in patients with severe and/or persistent hyperglycemia despite the subcutaneous scheme, insulin by infusion pump should be started^[9,33,34].

Several protocols to detect patients at risk of steroid-induced hyperglycemia are being studied. This is based on the hypothesis that abnormalities in insulin secretion and loss of beta cell function present in pre-diabetic individuals can be exacerbated in response to an increase in insulin requirements secondary to GC exposure. Abdelmannan *et al.*^[18] recently reported the use of a "stress test", in which the administration of 8 mg dexamethasone provides timely detection of increases in serum glucose, C-peptide, and insulin in at risk population, whereby one can predict this complication prior to the usual dose of the steroid. However, it is necessary to develop further studies to confirm its usefulness.

TREATMENT OF STEROID HYPERGLYCEMIA

Due to differences in steroid dose and the scheme used, the approach to hyperglycemia should always be individualized^[35]. A complete evaluation of the degree of pre-existing glucose intolerance, the patient's clinical condition, the degree of hyperglycemia, the type, dose and frequency of administration of the corticosteroid compound and the mechanism of action, pharmacokinetics and pharmacodynamics of the different hypoglycemic drugs must be made in order to determine the best treatment approach in each patient^[25]. When selecting the best treatment the first consideration to make is whether to use oral hypoglycemic drugs or insulin (Figure 1).

ORAL HYPOGLYCEMIC DRUGS

There is little information on the therapeutic efficacy of oral agents in steroid-induced hyperglycemia. In patients

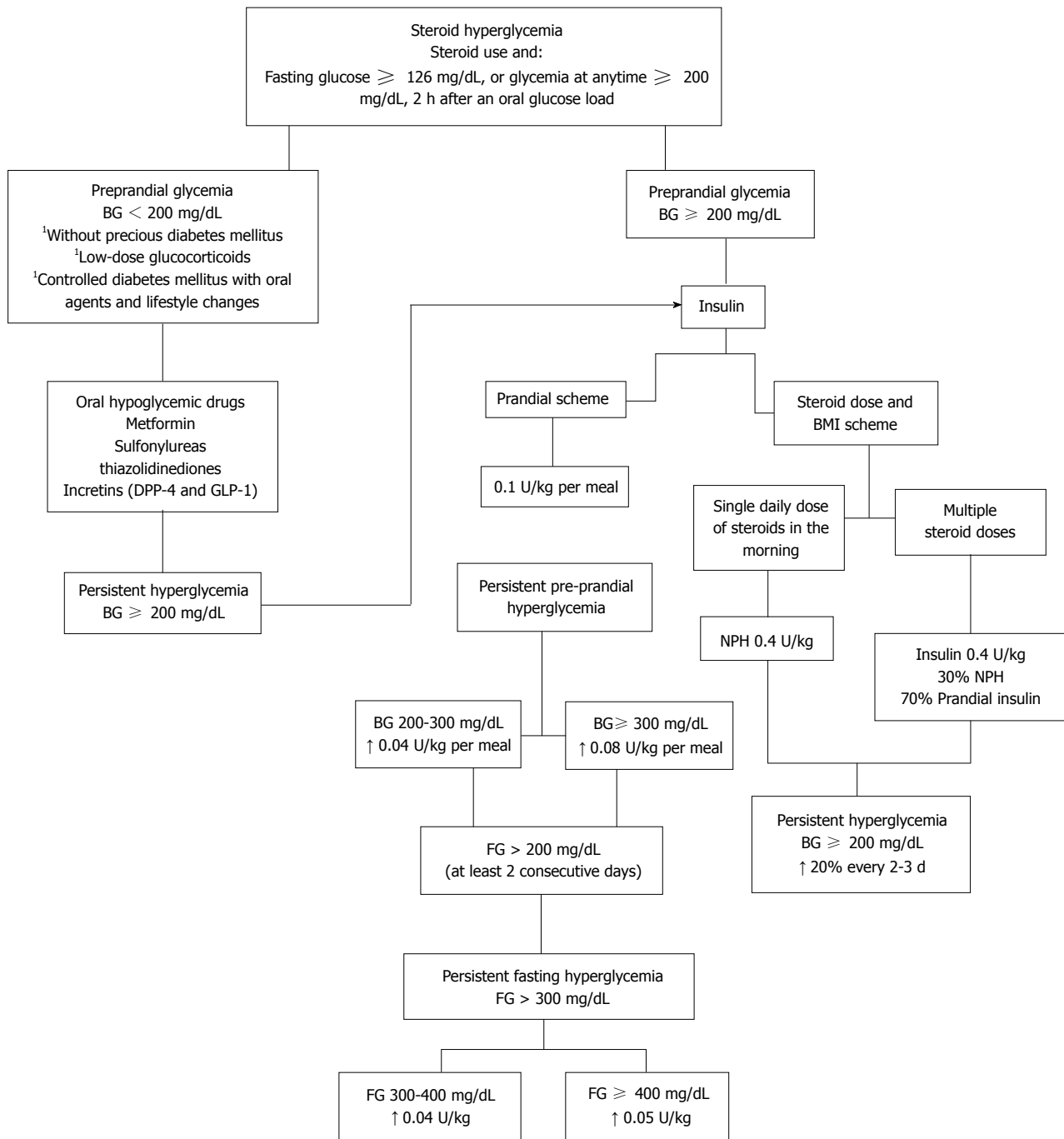


Figure 1 Algorithm for the management of glucocorticoids-induced hyperglycemia. Glargine and other analogues can be recommended in cases of nocturnal hyperglycemia associated with long-acting steroid use. ¹Calculation rule is: mg/dL × 0.0555 = mmol/L. NPH: Neutral protamine Hagedorn; BG: Blood glucose; FG: Fasting glucose; DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; BMI: Body mass index.

with fasting glucose levels below 200 mg/dL, without previous diabetes and given low-dose GCs, therapeutic emphasis should focus on exercise, diet therapy and oral antidiabetic agents^[6]. Most available oral hypoglycemic drugs have a slow onset of action and/or a very limited or null titration, giving them little capacity to adapt to major changes in requirements of hypoglycemic action. Furthermore, the action profile of oral hypoglycemic drugs throughout the day does not usually coincide with the pattern of GC induced hyperglycemia^[13,25].

Long lasting sulfonylureas were the first drugs used in

renal transplant patients, with a therapeutic response of 25%. They have the advantage of being strong inducers of insulin secretion from pancreatic β -cells and secondary by increasing glucose uptake in peripheral tissues^[36]. However, due to their narrow therapeutic window, prolonged use increases the risk of hypoglycemia with short-term steroids, especially where single morning doses of steroids are given^[14]. In patients where intermediate-acting GCs in two or more daily doses, by long-term preparations such as dexametasone, or by intra-articular GCs are used, long acting sulfonylureas may

be considered as a therapeutic option, always bearing in mind the risk of hypoglycemia in these type of drugs.

Metformin may be a good therapeutic option because of its direct effect on the improvement of insulin sensitivity; however, there are few articles that support its usefulness. On the other hand, many patients who are treated with steroids have significant co-morbidities associated with hypoxia and renal failure, that make the use of metformin contraindicated^[13,14].

Thiazolidinediones (TZDs) were used for long-term treatment in patients with steroid-induced hyperglycemia. They act as ligands for PPAR- γ receptors enhancing insulin action in skeletal muscle and adipose tissue, while having little effect on insulin secretion. However, their usefulness is limited by the risk of edema, heart failure, hepatotoxicity and possible cardiovascular effects^[37]. They have also been associated with increased risk of fractures, which together with the osteopenic effect of steroids is an important contraindication to their use^[1,14].

Selective inhibitors of the dipeptidyl peptidase 4 (DPP-4) enzyme and glucagon-like peptide-1 have shown effectiveness in the control of hyperglycemia since they promote enhanced release of glucose dependent insulin, inhibiting glucagon secretion and enhancing uptake into peripheral tissues, in addition to increasing the speed of gastric emptying, with decreased appetite and calorie intake^[13,32,38]. Regarding steroid hyperglycemia, DPP-4 have shown to decrease glycated hemoglobin in up to 24.6% as well as serum glucose levels in 32.6% from baseline^[32]. Continuous intravenous infusion of exenatide significantly improves GC-induced hyperglycemia in healthy individuals in association with restoration of initial insulin secretion and decreased glucagon concentrations. Additionally, exenatide has been associated with reduced hypoglycemia and the promotion of weight loss^[13]. Despite the benefits observed, their applicability in these patients is still under study. Nevertheless, they can be recommended in patients receiving intermediate-acting corticosteroids in a single morning dose because their immediate onset of action, their predominant effect on postprandial glycemia, and their lack of risk of hypoglycemia related to glucose-dependent effects^[25]. A new review has been published with this type of drugs^[33].

Glinides allow minimal dose titration and have an immediate onset of action and short duration of effect, which adapts to the hyperglycaemic profile of the corticosteroids and reduces the risk of hypoglycemia in the morning, coinciding with the disappearance of the hyperglycemic action of corticosteroids^[25].

Renal sodium-linked glucose transporter 2 inhibitors are new antidiabetic drugs with an insulin-independent mechanism of action. They pose one remarkable advantage compared with already established antidiabetics: increasing urinary glucose excretion without inducing hypoglycaemia, thereby promoting body weight reduction due to loss of approximately 300 kcal per day. Clinical

trials showed promising results: enhancing glycaemic control was paralleled by reducing body weight and systolic and diastolic blood pressure. Nevertheless, some safety concerns remain, such as genital mycotic infections, urinary tract infections and cardiovascular risks in vulnerable patients. However in Treatment of steroid hyperglycemia haven't been used^[39].

INSULIN

Insulin is the treatment of choice in patients with persistent hyperglycemia ≥ 200 mg/dL. Several therapeutic schemes have been used, among which the use of prandial insulin has been included, and also based on schemes of steroid dose and the body mass index of the patient^[14]. In general, hyperglycemia associated insulin resistance, present at the start of treatment with steroids, generates the need for large doses of insulin in early stages of treatment, which are gradually reduced once glucose levels are controlled^[1,12].

PRANDIAL SCHEME

The prandial insulin scheme is based on the observation that even though normal levels of fasting glucose can be present; serum glucose gradually increases throughout the day reaching a maximum concentration after meals, with a gradual reduction at night. This mechanism could be explained by defective postprandial insulin secretion^[14].

The scheme is based on the patient's weight, the total calories consumed during the meal, and the establishment of a food pattern. Regular insulin is recommended for people who usually eat snacks between meals and those with delayed gastric emptying; on the other hand, rapid insulin, LysPro and Aspart, are used in people who do not eat snacks between meals and who usually eat a high carbohydrate diet^[1,7].

The initial dose is calculated at 0.1 U/kg per meal, and is then modified depending on the glycemic response and the amount of supplementary insulin required to correct the pre-prandial hyperglycemia: 0.04 U/kg per meal with a glucose level between 200-300 mg/dL, 0.08 U/kg per meal if levels are above 300 mg/dL. If the patient continues with pre-prandial corrections the initial insulin dosage should be increased^[1].

The use of basal insulin is usually considered when using high doses of steroids are used or in those patients with characteristics of diabetes prior to the start of the steroid. If fasting glucose is above 200 mg/dL on at least two consecutive mornings, NPH should be initiated at 0.1 U/kg before bedtime. If hyperglycemia levels persist > 300 mg/dL despite preprandial corrections, 0.04 U/kg at levels of 300-400 mg/dL and 0.05 U/kg when > 400 mg/dL, can be added. Additionally, glargine can be recommended particularly in cases of nocturnal hypoglycemia^[1,34].

Table 2 List of most commonly used drugs in glucocorticoids-induced hyperglycemia and their adverse effects

Drug	Adverse effects
Metformin	Gastrointestinal distress, lactic acidosis, B12 deficiency, contraindicated in renal failure and interactions with other drugs
Insulin	Hypoglycemia, weight gain, cancer-related
Sulfonylureas and Glinides	Hypoglycemia, weight gain, cardiovascular risk
Incretins (DPP-4 inhibitors and GLP-1 agonists)	Gastrointestinal distress, heightened pancreatitis risk, heightened risk of cardiac insufficiency
Thiazolidinediones	Weight gain, liquid retention, heightened fracture risk

DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1.

SCHEME BASED ON STEROID DOSE AND THE BMI OF THE PATIENT

In patients who receive a single daily steroid dose, generally in the morning, NPH insulin in the morning is recommended, considering that the peak and duration of action of this insulin is similar to conventional intermediate-steroids (prednisone and prednisolone)^[35]. Clore *et al.*^[14] recommend using a scheme based on weight and steroid dose, using an initial dose of 0.4 U/kg of NPH, with subsequent adjustments depending on the response.

If multiple steroid doses are intended during the day, NPH insulin is usually not enough to maintain glycemic control due to postprandial hyperglycemia, therefore the dose can be divided into 30% basal insulin and 70% nutritional insulin^[34]. When using dexamethasone, NPH could be replaced by detemir or glargine due to their pharmacodynamic similarities^[14].

Inpatient treatment

In-hospital dose calculation is similar to outpatient doses, with some modifications. If the patient is known to have diabetes with insulin use prior to admission, the dose should be increased 20%. On the other hand, if high doses of steroids are used and the dose must be calculated empirically, the insulin dose will be calculated based on weight 0.7 U/kg per day.

In hospitalized patients receiving high doses of steroids with glucose levels above 400 mg/dL, an insulin infusion pump should be indicated. This indication is particularly important in patients receiving intravenous steroids pulses in which insulin requirements are difficult to predict^[2,6].

DOSAGE ADJUSTMENTS

The insulin dose must be adjusted according to capillary glycemia every 2-3 d, with increases and/or decreases around 20%. Additionally, insulin doses should be adjusted based on changes in steroid dose to prevent hyperglycemia and/or hypoglycemia^[34]. The percentage of insulin adjustment corresponds to half the percentage in steroid change; for example, if the steroid dose is reduced or increased by 50%, the insulin dose will be reduced or increased 25%, respectively^[19,26]. The control goals must be those recommended for patients with DM

according to the current criteria: preprandial glycemia 70-130 mg/dL, postprandial glycemia < 180 mg/dL, and HbA1c < 7%^[40].

The drugs and their most common adverse effects can be seen in Table 2.

SCIENTIFIC ADVANCES

The understanding of the molecular mechanisms of steroids has allowed the development of compounds that reduce unwanted metabolic effects in comparison to conventional steroids, at the same time maintaining the same anti-inflammatory and immunosuppressive effects. These new drugs are based on the finding of mechanisms by which steroids promote gene transcription (transactivation), differing from those models that inhibit gene transcription (transrepression). Mechanisms related to transrepression are responsible for the anti-inflammatory effects, while those which involve transactivation are associated with known metabolic effects^[4,19].

Furthermore, to date various compounds that inhibit the effects of 11 β -hydroxysteroid dehydrogenase type 1, which results in improved glucose tolerance, insulin sensitivity, and improvement in lipid profile are under evaluation^[4].

CONCLUSION

GCs are drugs that have been widely used in a variety of medical conditions. Despite their medical efficacy, steroid-induced hyperglycemia remains as a common potentially harmful problem that must be considered when using any type a dose of GC. Despite its frequency, little is known about the impact of hyperglycemia associated with steroid use on clinical comorbidity and mortality.

A proper understanding of the mechanisms involved in steroid hyperglycemia is needed, since this will allow early detection and effective treatment in these patients. Appropriate guidelines that establish the recommendations for the diagnosis and treatment of steroid diabetes are needed in order to prevent all de complications associated with the hyperglycemic state. In most cases insulin must be the treatment of choice, especially in cases of serum glucose > 200 mg/dL. Nevertheless an individualized approach must be taken in each patient in order to consider lifestyle modifications and oral hypoglycemic drugs as alternative therapeutic

options.

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

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Telephone: +1-925-223-8242

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Glucose control in critical care

Jeremy Clain, Kannan Ramar, Salim R Surani

Jeremy Clain, College of Medicine, Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, United States

Kannan Ramar, Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, United States

Salim R Surani, Division of Pulmonary, Critical Care and Sleep Medicine, Texas AM University, Aransas Pass, TX 78336, United States

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Correspondence to: Salim R Surani, MD, MPH, MSHM, FACP, FCCP, Associate Professor, Division of Pulmonary, Critical Care and Sleep Medicine, Texas AM University, 1177 West Wheeler Ave, Suite 1, Aransas Pass, TX 78336, United States. srsurani@hotmail.com
Telephone: +1-361-8857722
Fax: +1-361-8507563

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Abstract

Glycemic control among critically-ill patients has been

a topic of considerable attention for the past 15 years. An initial focus on the potentially deleterious effects of hyperglycemia led to a series of investigations regarding intensive insulin therapy strategies that targeted tight glycemic control. As knowledge accumulated, the pursuit of tight glycemic control among critically-ill patients came to be seen as counterproductive, and moderate glycemic control came to dominate as the standard practice in intensive care units. In recent years, there has been increased focus on the importance of hypoglycemic episodes, glycemic variability, and premonitory diabetic status as factors that contribute to outcomes among critically-ill patients. This review provides a survey of key studies on glucose control in critical care, and aims to deliver perspective regarding glycemic management among critically-ill patients.

Key words: Glycemic control; Critical care; Blood sugar in intensive care unit; Diabetes in intensive care unit; Glycemic control

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Core tip: Glucose control among critically-ill patients has been an area of active research and considerable controversy in the past 15 years. This review provides a practical guide to the evidence, with a survey of the key studies that have informed current perspectives and clinical guidelines related to glycemic management among the critically ill. The article shows why initial enthusiasm for tight glycemic control waned as evidence accumulated favoring more modest glucose goals. The article also summarizes recent work investigating the importance of hypoglycemic episodes, glycemic variability, and premonitory diabetic status on morbidity and mortality in the intensive care unit.

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INTRODUCTION

In 2001, van den Berghe *et al.*^[1] reported results from a single-center, prospective, randomized controlled trial in Leuven, Belgium, and changed the way that blood glucose was managed in intensive care units (ICUs) throughout the world. Prior to the publication of this first Leuven study, glycemic control among critically-ill patients received scant attention, either at the bedside or in academic journals. The overwhelmingly favorable results of the study - which, among critically-ill surgical patients, found a remarkable mortality benefit from the use of intensive insulin therapy targeting normoglycemia - sparked strong interest in glycemic management in the ICU. Intensive insulin therapy quickly became the standard of care in both medical and surgical ICUs. However, as has been the experience in many facets of critical care, promising initial single-center results were not duplicated in subsequent trials. The publication of the NICE-SUGAR trial in 2009, which reported that intensive insulin therapy may actually result in increased mortality among critically-ill patients, served as a major bookend to the era of tight glycemic control as a pillar of ICU management^[2].

Nonetheless, interest in defining optimal glycemic control among critically-ill patients has continued. In the years that have followed the publication of the NICE-SUGAR trial, investigations have focused on establishing which factors of glycemic control and dysregulation most affect patient outcomes in the ICU. It has been increasingly recognized that hypoglycemia, glycemic variability, and premorbid diabetic status are all important considerations to be taken into account when approaching the glycemic management of a critically-ill patient.

This review aims to provide a survey of the key studies that have informed the changes in thinking in the past 15 years as regards glucose control in critical care. It explores the basis of the initial enthusiasm for, and subsequent skepticism of, intensive insulin therapy in the ICU. It also aims to provide perspective regarding major issues of glycemic management among critically-ill patients: hyperglycemia, hypoglycemia, glycemic variability, and premorbid diabetic status.

HYPERGLYCEMIA

Elevated blood sugar levels are commonly seen among critically ill patients, including those without a known history of diabetes. There are many reasons why patients undergoing treatment for critical illness develop hyperglycemia, and these reasons include both effects of endogenous stress responses and byproducts of medical interventions. Inflammatory cytokines and stress hormones, including cortisol and epinephrine, serve to inhibit insulin release and promote insulin resistance, thereby naturally increasing blood glucose levels by stimulating gluconeogenesis and glycogenolysis while impeding glucose uptake by peripheral tissues^[3,4]. Many

medical therapies further promote hyperglycemia, including the administration of exogenous catecholamines and corticosteroids, the infusion of dextrose for the purpose of suspending intravenous medications or providing parenteral nutrition, and even bedrest, which in and of itself may serve to impair glucose uptake in skeletal muscles^[5,6].

Prior to the publication of the first Leuven trial^[1], many practitioners viewed moderately severe hyperglycemia among critically ill patients to be either an epiphenomenon or an adaptive response, not warranting significant concern or intervention^[7]. However, as observational studies accumulated linking hyperglycemia to negative in-hospital patient outcomes, this permissive attitude began to change^[8-11]. Hyperglycemia was coming to be seen as complication worthy of physician attention. For example, a retrospective study of 1826 patients admitted to a mixed ICU in Stamford, Connecticut serving medical, surgical, and coronary patients reported reduced survival among those with elevated mean blood glucose levels, with a stepwise effect resulting in higher mortality as mean blood glucose levels rose^[8]. Compared to patients who survived to hospital discharge, those who died had higher initial (175 mg/dL vs 151 mg/dL), mean (172 mg/dL vs 138 mg/dL), and maximum (258 mg/dL vs 177 mg/dL) blood glucose levels. In-hospital mortality was 9.6% among those with a mean blood glucose of 80-99 mg/dL, 29.4% among those with a mean blood glucose of 180-199 mg/dL, and 42.5% among those with a mean blood glucose greater than 300 mg/dL.

Observations such as these raised concern that acute hyperglycemia was itself contributing to poor outcomes, potentially by leaving affected patients susceptible to at least some of the consequences that have long been observed among chronic diabetics, including high infection rates, poor wound healing, and polyneuropathy^[1,5]. Laboratory studies have also raised concerns about the possible deleterious effects of acute hyperglycemia, as hyperglycemia has been shown to cause injury to a variety of cell types that exhibit insulin-independent glucose uptake, including endothelial cells, hepatocytes, and renal tubular cells^[12-16].

The repeated observation that hyperglycemia is associated with worse outcomes among critically ill patients, together with the theoretical harms of acutely elevated blood glucose levels, represents the basis for focusing on glycemic control in the intensive care setting. However, the possibility remains that elevated blood glucose levels are actually beneficial to the critically ill individual, and that stress hyperglycemia is an appropriate and adaptive response to life-threatening illness, as no randomized trial investigating glycemic control has studied the effect of truly permissive hyperglycemia^[17]. Potential benefits of hyperglycemia in the critically ill individual include promotion of glucose delivery in the face of ischemic insults (down an enhanced glucose diffusion gradient), with insulin resistance favoring redistribution of available glucose

stores toward cells of the immune and nervous systems, and away from peripheral tissues^[17]. Recent observational studies have provided some support for this view, reasserting the possibility that hyperglycemia is simply a marker of illness severity. For example, a recent retrospective study of 7925 consecutive critically ill patients admitted to three mixed ICUs in Australia showed that while hyperglycemia was associated with in-hospital mortality, once lactate levels were considered, there was no independent association between hyperglycemia and mortality^[18]. This finding was consistent with a previous retrospective study, which found that among a cohort of septic nondiabetic adult patients, hyperglycemia noted on initial presentation did not increase mortality risk unless accompanied by concurrent hyperlactatemia^[19]. Such observations present a useful reminder that our understanding of the effects of hyperglycemia remains incomplete.

Our ability to identify patients most likely to suffer harm from hyperglycemia also remains incomplete. Several studies have concluded that the association between hyperglycemia and in-hospital mortality is attenuated among those with pre-existing diabetes mellitus, with some even failing to demonstrate any association at all^[11,20-23].

MAJOR INVESTIGATIONS OF GLYCEMIC CONTROL IN CRITICALLY ILL PATIENTS

Concern about the potentially deleterious effects of hyperglycemia in critically ill patients has motivated multiple randomized controlled trials investigating glycemic management in ICUs^[1,2,24-32]. This section serves to review the major trials regarding this subject, exploring the evidence that underlay the initial enthusiasm for, and subsequent skepticism of, intensive insulin therapy for glycemic normalization among critically ill patients. Key features of the trials are summarized in Table 1.

The original Leuven study, reported by van den Berghe *et al.*^[1] in 2001, was the first major prospective trial to investigate the effects of tight glycemic control in critically ill patients. This was a prospective, non-blinded, randomized controlled trial of 1548 mechanically ventilated adult patients admitted to a single surgical ICU in Leuven, Belgium. A majority of the patients (63%) had undergone cardiac surgery. Prior to admission, 13% of patients had been diagnosed with diabetes mellitus, and 5% had been maintained on insulin therapy. Upon ICU admission, patients were randomly assigned to receive either "intensive" or "conventional" insulin therapy. For all patients, insulin was delivered *via* a continuous infusion, and glycemic monitoring was performed *via* measurements of whole-blood glucose of arterial blood samples, collected every one to four hours. For patients in the intensive insulin therapy group, insulin infusions were started if measures of blood glucose exceeded 110 mg/dL, and the infusions were titrated to maintain

blood glucose in the range of 80 to 110 mg/dL. By contrast, for patients in the conventional therapy group, insulin infusions were only started if measures of blood glucose exceeded 215 mg/dL, and the infusions were titrated to maintain blood glucose in the range of 180 to 200 mg/dL. All patients received intravenous glucose for the first 24 h of ICU admission, after which feeding continued *via* total parenteral, total enteral, or combined enteral and parenteral nutrition. All patients reverted to conventional blood glucose management upon discharge from the ICU. During their ICU stays, 98.7% of patients in the intensive insulin therapy group required insulin infusions, and the targeted blood glucose level was achieved, with a mean blood glucose of 103 mg/dL. Among patients in the conventional insulin therapy group, only 39.2% required insulin infusions, and the mean blood glucose was 153 mg/dL. The results of the study strongly favored the intensive insulin therapy group, with observed benefits in terms of both morbidity and mortality. In-ICU mortality was 4.6% in the intensive insulin therapy group compared to 8.0% in the conventional insulin therapy group ($P < 0.04$), and the survival benefit persisted to hospital discharge, with an absolute risk reduction of in-hospital mortality of 3.7% (7.2% vs 10.9%; $P = 0.01$), largely due to a reduction in deaths attributed to sepsis. Compared to patients in the conventional insulin therapy group, those receiving intensive insulin therapy also experienced reduced rates of renal replacement therapy, prolonged mechanical ventilation, and extended ICU stays. The overwhelmingly positive results from the first Leuven study were in many ways practice-changing, and it informed investigations into glycemic management of critically ill patients for the ensuing decade, and beyond.

The next major prospective trial came from the same group in Belgium, and was again a single-ICU study^[24]. In this second Leuven study, 1200 adult patients admitted to a medical ICU were studied. The study included only patients who were unable to take oral nutrition upon ICU admission, and who were anticipated to require at least 3 d of intensive care. Patients were randomized to intensive vs conventional insulin therapy groups, with stratification according to diagnostic categories. Thresholds for initiation of insulin therapy and target blood glucose levels for the two groups were identical to what had been used in the first Leuven study^[24]. In stark contrast to the findings of the previous trial, the second Leuven study showed no overall mortality benefit to intensive insulin therapy, as both ICU and in-hospital mortality rates were similar among patients in the intensive and conventional insulin therapy groups. However, the authors reported a statistical difference in in-hospital mortality among the subset of patients who actually received at least 3 d of ICU care, as had been intended at the time of their inclusion in the study. Among this subset of 767 patients who stayed in the ICU for at least 3 d (of whom 386 received intensive insulin therapy and 381 received conventional insulin therapy), in-hospital mortality was

Table 1 Summary of major randomized controlled trials investigating the use of intensive vs conventional insulin therapy in critically-ill patients

Trial	Study population	Number of patients enrolled	Target blood glucose in the intensive insulin therapy group (mg/dL)	Target blood glucose in the conventional insulin therapy group (mg/dL)	Key mortality findings	Key morbidity findings
First Leuven Trial, 2001 ^[1]	Single-center; surgical ICU	1548	80-110	180-200	Reduced ICU and in-hospital mortality in the intensive insulin therapy group	Reduced incidence of bloodstream infection, acute renal failure, red cell transfusion, and critical-illness neuropathy in the intensive insulin therapy group. Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Second Leuven Trial, 2006 ^[24]	Single-center; medical ICU	1200	80-110	180-200	No mortality difference	Reduced incidence of newly acquired kidney injury, reduced duration of mechanical ventilation, and reduced lengths of ICU and hospital stays in the intensive insulin therapy group. Increased occurrence of hypoglycemia in the intensive insulin therapy group
Arabi <i>et al</i> ^[25] , 2008	Single-center; mixed ICU, including medical, surgical, and trauma patients	523	80-110	180-200	No mortality difference	Increased occurrence of hypoglycemia in the intensive insulin therapy group
Brunkhorst <i>et al</i> ^[26] , 2008	Multicenter; mixed ICUs; all patients with severe sepsis or septic shock	537	80-110	180-200	No mortality difference	No difference in the mean score for organ failure. Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
De La Rosa Gdel <i>et al</i> ^[27] , 2008	Single center; mixed ICU, including medical, surgical, and trauma patients	504	80-110	180-200	No mortality difference	Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Preiser <i>et al</i> ^[28] , 2009	Multicenter; medical and surgical ICU patients	1078	79-110	140-180	No mortality difference	Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
NICE-SUGAR Trial, 2009 ^[21]	Multicenter; medical and surgical ICU patients	6104	81-108	144-180	Increased 90-d mortality in the intensive insulin therapy group	Similar between-group markers of morbidity, with the exception of an increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Annane <i>et al</i> ^[29] , 2010	Multicenter; all patients with septic shock	509	80-110	180-200	No mortality difference	Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Coester <i>et al</i> ^[30] , 2010	Single center; all patients with severe traumatic brain injury	88	80-110	< 180	No mortality difference	No difference in neurologic outcomes. Increased occurrence of hypoglycemia in the intensive insulin therapy group
Green <i>et al</i> ^[31] , 2010	Single center; mechanically-ventilated neurologic patients	81	80-110	≤ 150	No mortality difference	No difference in neurologic function at 90 d. Increased occurrence of hypoglycemia and severe hypoglycemia in the intensive insulin therapy group
Macrae <i>et al</i> ^[32] , 2014	Multicenter; medical and surgical pediatric patients	1369	72-126	180-216	No mortality difference	No difference in ventilator-free survival. Increased occurrence of severe hypoglycemia in the intensive insulin therapy group

ICU: Intensive care unit.

43.0% in the intensive therapy group, compared to 52.5% in the conventional therapy group ($P = 0.009$). While an interesting finding, this subset analysis suffered from a lack of real-world applicability (even the authors were unable to accurately predict which patients would require extended ICU stays) and a loss of balanced diagnostic categorization (likely biasing the results). While no mortality benefit to intensive insulin therapy was identified, secondary analyses of patient morbidity found reduced rates of acquired kidney injury, reduced durations of mechanical ventilation, and reduced lengths of ICU and hospital stay among patients in the intensive insulin therapy group compared to those in the conventional insulin therapy group.

The mortality benefits realized in the first Leuven study and the morbidity benefits realized in the second sustained considerable enthusiasm for tight glycemic control in critically ill patients for the next several years, with widespread adoption of intensive insulin protocols in medical and surgical ICUs, despite occasional voices urging caution^[33,34]. However, a series of studies published in 2008 and 2009, culminating with the NICE-SUGAR trial, severely tempered this enthusiasm^[2,25-28]. The first of these trials, reported by Brunkhorst *et al.*^[26], involved patients with severe sepsis or septic shock admitted to multidisciplinary ICUs in 18 academic tertiary hospitals in Germany. This was a two-by-two factorial trial, and patients were randomized to receive either intensive or conventional insulin therapy for glycemic control (with protocols similar to those used in the two Leuven studies^[1,24]) and either hydroxyethyl starch or modified Ringer's lactate for fluid resuscitation. The use of intensive insulin therapy was terminated after the first safety analysis, due to a nearly six-fold increased frequency of hypoglycemia in the intensive insulin group, including a high proportion of severe hypoglycemic events that were classified as life-threatening and requiring prolonged hospitalization. Among the patients studied, there was no documented benefit to intensive insulin therapy, as there were no statistical differences in rates of mortality, rates of acute renal failure or renal replacement therapy, use of vasopressor medications, number of ventilator-free days, or length of ICU stay.

Several subsequent studies conducted in a variety of settings similarly failed to demonstrate clear benefits to tight glycemic control in critically ill patients, but consistently highlighted an increased risk of hypoglycemia among patients treated with intensive insulin protocols^[2,25,27,28]. Arabi *et al.*^[25] reported a prospective trial wherein they randomized 523 medical, surgical, and trauma patients admitted to a single ICU in Riyadh, Saudi Arabia to intensive or conventional insulin therapy, and found no between-group differences in mortality, ICU or hospital lengths of stay, rates of renal replacement therapy, durations of mechanical ventilation, or frequencies of infectious complications, but patients in the intensive insulin group experienced much higher rates of hypoglycemia. Similar negative findings with respect to measures of mortality and morbidity were

reported by De La Rosa Gdel *et al.*^[27] in their study of 504 medical, surgical, and trauma patients admitted to a single ICU in Medellin, Colombia and randomized to intensive or conventional insulin therapy, though again, rates of hypoglycemia were much higher in the intensive insulin group. A subsequent multinational trial, involving patients admitted to 21 medico-surgical ICUs in 7 countries, also failed to identify meaningful benefits to tight glycemic control^[28]. This study, which again randomized patients to intensive or conventional insulin therapy, was ultimately underpowered, as it was prematurely stopped due to a high rate of unintended protocol violations. However, among the 1078 patients studied, there were no between-group differences in mortality, and the only differences in measures of morbidity were higher rates of hypoglycemia among patients in the intensive insulin therapy group and a slight reduction in vasopressor/inotrope use in the conventional insulin therapy group.

On the heels of these four consecutive negative studies^[25-28], the landmark NICE-SUGAR trial was reported, which remains the most comprehensive study of glycemic control strategies among ICU patients performed to date^[2]. The NICE-SUGAR study included 6104 medical and surgical patients admitted to ICUs at 42 hospitals in Australia, New Zealand, Canada, and the United States. All patients were anticipated to require at least 3 d of ICU care, were expected to be unable to eat for at least 2 d, and had an arterial line in place as part of their routine ICU management. As with previous studies, patients were randomized to intensive or conventional insulin therapy groups, but the target blood glucose range of the conventional insulin therapy group was lower than it had been in the Leuven studies^[1,24], based on updated practice surveys. In the intensive insulin therapy group, the target blood glucose range was 81 to 108 mg/dL, while in the conventional insulin therapy group, the target blood glucose was 180 mg/dL or less, with insulin administration reduced and then discontinued if blood glucose levels fell below 144 mg/dL. As had been the case in Leuven studies^[1,24], blood glucose monitoring was performed every one to four hours, and the use of arterial rather than capillary blood samples for this purpose was encouraged. The majority of patients in both treatment groups received insulin therapy (97.2% of those in the intensive insulin therapy group and 69.0% of those in the conventional insulin therapy group). The mean time-weighted blood glucose level in the intensive group was 115 mg/dL, while it was 144 mg/dL in the conventional group. The primary study endpoint was 90-d all-cause mortality, which was 2.6% higher in the intensive than in the conventional insulin therapy group (27.5% vs 24.9%, $P = 0.02$). Subgroup analyses suggested no differences in treatment effects for comparisons of medical and surgical patients, patients with and without preexisting diabetes, and patients with and without severe sepsis. With the exception of rates of severe hypoglycemia, markers of morbidity did not differ according to treatment groups,

as there were similar between-group ICU and hospital lengths of stay, durations of mechanical ventilation, frequencies and durations of renal replacement therapy, rates of new organ failure, and occurrences of positive blood cultures. Severe hypoglycemia (defined as a blood glucose level less than or equal to 40 mg/dL) occurred in 6.8% of the patients in the intensive insulin therapy group vs 0.5% of those in the conventional therapy group ($P < 0.001$).

Following the overwhelmingly negative results of the NICE-SUGAR study, Annane *et al.*^[29] reported on the use of intensive vs conventional insulin therapy in patients with septic shock being treated with corticosteroids, hypothesizing that this subset of ICU patients may benefit from intensive insulin therapy, even if a general ICU population does not. A total of 509 patients treated in 11 ICUs in France were randomized to intensive or conventional insulin therapy, according to the treatment protocols used in the first Leuven study^[1]. Here again, there were no between-group differences in measures of patient mortality or morbidity, with the exception of an increased rate of severe hypoglycemia among patients in the intensive insulin therapy group. Subsequently, randomized controlled trials investigating intensive insulin therapy among mechanically ventilated neurologic patients, patients with severe traumatic brain injuries, and critically ill pediatric patients have all failed to demonstrate a clinical benefit to tight glycemic control^[30-32].

In summary, following the publication of the two single-center Leuven studies^[1,24], the preponderance of evidence has strongly indicated that the use of intensive insulin treatment with the goal of tight glycemic management in critically-ill patients at best provides no benefit over moderate or lax glycemic control, and at worst results in markedly increased rates of severe hypoglycemia and possibly even increased mortality^[2,25-29].

HYPOGLYCEMIA

As clinicians and investigators have grappled with the results of the NICE-SUGAR trial and of other negative studies regarding the use of intensive insulin therapy in critically-ill patients^[2,25-32], several potential explanations have been proposed to account for the lack of demonstrable benefit for tight glucose control. The proposed explanations have targeted either the rationale for intensive insulin therapy (positing that hyperglycemia may be beneficial, or that exogenous insulin may be harmful), or the execution of the strategy (suggesting that the labor-intensive focus on tight glycemic control distracts from other considerations, or that the benefits of normoglycemia have been obscured by an inability to avoid hypoglycemia)^[4,35]. This final consideration—that hypoglycemic complications negate the potential benefits of tight glycemic control—has gained widespread acceptance, and has important implications for future study of glycemic management among critically-ill patients. Hypoglycemia has been a commonly-reported

occurrence among the patients treated with intensive insulin therapy in major trials, and severe hypoglycemia (defined as a blood glucose level less than 40 mg/dL) has occurred in up to 28% of these patients^[4]. It was not initially clear whether the increased rate of hypoglycemia experienced among patients treated with a tight glycemic control strategy was problematic. In the first Leuven study, severe hypoglycemia was reported to have occurred 6.6-fold more commonly among patients in the intensive insulin therapy group, but no clinically-significant outcomes were associated with its occurrence in any of the patients, and the issue of hypoglycemia was not addressed in the manuscript's discussion^[1].

By the time the NICE-SUGAR trial was reported, the frequency of hypoglycemic episodes among patients treated with intensive insulin regimens had become a significant concern. It was recognized that hypoglycemia could theoretically be harmful to patients by means of a number of different mechanisms, including irreversible neuronal damage, autonomic instability, cardiac arrhythmia, and alteration of inflammatory responses^[36,37]. The relationship between hypoglycemia and mortality was examined in a post-hoc analysis of the NICE-SUGAR trial^[37]. For the purpose of this analysis, severe hypoglycemia was defined as a recorded blood glucose level of 40 mg/dL or less, while moderate hypoglycemia was defined as a recorded blood glucose level in the range of 41 to 70 mg/dL. Among the 6026 patients analyzed, severe hypoglycemia occurred in 3.7% of individuals, while moderate hypoglycemia occurred in an additional 45.0%. Hypoglycemic episodes were much more common among those patients in the intensive insulin therapy group, with this group accounting for 93.3% of severe hypoglycemia and 82.4% of moderate hypoglycemia. The occurrence of hypoglycemia was strongly associated with an increased risk of death, with moderate hypoglycemia associated with a 40% increase in adjusted mortality risk, and severe hypoglycemia associated with a doubling of this risk. While these data do not prove a causal relationship between hypoglycemia and mortality, they do support the possibility that it was the increased frequency of iatrogenic hypoglycemic episodes that accounted in some measure for the excess mortality observed among patients treated with intensive insulin therapy in the NICE-SUGAR trial.

This possibility has been supported by other retrospective studies investigating the relationship between hypoglycemic episodes and mortality among ICU patients. In a review of 4946 patients admitted to two ICUs in Australia, Egi *et al.*^[38] found that 22.4% of patients experienced at least one episode of hypoglycemia, defined as recorded blood glucose of less than 82 mg/dL. The patients were analyzed in six bands, according to the level of their lowest recorded blood glucose, and it was shown that the severity of hypoglycemia was independently associated with in-hospital mortality. In a separate single-center review of 5365 consecutive patients admitted to a mixed medical-

surgical ICU, the occurrence of even one episode of severe hypoglycemia was seen to be independently associated with mortality, both by case-control and by multivariable logistic regression analyses^[39].

To a significant extent, a desire to avoid inducing hypoglycemia has motivated the move away from treating ICU patients with intensive insulin protocols^[40]. It should be noted that the focus on avoiding hypoglycemia leaves the door open to future reconsideration of the benefits of tight glycemic control. If the problem with intensive insulin therapy is mainly an inability to avoid hypoglycemic episodes, one can imagine that the development of better glucose monitoring technologies and glycemic control algorithms (if they allow for severe reductions in the incidence of hypoglycemia) could result in improved outcomes with a tight glycemic control strategy. In recent years, the development of continuous glucose monitoring systems has received significant attention along these lines, but the benefits of continuous glucose monitoring have not yet been established^[41-43].

GLYCEMIC VARIABILITY

In recent years, it has increasingly been recognized that glycemic variability is a dimension of significant importance among critically-ill patients, independent of the acute highs and lows of blood glucose measurements in the ICU. The potential significance of glycemic variability among ICU patients was first raised by Egi *et al.*^[44], in a retrospective observational study of 7049 patients who had been admitted to four hospitals in Australia. For the purposes of this study, a patient's glycemic variability was defined as the standard deviation of the arithmetical mean of the entire set of glucose measurements during that individual's ICU stay. The authors found that glycemic variability was an independent predictor of mortality, and that the glycemic variability was actually a stronger predictor of ICU mortality than the mean glucose concentration. A subsequent single-center retrospective observational study of 3252 ICU patients in the United States confirmed and extended these findings, again demonstrating that this measure of glycemic variability was a strong independent predictor of mortality, even after excluding patients who had experienced severe hypoglycemia^[45].

As glycemic variability has been further considered among ICU patients, definitions have changed. Defining glycemic variability as the standard deviation of the mean of all blood glucose measurements has fallen out of favor, as starkly different glycemic patterns can generate identical mean glucose and standard deviations^[46]. Multiple other measures of glycemic variability have been described, including coefficient of variation, glycemic lability index, mean absolute glucose change, and mean amplitude of glycemic excursion^[47,48]. No gold standard for measuring glycemic variability has been established, but multiple studies utilizing these more complicated metrics have confirmed that glycemic variability is

independently associated with mortality among ICU patients^[23,46,48,49].

Whether glycemic variability is a cause of poor patient outcomes or is simply a marker of severe illness is not known. However, several lines of evidence have suggested that glycemic variability causes oxidative stress, enhances cell apoptosis, and impairs endothelial function^[45,46]. Therefore, it is plausible that glycemic variability causes harm to critically-ill patients, and that optimal glycemic control in the ICU would aim to minimize glycemic variability. As with avoiding hypoglycemia in the ICU, it is hoped that advances in glycemic monitoring and corresponding glucose control algorithms will reduce the extent of glycemic variability, but at least one early study has failed to show that existing means of continuous glucose monitoring would reduce glycemic variability^[47].

PREMORBID DIABETIC STATUS

From the first Leuven study to the NICE-SUGAR trial, all of the major investigations of intensive insulin therapy in critically-ill patients utilized glycemic-control protocols that did not differentiate between diabetic and nondiabetic patients^[1,2,24-28]. Similarly, recent guidelines regarding the use of insulin infusions in the ICU do not advocate altering the approach to glycemic management on the basis of patients' premorbid diabetic status^[40]. However, there is growing evidence that diabetic and nondiabetic patients respond differently to dysglycemia experienced in the ICU.

Krinsley *et al.*^[49] performed a retrospective observational study of 44964 patients admitted to 23 ICUs in 9 countries to determine how diabetic status affected the associations of hyperglycemia, hypoglycemia, and glycemic variability with mortality. While hypoglycemia was associated with an increased risk of mortality among all patients, the diabetic status modulated the impact of both hyperglycemia and glycemic variability. In nondiabetic patients, maintenance of euglycemia was independently associated with a reduced mortality risk, but among diabetic patients, those with a mean glucose of 80 to 110 mg/dL actually had an increased risk of mortality, even when compared only to those with a mean glucose greater than 179 mg/dL. The significance of glycemic variability also seemed to differ according to diabetic status, as a high level of glycemic variability (defined as a coefficient of variability greater than 20%) was independently associated with an increased risk of mortality among nondiabetic patients, but not among those with diabetes.

Similar findings were reported in a subsequent single center retrospective observational study that analyzed glucose and outcome data from 10320 ICU patients^[23]. Again, hypoglycemia was associated with mortality in both diabetic and nondiabetic patients, but outcomes associated with hyperglycemia and glycemic variability differed according to premorbid diabetic status. While hyperglycemia was associated with increased mortality

among the nondiabetic patients, no clear pattern relating elevated mean glucose levels with mortality could be found among the diabetic patients. In addition, glycemic variability (as measured by mean absolute glucose change) was only associated with increased mortality among the nondiabetic patients.

Such differences among diabetic and nondiabetic patients have raised the possibility that future glycemic control protocols for critically-ill patients will differ according to premorbid diabetic status, or other markers of insulin resistance, such as metabolic syndrome or non-alcoholic fatty liver disease^[50,51]. However, further studies are needed to better define optimal glycemic management among diabetic patients in the ICU.

CONCLUSION

In the past two decades, glycemic management among critically-ill patients has been a topic of extensive study, leading to significant changes in clinical practice. Intensive insulin therapy was widely adopted following the publication of the first Leuven study^[1], only to be largely abandoned as further knowledge accumulated questioning the benefits of this approach, ultimately culminating with the NICE-SUGAR trial, which found an increased risk of mortality among patients treated with tight, as compared to moderate, glucose control strategies^[2]. Current guidelines regarding glycemic management of critically-ill patients advocate initiating insulin infusions for blood glucose measurements in excess of 150 mg/dL, with the goal of maintaining blood glucose less than 180 mg/dL^[40]. While targeting a blood glucose level less than 180 mg/dL is now widespread (and consistent with the control group in NICE-SUGAR), it should be noted that evidence supporting this goal, as opposed to an even more permissive glycemic control strategy, is lacking.

In recent years, there has been an increased focus on the potential deleterious effects of glycemic variability, though it remains unclear how best to avoid fluctuations in blood glucose levels. In addition, there has been increasing attention given to differences among the glycemic control needs of diabetic and nondiabetic patients.

In coming years, we expect that new glucose monitoring systems will emerge, and that new strategies for maintaining euglycemia (while avoiding hypoglycemic episodes and glycemic variability) will follow. Glycemic management among critically-ill patients remains an area of unsettled medicine.

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Interpretation of cardiovascular outcome trials in type 2 diabetes needs a multiaxial approach

Odd Erik Johansen

Odd Erik Johansen, Boehringer Ingelheim Norway KS, 1373 Asker, Norway

Odd Erik Johansen, Department of Medical Research, Bærum Hospital, Vestre Viken Hospital Trust, 1309 Rud, Norway

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Correspondence to: Odd Erik Johansen, MD, PhD, Medical Director, Boehringer Ingelheim Norway KS, P.O. Box 405, 1373 Asker, Norway. odd_erik.johansen@boehringer-ingelheim.com
 Telephone: +47-91817674
 Fax: +47-66761330

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Abstract

In cardiovascular (CV) diabetology a "one-size fits-all" approach needs caution as vasculopathy and CV manifestations in patients with type 2 diabetes (T2D) with short disease duration are different as compared to those with longer duration. This is of relevance when

interpreting results of CV outcome trials as responses to any intervention aimed to reduce CV risk might be different in patients with established vasculopathy as compared to those without, where also the duration of the intervention may play a role. Additionally, the mode-of-action of the intervention and its assumed time to peak CV risk modulation need to be taken into account: an intervention with possibly immediate effects, like on blood pressure or other direct functional dynamic parameters such as endothelial function or renal hemodynamics, could likely provide a meaningful impact on CV outcomes over a shorter time span than interventions that primarily target pathways that work on atherosclerotic processes, organ-remodelling, or vessel integrity. We are now faced with CV outcome results to interpret from a plethora of outcomes trials in T2D, some of which are testing the CV risk modulation predominantly beyond glucose lowering, *e.g.*, as is the case for several trials testing the newer therapy classes di-peptidyl peptidase-4 inhibitors, glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors, and this paper reviews the data that support a call for a multiaxial approach to interpret these results.

Key words: Type 2 diabetes; Pharmaceutical; Risk reduction; Outcomes; Cardiovascular

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Core tip: Vasculopathy and cardiovascular (CV) manifestations in patients with type 2 diabetes differ dependent on disease duration. This literature review supports that it is necessary to contextualize results of CV outcome trials in diabetes to diabetes duration as well as duration and mode of action of the intervention, which may be of particular relevance for those interventions that primarily target pathways related to atherosclerotic processes, organ-remodelling, or vessel integrity. Several CV outcome trials testing newer therapy classes (*i.e.*, di-peptidyl peptidase-4 inhibitors,

glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors) are now due to report and a multi-axial approach to interpret these results is needed.

Johansen OE. Interpretation of cardiovascular outcome trials in type 2 diabetes needs a multi-axial approach. *World J Diabetes* 2015; 6(9): 1092-1096 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i9/1092.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i9.1092>

INTERPRETATION OF CARDIOVASCULAR OUTCOME TRIALS IN TYPE 2 DIABETES

The human mind is a master in pattern recognitions. A flip-side to this profound ability in predicting cause-and-effects surfaces however in dealing with complex questions where a "one-size fits-all" approach not necessarily longer applies. Cardiovascular (CV) diabetology is one example of a complex system where a "one-size fits-all" approach needs caution. For example, vasculopathy and CV manifestations in patients with type 2 diabetes (T2D) with short disease duration are different as compared to those with longer T2D duration. Further, the response to any intervention aimed to reduce CV risk might be different in patients with established vasculopathy as compared to those without, where also the duration of the intervention may play a role for a successful risk reduction. The last point is however also dependent on the mode-of-action of the intervention, since an intervention with possibly immediate effects, like on blood pressure or other direct functional dynamic parameters such as endothelial function or renal hemodynamics, likely could provide a meaningful impact on outcomes over a shorter time span than interventions that primarily targets pathways that work on atherosclerotic processes, organ-remodelling, or vessel integrity. These are all important considerations that need to be taken into account when we soon will be faced with results to interpret from a plethora of outcomes trials in T2D, some of which are testing the CV risk modulation potential predominantly beyond glucose lowering, *e.g.*, as is the case for the newer therapy classes di-peptidyl peptidase (DPP)-4 inhibitors, glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors (Figure 1).

THE RELEVANCE OF CONTEXTUALIZING OUTCOME TRIAL RESULTS TO T2D DURATION AND PRESENCE OR ABSENCE OF CV COMPLICATIONS?

T2D is a progressive complex metabolic disease^[1] leading to disturbances in several pathways (*e.g.*,

hyperglycemia, insulin resistance, inflammation, oxidation, endothelial dysfunction, dysfunctional adiposity) involved in vasculo-biopathology and CV complications^[2]. With this in mind, what could possibly explain differing impact on CV risk of an intervention given early in the T2D disease course vs late? One element relates to that longer-standing T2D is associated with silent vasculopathy, as illustrated by *e.g.*, approximately 20% of clinically asymptomatic patients with T2D having significant coronary artery disease, either by invasive coronary angiography^[3] or by photon emission-computed tomography myocardial perfusion imaging^[4]. Further, since longer duration of the disease and advancing age typically lead to an accumulation of subclinical [such as vascular stiffness^[5], coronary artery calcifications (CAC)^[6], or myocardial dysfunction^[7]] or clinical manifestations of CV complications (*i.e.*, myocardial infarction), or microvascular complications (which is an emerging risk factor for CV complications^[8]), it might be conceivable that if the patient population being studied has advanced vasculopathy, the likelihood to influence the disease course could be lower. In particular if end-stage complications have manifested, *e.g.*, as observed in patients on dialysis where statins apparently do not reduce CV risk^[9], since these patients may be less sensitive to improvement in CV risk factors.

In longer-term outcome trials in T2D, where different strategies to intensively improve glucose control were tested, this point, to a certain degree, was illustrated by different results on outcomes as observed in the United Kingdom Prospective Diabetes Study (UKPDS); a study^[10] that recruited newly diagnosed patients with T2D with a low CV disease burden, and the ORIGIN trial^[11], which recruited patients with 5-6 years of diabetes duration of whom approximately 60% had prior CV complications (Figure 2). Although both studies achieved meaningful differences in glucose control between treatment arms, only those patients with newly diagnosed T2D without prevalent CV disease in UKPDS, achieved outcome benefits. Whether this was related to the short diabetes duration and low vasculopathy burden at the start of the intervention, a long treatment duration, or mode of action of the different interventions, is not known. The potential differing response to preventive therapies in patients with short vs long standing diabetes was also illustrated in a subanalysis of the recent CV outcome trial comparing outcomes of placebo or alogliptin superimposed on standard of care in patients with T2D and acute coronary syndrome (the EXAMINE trial)^[12]. Overall the glycemic differences between the treatment arms were small and the primary outcome was neutral, however, patients with shorter diabetes duration (*i.e.*, less than 5 years) had reduced risk [hazard ratio (HR) = 0.74 (95%CI: 0.54, 1.01)] for the composite primary CV endpoint as compared to those with longer disease duration [5-10 years HR = 0.81 (95%CI: 0.58, 1.13); > 10 years HR = 1.22 (95%CI: 0.98, 1.53); interaction with treatment *P*-value 0.014]. Another interesting observation in the context

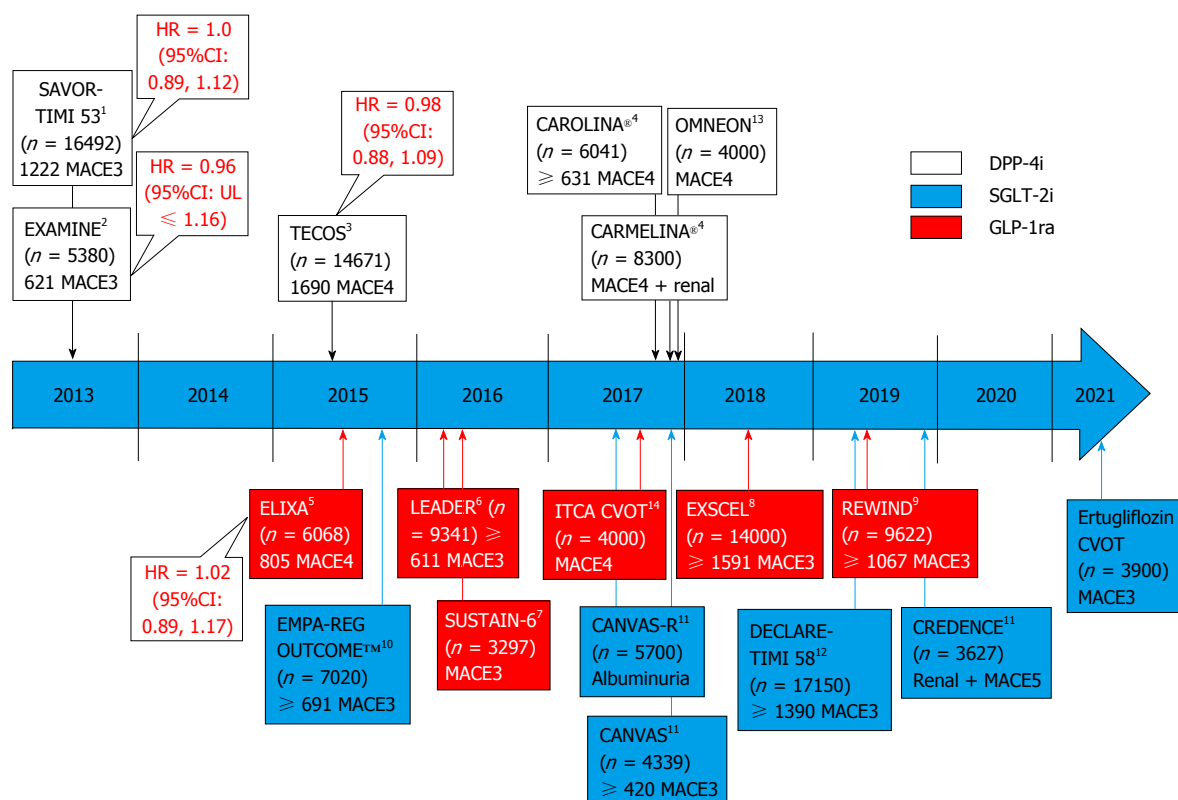


Figure 1 Anticipated ending of outcome trials in type 2 diabetes and their primary outcomes and patient/event numbers involving di-peptidyl peptidase-4 inhibitors, glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors. Superscript note indicate study drug(s) in testing. All trials are placebo controlled except CAROLINA⁴ that compared vs the sulfonylurea glimepiride. ¹Saxagliptin, Astra Zeneca; ²Alogliptin, Takeda; ³Sitagliptin, Merck; ⁴Linagliptin, Boehringer Ingelheim/Eli Lilly; ⁵Lixisenatide, Sanofi Aventis; ⁶Liraglutide, Novo Nordisk; ⁷Semaglutide, Novo Nordisk; ⁸Exenatide, Astra Zeneca; ⁹Dulaglutide, Eli Lilly; ¹⁰Empagliflozin, Boehringer Ingelheim/Eli Lilly; ¹¹Canagliflozin, J and J; ¹²Dapagliflozin, Astra Zeneca; ¹³Omarigliptin (once weekly tablet), Merck; ¹⁴ITCA 650 [once/twice yearly exenatide via subcutaneous mini-pump (Duros device)], Intarcia Therapeutics. DPP-4: Di-peptidyl peptidase-4; GLP-1: Glucagon-like protein-1; SGLT-2: Sodium glucose co-transporter-2; MACE3: Composite endpoint of cardiovascular death, non-fatal myocardial infarction and non-fatal stroke; MACE4: MACE3 plus hospitalized unstable angina pectoris; MACE5: MACE4 plus hospitalized congestive heart failure.

of degree of vasculopathy as a potential determinant for the effect of an intervention stems from the veterans affairs diabetes trial (VADT)^[13]. The VADT tested whether intensive glucose control (targeted/achieved HbA1c < 6.0%/6.9%) vs conventional (targeted/achieved HbA1c < 9.0%/8.9%) could reduce CV risk in 1791 patients with long-standing T2D^[13]. Although intensive glucose-lowering therapy did not significantly reduce CV events in the study cohort as a whole, there was evidence that the response was modified by baseline CAC. They observed, *e.g.*, that among those randomized to intensive treatment, in the subgroup with CAC > 100, 11 of 62 individuals had events, while only 1 of 52 individuals with CAC ≤ 100 had an event (significant risk reduction), indicating that intensive glucose lowering reduced CV events only in those with less extensive calcified coronary atherosclerosis^[14].

WHY IS IT IMPORTANT TO CONTEXTUALIZE OUTCOME TRIAL RESULTS TO DURATION OF INTERVENTION?

In order for an intervention to reduce CV risk it has

to interfere with the cascade of events that lead to complications. Since T2D is a CV risk entity by itself, where CV risk typically is further magnified in the presence of CV complications, any intervention that targets outcomes like myocardial infarction or hospitalization for angina pectoris primary related to atherosclerosis, likely have to be of sufficient duration since the biopathological processes typically might evolve over decades^[15,16]. Although the targeted study outcome as well as the mode of action of the intervention certainly plays an important role here, one important question is when the effects of an intervention are assumed to peak. This was illustrated, for example, by the PRO active trial^[17], comparing pioglitazone vs placebo as secondary CV prevention: at study end the primary endpoint just missed the significance level, but as the survival curves separated in favour of pioglitazone towards study end, it was speculated that the trial result could have looked different if the trial had run longer^[18]. At this point it is only speculations if the two other recent neutral outcome trials involving DPP-4 is, a class that in animal studies have been implied to reduce several pathways leading to atherosclerosis^[19], namely SAVOR-TIMI53^[20], EXAMINE^[12], and TECOS^[21] would have showed different results if ran longer than

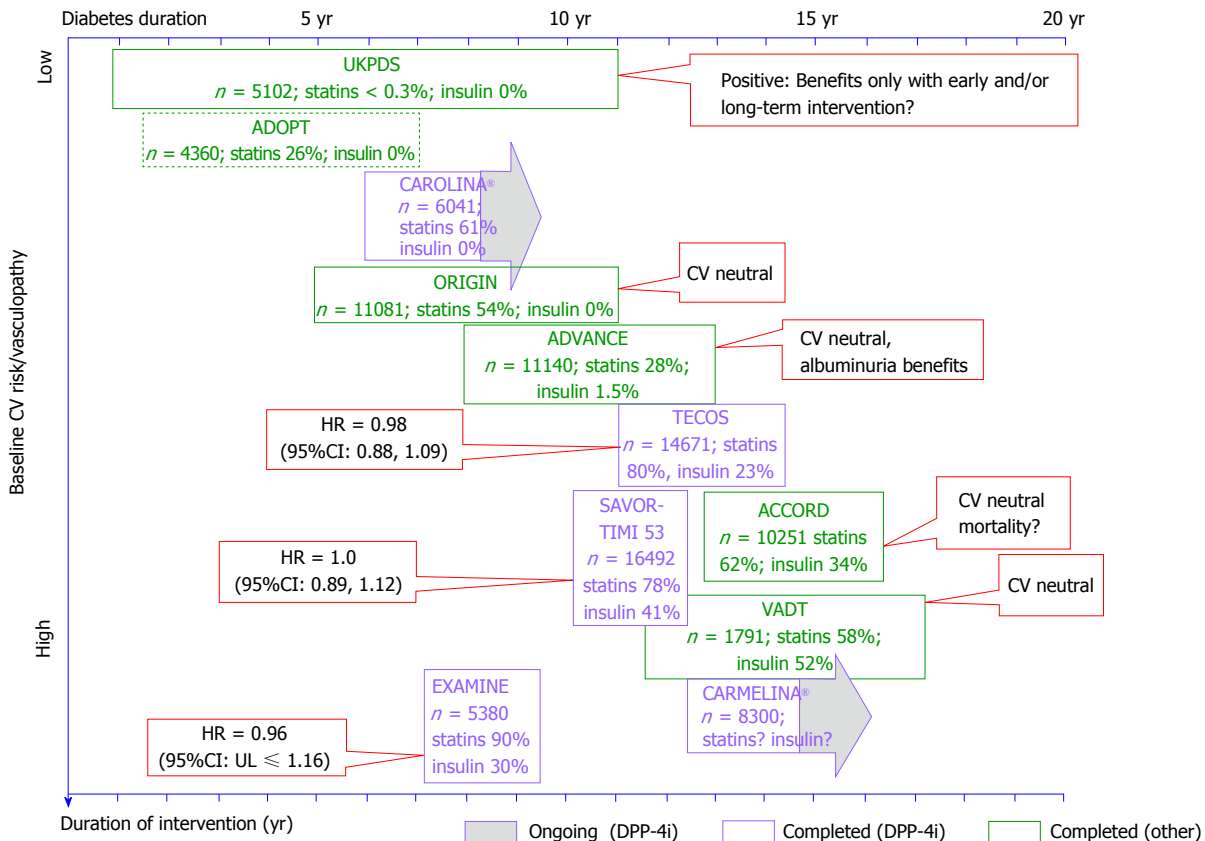


Figure 2 Selected outcome trials in type 2 diabetes with a focus on di-peptidyl peptidase-4 inhibitor studies, and their results, in the context of the duration of intervention and the study population's diabetes duration and baseline cardiovascular risk. CV: Cardiovascular; UKPDS: United Kingdom Prospective Diabetes Study; VADT: Veterans affairs diabetes trial.

their median duration of respectively 2.1, 1.5 and 2.8 years. Obviously this needs further clarification in trials of longer duration.

RESULTS OF CV OUTCOME TRIALS IN T2D NEED TO BE INTERPRETED IN A MULTIDIMENSIONAL FRAME

Over the next years, with several CV outcome trials due to report (Figure 1)^[22-29], an opportunity for great learnings is at our doorsteps. Since some trials might even contribute to paradigm shifts in our approach to T2D management, it is important to contextualize the results to the study populations in scope taking into account T2D disease duration, degree of vasculopathy, duration of the intervention, and the mode of action of the intervention (Figure 2). Only this will fully support and facilitate an optimized patient centered approach to T2D care and CV risk management^[30].

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Aloesin as a medical food ingredient for systemic oxidative stress of diabetes

Mesfin Yimam, Lidia Brownell, Qi Jia

Mesfin Yimam, Lidia Brownell, Qi Jia, Unigen, Inc., Seattle, WA 98121, United States

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Correspondence to: Mesfin Yimam, DVM, MS, Unigen, Inc., 3005 1st Ave, Seattle, WA 98121, United States. myimam@unigen.net
Telephone: +1-360-4868200
Fax: +1-360-4139135

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Abstract

Diabetes is a chronic disease that requires a long term management where oxidative stress plays a pivotal role in disease progression and intensifying secondary complications. In spite of all the research on diabetes and recent advances in diabetes treatments, the reality is that there is no cure for diabetes and its devastating

complications. While currently available anti-diabetic therapies are effective in reducing blood glucose level, they are not without associated side effects when they are used for a long term applications. As a result, physicians and patients are inclining more towards a safer therapy with less serious side effects in the form of medicinal foods and botanical alternatives that are suitable for chronic usage. Aloesin, an Aloe chromone, has previously been formulated with an aloe polysaccharide to give a composition called Loesyn, where it showed significant impact in reducing glycosylated hemoglobin, fasting blood glucose, fructosamine and plasma insulin level in humans. Radical scavenging activities of chromones and polysaccharides from Aloe have also been reported. Here we rationalize the relevance of use of Aloesin alone or in a standardized blend with Aloe polysaccharides, as a potential medical food to manage systemic oxidative stress and/or high blood glucose of diabetes.

Key words: Aloesin; Aloe polysaccharides; Diabetes; Oxidative stress; Medicinal food; Aloe chromone

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Core tip: Diabetes has become epidemic in industrialized countries; Diabetes is a chronic disease with no cure; Oxidative stress plays a pivotal role in diabetes complication; Aloesin and aloe polysaccharides have strong free radical scavenging activities; Aloesin formulated with aloe polysaccharides has shown merits in diabetes management in human clinical trials; Aloesin formulated with aloe polysaccharides could have potentials in combating diabetes associated oxidative stress or to be used as an adjunct to pharmaceutical drugs.

Yimam M, Brownell L, Jia Q. Aloesin as a medical food ingredient for systemic oxidative stress of diabetes. *World J Diabetes* 2015; 6(9): 1097-1107 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i9/1097.htm> DOI: <http://>

INTRODUCTION

The diabetes epidemic that continues to sweep across the United States has left an estimated 29.1 million Americans in 2012 struggling with the disease. Currently, 387 million people worldwide are affected by diabetes mellitus and are predicted to reach 592 million in 2035. A staggering number, 4.9 million deaths were directly caused by diabetes in the year 2014 highlighting the death of a human being every seven seconds due to the seriousness of the disease. It is also predicted to be the 7th leading cause of death by the year 2030^[1]. There are 86 million people in the United States who have elevated blood glucose levels and worldwide, more than 300 million people were estimated to have this pre-diabetic condition^[2]. Based on the survey carried out in the years between 2009-2012, and fasting glucose or glycosylated hemoglobin (HbA1C) levels, 37% of United States adults age ≥ 20 years had pre-diabetes. Correcting this percentage to the entire United States population, in 2012 there were an estimated 86 million Americans age 20 years or older with pre-diabetes. Worldwide, by 2025, the pre-diabetic population number is expected to reach over 500 million people, but even more alarming is the fact that between 29%-68% of people with pre-diabetes develops type II diabetes over the course of 3-5 years^[3]. Diabetes can affect many parts of the body where oxidative stress induced by hyperglycemia is involved in both the development and progression of the disease and can lead to serious complications such as blindness, kidney damage, lower-limb amputations, and cardiovascular diseases.

According to the National Diabetes Statistics Report, 2014, diabetes in the fiscal year of 2012 cost the United States \$245 billion as a result of direct medical care (176 billion) and indirect costs (69 billion) due to disability, work loss, and premature death which accounts for more than 10% of all United States health care spending by the government and public. This is a 41% increase from previous estimate of \$174 billion in 2007. In 2012, it was estimated that after adjusting for population age and sex differences, average medical expenditures among people with diagnosed diabetes were 2.3 times higher than people without diabetes^[4].

Diabetes is one of the largest therapeutic segments of global pharmaceutical sales. It has been projected that the overall annual global spending on medicines will reach nearly \$1.2 trillion by 2016 where the top 20 therapy areas will account for 42% of global spending, led by cancer, diabetes and asthma/Chronic Obstructive Pulmonary Disease from which spending on conventional medicines for diabetes expected to range \$48-53 billion^[5]. Overall, anti-diabetic drugs sales are projected to grow dramatically over the coming years as the addressable patient population continues

to increase and new premium priced products enter the market to address high unmet clinical needs. While Food and Drug Administration approved effective drug therapies are currently available, their chronic usages are limited by serious side effects for managing long-term condition of the disease. Hence, both physicians and patients are increasingly seeking safer therapy with less serious side effects in the form of medicinal foods and botanical drugs that are suitable for long term chronic usage to help manage their blood sugar levels. Such safer alternatives would also be appropriate interventions at the pre-diabetic condition to halt or slow progression to full blown type 2 diabetes. Here we describe the relevance of use of an Aloe chromon, Aloesin by itself or in a standardized blend with Aloe polysaccharides as potential medical food ingredients to manage systemic oxidative stress of diabetes and/or mitigating the primary causes as a partial fulfilment to the unmet needs of botanical interventions.

SYSTEMIC OXIDATIVE STRESS IS ASSOCIATED WITH DIABETES AND ITS COMPLICATIONS

Principally, it is recognized that oxidative stress is an imbalance between the production of free radicals and the inherent capacity of the body to counteract or neutralize their harmful effects through interaction with various reducing and sequestering endogenous antioxidant defense networks. Reactive oxygen species (ROS) are heterogeneous population of molecules that include oxygen related free radicals, and non-radical species. Normally, ROS can be generated as by-products of glucose or free fatty acid metabolic processes in the mitochondria. In mitochondrial respiration process, between 0.4%-4% of all oxygen consumed in metabolism of glucose is converted into the free radical superoxide (O_2^-). Additionally, ROS can also be generated from food additives, environmental sources, (e.g., ultraviolet radiation) and tobacco smoke, and many other environment pollutants. When there is a lack of an appropriate adaptation by the body antioxidant defense system, ROS buildup will lead to the activation of stress-sensitive intracellular signaling pathways that, in turn, promote cellular damage and contribute to the diabetic complications development and progression.

Currently there are considerable indications that multiple biochemical pathways are activated by hyperglycemia, and are associated with the generation of ROS, which ultimately lead to increased oxidative stress. Primarily, chronic elevation of glucose in association with free fatty acid (FFA) can cause oxidative stress due to increased production of mitochondrial ROS, non-enzymatic glycation of proteins, glucose oxidation, increased mitochondrial uncoupling and beta-oxidation. The oxidative stress from both metabolism of glucose and FFA can activate signaling pathways such as nuclear factor- κ B (NF- κ B), p38 mitogen-activated

protein kinase (MAPK) and NH2-terminal c-Jun kinases. These stress activated pathways, in turn, can lead to insulin resistance, beta-cell dysfunction and impaired insulin secretion proceeding to further damage of the eye, kidney, nerve, cardiovascular system and other complications of type-2 diabetes^[6]. This fact holds true even for type-1 diabetes, where systemic oxidative stress is also present^[7]. For example, under a clinical study, patients with diabetes mellitus showed a positive correlation of NF- κ B activation in peripheral blood mononuclear cells with poor glycemic control.

Under normal circumstances, cells have specific mechanisms to preserve homeostasis^[8] that include the synthesis and recycling of γ -glutamyl-cysteinyl-glycine (Glutathion GSH) and enzymes, such as superoxide dismutase (SOD), GSH peroxidase and catalase^[9]. However, changes in diet, lifestyle, and aging could result in imbalance between the generation and clearance of ROS. Such excess formation and insufficient removal of the mitochondrial ROS expose the intracellular environment to subsequent oxidative stress challenge.

One of the intracellular mechanisms in response to oxidative stress is the activation of the transcriptional factors, such as NF- κ B and activator protein 1, which contribute to changes in many gene responses^[10] and play very important roles in mediating immune and inflammatory responses and apoptosis^[11]. NF- κ B regulates the expression of large number of genes, including pro-inflammatory cytokines, vascular endothelial growth factor, and multiple serine kinase cascades, such as p38 MAPKs which play a significant role in diabetes progression and complications. For instance, insulin receptor (IR) and the IR substrate (IRS) family of proteins are potential targets for the elevated serine kinase. Their involvement was demonstrated in muscle cell model, where activation of p38 MAPK by oxidative stress was found to be linked to the ROS-mediated inhibition of insulin-stimulated glucose transport^[12]. In fact, inhibition of insulin signaling was reversed by a specific inhibitor of p38 MAPK.

Oxidative stress in diabetes mellitus causes several adverse effects on the cellular physiology where it is particularly relevant and critical for those tissues that have lower levels of intrinsic antioxidant defenses such as islets. Pertaining to blood glucose level signaling and insulin secretion mediations, β -cells are particularly susceptible to the damages inflicted by oxidative stress due to the fact that ROS cascade eventually will cause induced auto-immune attack, which further accelerate the dysfunction and destruction of β -cells^[13] that lead both insulin resistance and impaired insulin secretion^[14].

Diabetic peripheral neuropathy is the most common complication of long-standing diabetes mellitus. Neuropathy frequently results in clinically significant morbidities, such as pain, loss of sensation, foot ulcers, gangrene and amputations^[15]. It now seems that the pathogenesis of diabetic neuropathy is heterogeneous with causative factors, including microvascular insufficiency, oxidative stress, nitrosative stress, defective neurotrophism,

and autoimmune-mediated nerve destruction. As such, oxidative stress has been viewed as a core and fundamental causing factor in the pathogenesis of diabetic neuropathy. Studies have showed proteins that are damaged by oxidative stress have decreased biological activity leading to loss of energy metabolism, cell signaling, transport, and, ultimately, to cell death^[16]. Those oxidative stress induced damages have been demonstrated on cell based^[17], *in vivo* animals^[18], and human clinical studies^[19]. Under clinical observations, the impaired glucose tolerance^[20] and advanced glycation end products^[21] are positively associated with the development and progress of the oxidative stress and neuropathy. As a result, new therapies are aimed at the underlying pathogenesis as well as the symptom complex^[22]. For example, anti-oxidants, such as alpha-lipoic acid^[23,24], dietary glutathione^[25], and polyphenols from grape seeds^[26] have shown beneficial clinical effects in management of peripheral nerves function in diabetic rats and human subjects.

One of the common microvascular complications of diabetes, diabetes retinopathy is classified as proliferative and nonproliferative diabetic retinopathy, mainly characterized by retinal neovascularization leading to blindness. It has been estimated that, ones diagnosed, nearly all patients with type 1 diabetes and more than 60% of patients with type 2 diabetes are expected to experience some form of retinopathy by the their first decade^[27]. The pathophysiology of diabetic retinopathy has been thought to incorporate multiple intertwined biochemical pathways as key contributors in the development of the disease. Among these, an oxidative stress induced by hyperglycemia has been identified as one of the key players in both the development and progression of the disease^[28]. Research has shown that in diabetes patients, besides the increased generation of mitochondrial reactive species (oxygen and nitrogen), the level of antioxidant defence enzymes responsible for scavenging free radicals and maintaining redox homeostasis such as SOD, glutathione reductase, glutathione peroxidase, and catalase were found reduced in the retina^[29].

Recently Fiorentino *et al.*^[30] have summarized the association of diabetes induced ROS as a risk factors for the development of cardiovascular disease. In this review, hyperglycemia was identified as the core of the primary disease and its secondary complications. They propose multiple mechanisms *via* activation of protein kinase C, polyol and hexosamine pathways, and advanced glycation end products production. These pathways, together with hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, causes ROS buildup which, in turn, cause cellular damage and contribute to the diabetic complications development and progression^[30].

Currently, diabetic nephropathy is largely considered as the leading cause of end-stage renal disease in the western world. Hyperglycemia-mediated alterations of intracellular metabolism, including oxidative stress

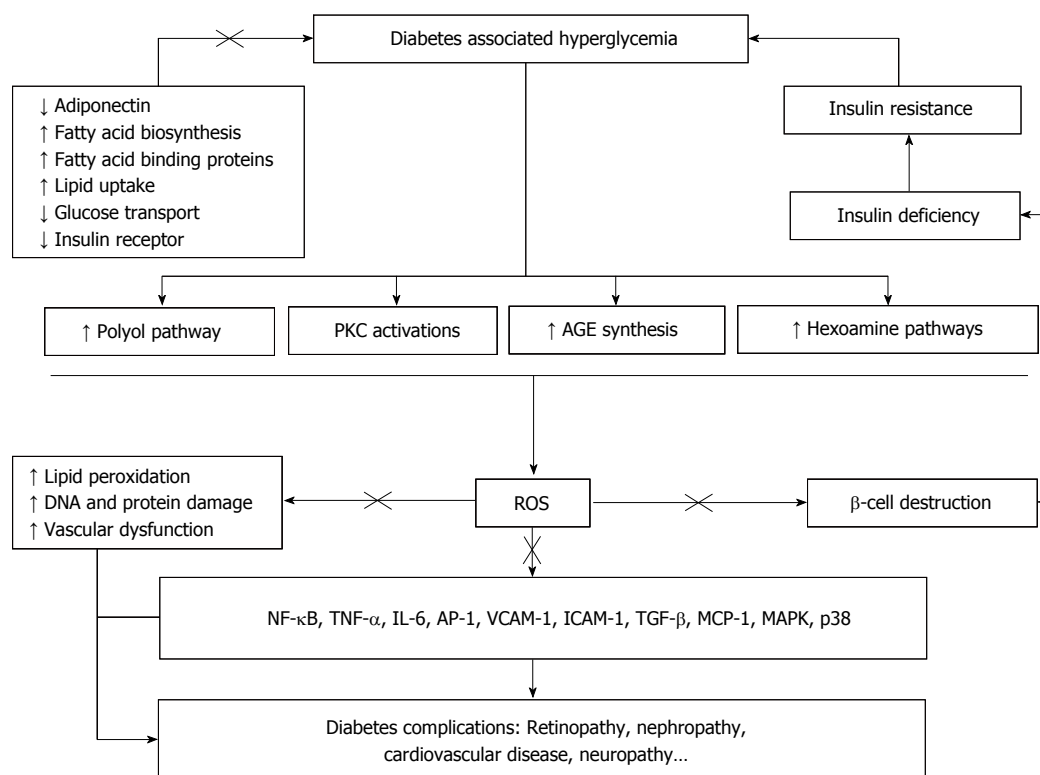


Figure 1 Oxidative stress and its possible pathways leading to diabetes complications. “X” potential sites where aloesin may likely interfere. PKC: Protein kinase C; AGE: Advanced glycation end-products; ROS: Reactive oxygen species; NF-κB: Nuclear factor-kappaB; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin 6; AP-1: Activating protein-1; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular adhesion molecule-1; TGF-β: Transforming growth factor beta; MCP-1: Monocyte chemoattractant protein-1; MAPK, p38: Mitogen-activated protein kinases, p38.

are major contributing factors to the pathogenesis of diabetic nephropathy. Despite the fact that interventions such as intensive lifestyle modification coupled with aggressive therapeutic management of glycemic control, blood pressure control, and inhibition of the renin-angiotensin-aldosterone system have shown promise to slow down progression of the disease, the number of patients with diabetes that ultimately develop end-stage renal disease have become significantly high. These highly predictive consequences suggest that there still is an urgent need to further understand the pathogenesis of the disease in order to establish new therapeutic strategies and promote enhanced clinical management for a better prognosis. In this respect, in the past few years, significant evidences from pre-clinical and clinical studies have been documented to link impaired autophagic activity in the pathogenesis of diabetic renal disease^[31]. Autophagy is a fundamental homeostatic cellular process that plays a critical role in maintaining functional integrity during normal or diseased state^[32]. It is believed that increase in ROS can induce autophagy, presumably as an adaptive response to cellular stress, and in turn autophagy could lead to reduction of ROS to protect the kidney under diabetic conditions. In fact a recent study has shown this association in a way that exposure of podocytes to a high glucose challenge resulted in an increase in ROS generation and hence autophagy inductions within 24 h. Interestingly, treat-

ment with antioxidant acetylcysteine inhibited the high glucose-induced autophagy^[33].

Overall, it has been considered that oxidative stress as a “unifying mechanism” which connects almost all of the complicated destructive biochemical pathways induced by hyperglycemia in diabetic patients^[34]. The hypothesis details that besides inducing NF-κB dependent pro-inflammatory and pro-coagulant pathways, mitochondrial-derived ROS to cause breaks in DNA strand which in turn activates poly-(ADP-ribose)-polymerase (PARP). The activation of PARP inhibits glyceraldehyde phosphate dehydrogenase activity which causes the accumulation of glycolytic intermediates. The intermediates then flux into the advanced glycation endproducts, protein kinase C, polyol, and hexosamine pathways, in part, are the major biochemical pathways of diabetes complications development and progression. The possible pathways have been summarized in Figure 1.

With the strong scientific and clinical evidence to link the impaired insulin sensitivity, beta-cell dysfunction, and diabetes complications directly with oxidative stress, new therapeutic approaches by administration of antioxidants^[35] or modulation of the oxidative-inflammatory cascade^[36] have been proposed. It is likely a promising approach to incorporate systemic oxidative stress management into clinical practice in order to control the contributing factor of diabetes and its complications^[37].

DISTINCTIVE NUTRITIONAL REQUIREMENTS TO MANAGE THE OXIDATIVE STRESS

Anti-oxidant defense systems are species specific and are prone to changes in nutrition; for example ascorbic acid and α -tocopherol cannot be synthesized by humans and therefore, needs to be acquired from consumed diet^[38]. Vitamins, minerals, amino acids, phenolic acids, flavanoids, anthrocynadines, pycnogenol, coumarine derivatives, polyphenols and many different types of herbal extracts^[39] have been promoted as types of antioxidant products. In functional specificity: (1) Dietary antioxidants: The beneficial effects of dietary antioxidants, such as resveratrols^[40] and alpha-lipoic acid^[41] in reducing the incidence of coronary heart diseases; butylated hydroxytoluene and β -carotene in photocarcinogenesis^[42] have been documented. Nevertheless, while antioxidants may reduce free radicals generated by radiotherapy and chemotherapy, clinical evidences are limited to show their significant applications in reducing systemic oxidative stress, even at higher dosages^[43,44]; (2) Vitamins and Minerals: Common antioxidants, such as vitamins A, C, E, mixed carotenoids, Co-Q10, α -lipoic acid, bio-flavonoids, antioxidant minerals (copper, zinc, manganese and selenium) and other cofactors (folic acid, vitamins B1, B2, B6, and B12) have been evaluated in streptozotocin and alloxan induced diabetes models^[45]. Increased glutathione, catalase and SOD activities, reduced lipid peroxidation, and reduced oxidative stress markers functions on experimentally induced diabetic animal models have been reported^[46]. Despite the significant findings from animal diabetes models, clinical trials conducted to date failed to provide adequate support for the use of antioxidants such as vitamin E, vitamin C, beta-carotene, selenium in a period of 7.5-12.5 years to reduce the risks of diabetes and to prevent its complications in randomized placebo-controlled clinical trials^[47]. The failure to deliver the perceived reduction of systemic oxidative stress from supplement of simple anti-oxidant vitamins may be due to the sub-optimum dosages, poor bioavailability, and lacks of organ/tissue specificity from the antioxidants. Another factor that has to be taken into consideration is how to better control the macronutrients that induce oxidative stress^[48]. In fact, a study conducted using foods selected based on total antioxidant capacity without standardization was failed to achieve the reductions of oxidative stress markers in a crossover two weeks intervention study^[49]; and (3) Polyphenols: Polyphenols are classes of natural anti-oxidants that exist in fruits, vegetables, nuts, different plant part as free radical scavengers, that prevent free radical chain reactions by counteracting existing free radicals and/or upholding a reducing environment around the cells^[50]. To deliver natural polyphenols in medical foods and in order to meet distinctive nutritional requirements, managing the oxidative stress has unique advantage than administration of classical anti-oxidation

vitamins. Natural polyphenols have a great structural diversity with anti-oxidation capacities higher than vitamin C and E^[51]. The food sources, daily intakes and related bioavailability of polyphenols have been very well documented^[52]. The polyphenols in foods can be quantitatively analyzed using modern analytical tools with the complement test of free radical scavenging activity using diphenylpicrylhydrazyl (DPPH) assay^[53]. However, both the complicated polyphenol compositions in food matrix^[54] and changes of the chemically active polyphenols into polymerized or decomposed compounds in food processing and storing make the delivery of standardized polyphenols with consistency a very challenging task^[55]. Those challenges may give explanations for the observations in two prospective human clinical studies that showed daily intake from 8.85 to 47.2 mg total flavonoids from flavonoid-rich foods, such as apple, tea, berries, citrus, broccoli, red wines, were not associated with the risk of type 2 diabetes^[56]. Quercetin, a high polarity but low bio-available flavonoid glycoside, was the major contributor to the total flavonoids (72%) in the foods.

On the other hand, in another clinical trial, 30% lower risk of developing type-2 diabetes from women who ate more than 1 apple per day or had more than 4 cups of tea than those who consumed no apple or tea were observed which shined a promising light^[57] for the potential use of antioxidants in diabetes prevention. This leads the possibility of selecting specific types of polyphenols with an improved bioavailability, potent anti-oxidation properties and standardized dosage level to deliver the perceived health benefits to diabetic patients by managing systemic oxidative stress.

SCIENTIFIC EVIDENCE TO SUPPORT THE POTENTIAL USAGE OF ALOESIN AND ALOE POLYSACCHARIDES AS A MEDICAL FOOD INGREDIENTS TO MEET THE DISTINCTIVE NUTRITIONAL REQUIREMENTS OF DIABETES

Aloe plants and extracts have been utilized for diabetes
Aloe vera (*Aloe barbadensis* Miller) is a perennial cactus like succulent plant belonging to the Xanthorrhoeaceae family. It is a biochemically complex plant that includes more than 300 species comprising many biologically active substances with diverse applications^[58]. The major components of *Aloe vera* such as chromones, anthraquinones, polysaccharides, vitamins, enzymes, and low molecular weight substances, such as organic acids and minerals, collectively, have been reported to possess immunomodulatory, anti-inflammatory, ultraviolet radiation protective, antiprotozoal, and wound/burn-healing promoting properties^[59]. While polysaccharides, in specific, have been described to show anti-inflammation, anticancer, and immunomodulation

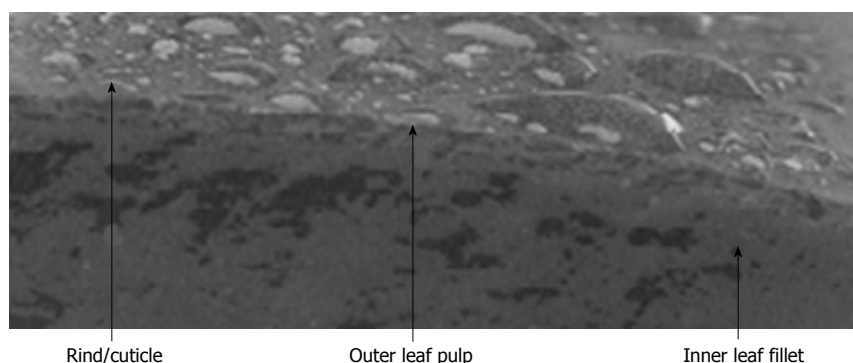


Figure 2 Cross-section of Aloe.

activities, biological activities such as cell growth stimulation, melanin synthesis inhibitions and antioxidant functions were documented for aloesin^[60]. Structurally, the aloe whole leaf encompasses three main distinctive sections each with specific function. These parts are categorized as the green rind or cuticle, the outer leaf pulp and the gel fillet (Figure 2). Polysaccharides are mainly located within the mucilaginous gel from the parenchymatous tissue whereas aloesin is housed inside the exudate of the leaf pulp.

Significant animal studies have reported beneficial effects of *Aloe vera* including reduced fasting blood glucose levels in alloxan-induced diabetic mice^[61], improved glucose tolerance in glucose-loaded rats^[62], decreased glucose levels^[63] and, enhanced liver gluconeogenesis in streptozotocin-induced diabetic rats^[64], decreased oxidative damage in the brains of streptozotocin-induced diabetic mice^[65]; decreased lipid peroxidation in diabetic rat kidney^[66] and liver^[67]; and, in streptozotocin-induced diabetic rats, decreased fasting glucose, normalization of lipids and liver and kidney fatty acid composition with reduced liver transaminases, and improved plasma insulin levels^[63]. Articles on systematic review of herbs and dietary supplements for glycemic control in diabetes and a systematic review of aloe's clinical effectiveness give substantial information regarding use of aloe in diabetes^[68,69].

In contrast to animal studies, until recently few human clinical trials were found in the literature. The two studies most frequently cited to support the use of *Aloe* for human diabetes^[70,71] contain methodological flaws, which unfortunately bring the significance of the results into question. A third study, evaluating the effects of bread prepared with *Aloe* gel consumed twice daily for 3 mo, reported an incidental finding of decreased fasting and post-prandial blood glucose levels in the subjects diagnosed with diabetes^[72]. Recently, Huseini *et al.*^[73], reported a study that evaluates the effects of *Aloe vera* gel in hyperlipidemic type 2 diabetes subjects and documented that Aloe gel significantly lowered fasting blood glucose, HbA1c, total and low-density lipoprotein cholesterol levels with no other side effects when administered twice a day at a dose of 300 mg for

8 wk^[73].

Aloe chromone, a special type of polyphenol isolated from aloe leaves, and Aloe polysaccharides have well documented biological and anti-oxidation functions

Chromones isolated from various Aloe species have been reported to have diverse biological activity. A c-glycosyl chromone isolated from *Aloe barbadensis* demonstrates anti-inflammatory activity^[74] and antioxidant activity similar to that of alpha-tocopherol based on a rat brain homogenates model^[60]. The chemical components of *Aloe ferox* leaf gel were thoroughly analyzed with potent anti-oxidation properties reported and potential usages in alleviating symptoms and/or preventing diabetes were speculated^[75]. Aloesin is a C-glucosylated 5-methylchromone with a potent anti-oxidation activity^[76,77]. *In vitro*, aloesin is a strong inhibitor of tyrosinase activity^[78] and up-regulates cyclin E-dependent kinase activity^[79].

In a recent study where the phytochemical profile of *Aloe barbadensis* was investigated using colorimetric assays, triple quadrupole and time-of-flight mass spectrometry, focusing on phenolic secondary metabolites in the different leaf portions, the outer green rind that contains aloesin was identified as the most active in radical scavenging activity, than the inner parenchyma in stable radical DPPH test and oxygen radical absorption capacity (ORAC) assay. Further tests using isolated pure secondary metabolites confirmed as the 5-methylchromones aloesin were among the most active chromones^[80].

Specifically, Aloesin was tested for ORAC relative to green tea extract and grape seed extract using the experimental procedures described in two publications^[81,82]. It was found that Aloesin has an ORAC value (5331, 419 and 3221 for whole, 95% and 50% ORAC, respectively) that is much higher than the high purity polyphenols in green tea (2945, 481 and 1838 for whole, 95% and 50% ORAC, respectively) and grape seed extracts (3213, 312, 411 for whole, 95% and 50% ORAC, respectively). For comparison, the well-known antioxidants pure vitamin C and vitamin E have reported ORAC values of 2000 and 1162 $\mu\text{mol TE/g}$ ^[83],

respectively.

Moreover, polysaccharides, the major constituents of *Aloe vera* gel, have been utilized for varieties of human disease and suggested for diabetes management, in part, because of their antioxidant activities. For instance, strong antioxidant activities have been reported for purified polysaccharides from *Aloe barbadensis* gel when tested in DPPH, hydroxyl and alkyl radical scavenging assays^[84]. Similarly, in *Aloe* plant age and function related study, polysaccharides from three-years-old *aloe* extract were found showing the strongest radical scavenging activity (72.19%) which was significantly higher than that of synthetic antioxidants butylated hydroxytoluene (70.52%) and α -tocopherol (65.20%) at the same concentrations of 100 mg/L *via* DPPH assay^[85]. Polysaccharides isolated from *Aloe vera* have also been found to possess high antioxidant efficiency as demonstrated with a decrease in the oxidative stress marker malondialdehyde and an increase in the hepatic non-enzymatic antioxidant GSH and enzymatic antioxidant SOD *in vivo* in chronic alcohol-induced hepatotoxicity in mice^[86].

Therefore, these strong antioxidant activities of both Aloesin and *aloe* polysaccharides suggest their potential indications in diabetes to curve its devastating complications.

Aloesin can increase adiponectin production from adipocyte

Adiponectin - an adipocyte-derived plasma protein is exclusively produced by fat cells and its blood levels inversely correlates with insulin sensitivity and are thought to be predictive of susceptibility to type 2 diabetes^[87]. It is believed that the key adipokine marker protein - adiponectin can modulate other glucose and fatty acid key metabolic pathways, improve directly and indirectly insulin resistance and glucose intolerance. The anti-atherosclerotic and anti-obesity effects of adiponectin have been well established. Recently Adiponectin has been discovered with suppression of high-glucose-induced ROS based on an *in vitro* model^[88]. Therefore, finding a compound that can up regulate the production of adiponectin from adipocytes is a potential approach to managing the causal factor of diabetes and its complications.

Previously, we carried out a random screening of 2059 botanical extracts to identify natural substances that increase adiponectin production by adipocytes, *i.e.*, fat cells^[89]. The initial screening yielded 139 positive hits. As a result of the subsequent verification assays and secondary screening, one active extract from leave exudates of *Aloe ferox*, designated as P0017, showed a consistent up modulating adiponectin level in the media. That led to the isolation and identification of Aloesin as the active component in the *Aloe ferox* extract. Aloesin tripled the adiponectin concentration in the culture media that was determined with an ELISA kit. In comparison, indomethacin at 10 μ mol/L increased adiponectin production by 7-folds.

Gene expression study showed that a standardized composition containing Aloesin formulated with Aloe polysaccharides can down regulate fatty acid biosynthesis, and up regulated multiple key genes in the IR signaling cascade

It has also been shown that microarray analysis of gene expression modifications in white adipose tissue (WAT) and liver isolated from high fat diet induced pre-diabetes mice that were administered orally with Aloesin in *Aloe vera* gel powder (also known as Loesyn or UP780) to regulate fatty acid biosynthesis and up regulated multiple key genes in the IR signaling cascade. Specifically in liver, microarray analysis suggested that Loesyn modified multiple metabolic pathways for lipid metabolism such as decreased fatty acid biosynthesis, increased fatty acid binding proteins, decreased lipid uptake, and increased bile biosynthesis. These findings were also corroborated by quantitative polymerase chain reaction that showed Loesyn to cause coordinated increases in gene expression for multiple key genes in the IR signaling cascade such as up-regulation of IR (INSR), IRS1, and glucose transporter 4. The combined modifications to lipid metabolism in liver and insulin response in WAT suggested Aloesin delivered in *Aloe vera* gel powder can reduce the systemic oxidative stress by improving the glucose transportation and usage with enhanced insulin sensitivity and by reducing fatty acid synthesis^[90].

Aloesin delivered as a pure compound or formulated within Aloe gel powders reduced fasting glucose, improved glucose tolerance and insulin sensitivity of diabetic animals

Impaired insulin sensitivity, glucose tolerance and metabolic disorders were induced in C57BL/6J mice by feeding the animals a high fat diet for 8 wk. The mice were then treated intraperitoneal with Aloesin at a dose of 100 mg/kg and a reference compound GW1929 at a dose of 5 mg/kg for 4 wk. Glucose and insulin tolerance tests were carried out on day 18 and day 24, respectively. Animals treated with Aloesin showed a significant improvement of glucose clearance and/or utilization in both tests compared to the vehicle treated animals. The insulin sensitizing activity of Aloesin was also further demonstrated by the ability of the compound in lowering the plasma insulin levels in the treated animals. The reference compound, GW1929 [the Active Pharmaceutical Ingredient for the AvandiaTM (GSK) insulin sensitizer drug]^[91] induced a 50.2% reduction in plasma insulin compared to vehicle, as expected. Similarly, Aloesin showed 37.9% decreased in plasma insulin levels compared to that of the vehicle treated mice. In a subsequent study using high-fat diet induced diabetes mice, administered orally with chromone enriched *aloe* composition (UP780) at a dose of 200 mg/kg for 10 wk, showed a 30.3% decrease in fasting blood glucose levels and 32.2% reductions in plasma insulin with significant improvement in blood glucose clearance. Additionally, in *db/db* mice, the

composition also showed a 33.7% and 46.0% decrease in fasting triglyceride and plasma glucose levels after 10-wk oral treatment, respectively, when compared to vehicle.

Substantiating the above findings, administered orally at a dose of 2 g/kg, the composition UP780 has also showed reduced blood glucose and triglyceride, improved blood glucose clearance and plasma insulin level in alloxan induced insulin dependent mouse diabetes model^[92].

In a double-blind, placebo controlled human clinical trial, Aloesin delivered within Aloe vera gel powder (referred as Loesyn) improved commonly monitored diabetic associated markers

Human clinical trial was carried out for Loesyn against placebo control by a third party University hospital for 8 wk following institutional review board approved protocol^[93]. Subjects were given Loesyn at a dose of 500 mg capsules BID (*bis in die*) orally for a total daily dose of 1 g/d and equally matched in appearance placebo capsules for the duration of the study.

Inclusion criteria for pre-diabetes subjects were: fasting plasma glucose 100-125 mg/dL (5.55-6.94 mmol/L), waist circumference > 35 in (88.9 cm) females, > 40 in (101.6 cm) males, 2 h oral glucose tolerance test 149-199 mg/dL (8.27-11.05 mmol/L), HbA1c 5.0%-7.0%, Age > 25 years, No history of diabetes, or insulin or other diabetes medications, no cholesterol lowering or high dose antioxidants/anti-inflammatory medication or other concurrent dietary supplements, diet aids, weight loss programs, no other chronic conditions (heart disease, renal failure, or abnormal CBC).

A total of 30 subjects with impaired fasting glucose or impaired glucose tolerance were randomized to either placebo or Loesyn 500 mg BID for a period of 8 wk. After 8 wk of oral treatment, there were no significant changes in the placebo group on any of the parameters. On the other hand, indicators of improved glycemic control such as significant reductions in HbA1C as well as fasting glucose and fructosamine levels, were observed in the Loesyn treated subjects. The fasting glucose, HbA1C and fructosamine decreases were statistically significant ($P < 0.05$) for this group in comparison with placebo. Moreover, significant reduction in oxidative stress marker - urinary f2-isoprostanes was noted for subjects treated with Loesyn when compared to baseline.

There was no reduction of total cholesterol and triglycerides levels for subjects received either the composition or the placebo group. No side effects were reported or observed and there were no significant baseline differences between the composition and placebo groups. Similarly, no changes were observed on the safety evaluation parameters, cardiovascular variables (systolic and diastolic blood pressure), Complete Blood Count, chemistry profile, and liver function tests.

In a similar double-blind randomized controlled trial, a total of 136 subjects were recruited based on inclusion

criteria such obesity (body mass index ≥ 25 kg/m²) or abdominal obesity (waist circumference ≥ 90 cm for men or ≥ 85 cm for women), impaired fasting blood glucose FBG (≥ 100 mg/dL) or impaired glucose tolerance (2-h oral glucose tolerance test ≥ 140 mg/dL), and subjects that would more likely to ensure a lifestyle modification to control blood sugar levels (FBG < 180 mg/dL and HbA1c < 8.0%). Such equally divided subjects received aloe vera gel complex containing Aloesin or Placebo at a dose of 700 mg/kg twice a day for 8 wk. Parameters were evaluated at baseline, week 4 and week 8. After 8 wk of repeated daily oral treatment, statistically significant reduction in body weight, body fat mass, and fasting blood glucose were observed for subjects with intervention. Homeostasis model of assessment - insulin resistance and serum insulin level were also found statistically significant at week 4 in these subjects compared to baseline^[94].

CONCLUSION

Collectively, hyperglycemia in diabetes can induce imbalance of ROS by multiple metabolic pathways through increased flux of glucose *via* the polyol pathway, increased formation of AGEs and activation of their receptors, activation of PKC isoforms, over activity of hexosamine pathway, and decrease of antioxidant defenses. While hyperglycemia is a platform, ROS is the pivotal axis for diabetes and its complication development and progression. Appropriate glycemic management in associate with ROS balance control through antioxidants may counteract the complications of diabetes mellitus. Aloesin is a natural polyphenol originated from Aloe plants. Aloesin and/or aloe polysaccharides can reduce systemic oxidative stress by acting directly as a potent anti-oxidant and also indirectly by regulating the productions of adiponectin and gene expressions pathways related to insulin sensitivity, glucose transportation and fatty acid biosynthesis. The health benefits of supplementing a standardized composition containing Aloesin formulated with Aloe polysaccharides were demonstrated by animal studies on high fat diet and alloxan-induced as well as db/db diabetic mice models. Besides reducing fasting glucose, improving glucose tolerance, and enhancing insulin sensitivity, in a human clinical trial, the composition Loesyn also reduced oxidative stress marker in urine after 8 wk of oral supplements. Therefore, Loesyn formulated in foods could potentially be used either as over the counter or under the supervision of a physician for managing systemic oxidative stress of diabetes^[95] and/or lowering blood glucose. This approach could likely make this inexpensive, safe and efficacious medical food product available quickly to the growing pre-diabetic and diabetic population worldwide.

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Limited joint mobility syndrome in diabetes mellitus: A minireview

Esther G Gerrits, Gijs W Landman, Leonie Nijenhuis-Rosien, Henk J Bilo

Esther G Gerrits, Department of Internal Medicine, Maastricht University Medical Center, 6229 HX Maastricht, The Netherlands

Gijs W Landman, Leonie Nijenhuis-Rosien, Henk J Bilo, Diabetes Centre, Isala, 8025 AB Zwolle, The Netherlands

Gijs W Landman, Department of Internal Medicine, Gelre Hospital, 7334 DZ Apeldoorn, The Netherlands

Leonie Nijenhuis-Rosien, InnoFeet Voetencentrum Nijenhuis, 8013 NA Zwolle, The Netherlands

Henk J Bilo, Department of Internal Medicine, Isala, 8025 AB Zwolle, The Netherlands

Author contributions: Gerrits EG designed and wrote the manuscript; Landman GW, Nijenhuis-Rosien L and Bilo HJ contributed equally to the writing of the manuscript.

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Correspondence to: Esther G Gerrits, MD, PhD, Department of Internal Medicine, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands. esther.gerrits@mumc.nl
Telephone: +31-43-3877005
Fax: +31-43-3875006

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Abstract

Limited joint mobility syndrome (LJMS) or diabetic cheiroarthropathy is a long term complication of diabetes mellitus. The diagnosis of LJMS is based on clinical features: progression of painless stiffness of hands and fingers, fixed flexion contractures of the small hand and foot joints, impairment of fine motion and impaired grip strength in the hands. As the syndrome progresses, it can also affect other joints. It is important to properly diagnose such a complication as LJMS. Moreover, it is important to diagnose LJMS because it is known that the presence of LJMS is associated with micro- and macrovascular complications of diabetes. Due to the lack of curative treatment options, the suggested method to prevent or decelerate the development of LJMS is improving or maintaining good glycemic control. Daily stretching exercises of joints aim to prevent or delay progression of joint stiffness, may reduce the risk of inadvertent falls and will add to maintain quality of life.

Key words: Diabetic cheiroarthropathy; Limited joint mobility; Diabetes mellitus; Joint stiffness; Advanced glycation endproducts

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Core tip: "Limited joint mobility syndrome in diabetes mellitus: A minireview" is an article about limited joint mobility syndrome in diabetes mellitus that is an underreported complication, associated with micro and macrovascular complications. From a clinical perspective, a good glycemic control and daily exercising are the main and the base of prevention. Treatment options include symptomatic therapies and surgical correction. Medical treatment targeting the

formation of glycosylated end products accumulating on collagen and other connective tissues are unsuccessful for this complication. This mini-review analyzes all the aspects of a forgotten complication of diabetes mellitus.

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INTRODUCTION

Musculoskeletal disorders such as Achilles tendon pathology, trigger finger, Dupuytren, limited joint mobility syndrome (LJMS), carpal tunnel syndrome, frozen shoulder and plantar fasciitis have been found to occur more often in subjects with diabetes compared to those without diabetes^[1-5]. With the increasing number of patients known with diabetes and - consequently - an increase in incidence and prevalence of diabetes related complications along with increasing age of these patient group, it is important to pay attention to the topic of musculoskeletal disorders in order to recognize and diagnose these disorders in clinical practice as early as possible. LJMS is one of the musculoskeletal disorders and is rather underexposed and underdiagnosed compared to the well-known micro- and macrovascular complications of diabetes. Due to their relative relationship to mortality, more attention is paid towards the complications of diabetes such as nephropathy, neuropathy and cardiovascular disease. Less attention is paid to LJMS, although it is associated with neuropathy and other microvascular complications and it can influence patients' health-related quality of life quite dramatically^[1,4-9]. In this mini-review, we will exclusively focus on LJMS as a musculoskeletal complication of diabetes. It provides an overview of the pathophysiology, the importance of diagnosing LJMS, the practical implications of the diagnosis and future expectations on this topic.

LJMS

Epidemiology

Stiff hands in long-term diabetes has been described for the first time by Lundbaek^[10] in 1957. Less reports have been published about LJMS until 1974, when Rosenbloom *et al.*^[11] exhibited renewed interest in this syndrome. Joint stiffness and contractures were described as a common feature in children with type 1 diabetes mellitus^[11-13]. Currently, we define LJMS as a long term complication of diabetes mellitus, but it can also develop in patients without diabetes. The reported prevalence in diabetes mellitus apparently varies between 8%-58%, depending on the different

diabetes patients cohorts and the applied definitions of LJMS^[5-9,12,14-16]. The prevalence of LJMS in subjects without DM is difficult to estimate and may vary between 4%-26%^[2,4,5,9]. Generally, no clear gender or racial preferences have been found in the development of LJMS in diabetes.

Symptomatology and diagnosis

LJMS of the hands and fingers, also called cheiroarthropathy, is characterized by several clinical features which enhance painless stiffness of hands and fingers, fixed flexion contractures of the small hand joints, impairment of fine motion and impaired grip strength. Ultimately, these features will result in the impairment of joint mobility, especially of the small joints of the hands and may become painful. The "prayer sign" and the "tabletop sign" are clinical tests strongly supporting the diagnosis, which can only be used in the absence of previous hand injury or hand surgery^[17]. Under normal conditions, both hands will have contact for the total opposing hand surface parts, when the hands are pressed flat to each other, as making a "prayer sign". If this proves to be impossible, it means there are flexion contractures of the fingers and the sign is considered positive. With the "tabletop sign" one has to put the hands flat on the table with the fore arm in a 90 degree angle. If one hand doesn't make contact with the table at one spot, it means that there are contractures of the small hand joints suggesting the test positive.

Natural course: Besides joints involvement of hands, LJMS can also occur in the small joints of the feet and in the long term progression of disease can also result in impairment of other joints such as the shoulder, hip, ankle, spine and all other joints. Consequently, on the long term, LJMS might increase the risk of falling^[18]. A limb threatening situation might occur when the impairment of mobility of toes and feet joints is seen in combination with the presence of neuropathy. The combination can lead to serious plantary pressure points, which translates into a great risk for diabetic foot ulcer^[19-21]. When peripheral arterial disease is present, this might even result into an enhanced amputation risk. Conceivably, all these features and complications of LJMS can be accompanied by a significant reduced quality of life.

Differential diagnosis

Sometimes, LJMS is difficult to distinguish from other joint complaints in diabetes patients. Certain musculoskeletal conditions occur more frequently in diabetes patients compared to the general population which include Dupuytren, tenosynovitis and palmar/plantar fasciitis. Complex regional pain syndrome and scleroderma are also part of the differential diagnosis of LJM. The specific clinical features of each different disorder with or without supplementary laboratory and radio- or ultrasonographic evaluation confirm the diagnosis^[22-24].

One should keep in mind that any supplementary diagnostic evaluation is quite unspecific, so the diagnosis of limitation of joint mobility mainly relies on the clinical features.

Considering a prevalence of up to 50% and the LJMS accompanied microvascular and limb threatening complications, screening for LJMS in diabetes patients is important, and has to be part of the annual check up or more often when indicated.

PATHOGENESIS

The apparently higher prevalence of LJMS in subjects with diabetes compared to nondiabetic subjects is assuming that there is a correlation between diabetes mellitus and LJMS, but good literature to support this correlation is lacking. As the presence of LJMS is associated with nephropathy, retinopathy and neuropathy, it is not only important to diagnose LJMS *per se*, but also because it can be an early warning signal of the possible presence of one or more of the microvascular complications^[1,4-9]. In some cases it might be the first feature of tissue damage in diabetes which should alert physicians to actively screen or search for the presence of microvascular complications as well.

In general, the chances to develop LJMS are associated with age, diabetes duration and degree of glycemic control^[1,4-6,9,14]. Theoretically, good glycemic control should diminish the risk of LJMS in an identical fashion as the development of other diabetic complications. Eventually, a combination of factors will contribute to the development and progression of diabetic complications including LJMS.

Besides a variable genetic susceptibility, high oxidative stress levels seem to be one of the factors involved. Intracellular hyperglycemia will cause high levels of oxidative stress and the formation of advanced glycation endproducts (AGEs). These AGEs are damaging glycosylation products, nonezymatically formed under circumstances of hyperglycemic and oxidative stress. In such an unfavourable environment, increased production of reactive oxygen species will be induced that can initiate the inflammatory cascade leading to the production of several cytokines and growth factors causing the hyperglycemia-induced cellular damage^[25,26].

Furthermore, besides their damaging effects on the vascular endothelium, these accelerated formed AGEs also form cross-links with long-lived proteins such as skin collagen, tendons and ligaments altering their biological structure and function^[27-29]. Collagen has a long half life, which means that collagen degradation will take a long time: for more than ten years. Therefore, the AGE-cross-links to collagen will extensively accumulate in the skin, tendons and ligaments and are considered to play an important role in the development of LJMS.

Genetic susceptibility in combination with other factors such as a hyperglycemic and highly oxidative

stress environment will add to the development of LJMS.

THERAPEUTIC OPTIONS

LJMS seems to be an irreversible disorder with no specific curative treatment options. There are no drugs available which directly target LJMS. Only symptomatic therapy, such as analgesics, non-steroidal anti-inflammatory drugs or local corticosteroid injections can be given as a relief and in case of tendinitis or flexor tendon contractures. Surgery is indicated in case of severe contractures. Exercising, which include daily stretching exercises of the palm of the hand and sole of the foot, will also help to prevent or further delay the development of progressive joint stiffness in case of limited joint mobility^[30]. In case of limited lower limb joint mobility with or without the presence of neuropathy, professional foot care and rocker bottom shoes are indispensable in order to prevent the development of diabetic foot ulcers^[31].

In general, as LJMS is associated with glycemic control and diabetes duration, just like all other diabetic complications, the best way to prevent LJMS is to strive for good glycemic control from the onset of diabetes diagnosis.

NOVEL STRATEGIES

During the past 20 years, research has been performed to find efficient agents with AGE inhibitory properties without toxicity, meant for safe application in humans. Targeting AGE cross-links with alagebrium (ALT-711) in experimental settings have clearly shown beneficial effects, but in human trials there seems to be a safety concern and alagebrium still has to be proven to be beneficial^[32-34]. Aminoguanidine with a preventive effect on the formation and accumulation of AGEs in experimental studies, but not recommended for daily clinical use because of safety concerns and lack of evidence in human^[34,35]. Anti-oxidant agents with specific AGE-inhibiting effects (*e.g.*, pyridoxamine, benfotiamine) have shown beneficial effects in animal models, but still have to be proven as an effective therapy in human^[34].

With all these unsuccessful strategies, newly developed targeted drugs are needed in order to prevent or delay the onset of LJMS.

CONCLUSION

LJMS is an underreported complication of diabetes, along and associated with micro- and macrovascular complications, which should be assessed during the annual check up of diabetes care. From a practical perspective, both a good glycemic control and daily exercising are the main and actually only pillars of prevention. Treatment options include symptomatic therapies and surgical correction. Medical treatment targeting the

formation of glycosylated endproducts accumulating on collagen and other connective tissues that are said to be responsible for the development of LIMS, have so far proved to be unsuccessful. Newly developed targeted drugs are needed in order to prevent or delay the onset of LIMS, to reduce the risk of inadvertent falls and to maintain quality of life of subjects with diabetes.

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Prospective Study

Epigenetic profiles of pre-diabetes transitioning to type 2 diabetes and nephropathy

Thomas A VanderJagt, Monica H Neugebauer, Marilee Morgan, Donald W Bowden, Vallabh O Shah

Thomas A VanderJagt, Monica H Neugebauer, Vallabh O Shah, Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque, NM 87131, United States

Marilee Morgan, MIND Institute, Albuquerque, NM 87106, United States

Donald W Bowden, Center for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, NC 27157, United States

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Correspondence to: Vallabh O Shah, PhD, FASN, Professor and Sr Fellow New Mexico Center for the Advancement of Research, Engagement, and Science on Health Disparities (NM CARES HD), Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, 2211 Lomas Blvd, NE, Albuquerque, NM 87131, United States. vshah@salud.unm.edu
Telephone: +1-505-2729615
Fax: +1-505-2722614

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Abstract

AIM: To examine DNA methylation profiles in a longitudinal comparison of pre-diabetes mellitus (Pre-DM) subjects who transitioned to type 2 diabetes mellitus (T2DM).

METHODS: We performed DNA methylation study in bisulphite converted DNA from Pre-DM ($n = 11$) at baseline and at their transition to T2DM using Illumina Infinium HumanMethylation27 BeadChip, that enables the query of 27578 individual cytosines at CpG loci throughout the genome, which are focused on the promoter regions of 14495 genes.

RESULTS: There were 694 CpG sites hypomethylated and 174 CpG sites hypermethylated in progression from

Pre-DM to T2DM, representing putative genes involved in glucose and fructose metabolism, inflammation, oxidative and mitochondrial stress, and fatty acid metabolism. These results suggest that this high throughput platform is able to identify hundreds of prospective CpG sites associated with diverse genes that may reflect differences in Pre-DM compared with T2DM. In addition, there were CpG hypomethylation changes associated with a number of genes that may be associated with development of complications of diabetes, such as nephropathy. These hypomethylation changes were observed in all of the subjects.

CONCLUSION: These data suggest that some epigenomic changes that may be involved in the progression of diabetes and/or the development of complications may be apparent at the Pre-DM state or during the transition to diabetes. Hypomethylation of a number of genes related to kidney function may be an early marker for developing diabetic nephropathy.

Key words: Epigenetic changes; Pre-diabetes; Diabetes; Nephropathy

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Core tip: Many independent predictors of diabetes including markers of metabolic dysfunction (high body mass index, hypertension, low HDL and smoking) were significantly increased early on in pre-diabetes mellitus (Pre-DM) and sustained in diabetes groups. The innovation in high-throughput epigenome of DNA methylation studies suggests that some epigenomic changes that may be involved in the progression of diabetes and/or the development of complications may be apparent at the Pre-DM state or during the transition to diabetes.

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INTRODUCTION

Pre-diabetes mellitus (Pre-DM) is a condition characterized by elevated blood glucose concentrations that denote the incipient development of type 2 diabetes mellitus (T2DM), along with its co-morbid conditions of cardiovascular disease and renal disease. The most common definitions of Pre-DM refer to impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). IGT and IFG are assumed to define categories of glycemia associated with an increased risk of developing diabetes^[1-3]. It is estimated that roughly 86 million individuals in the United States aged 21 years

and older have Pre-DM. People with Pre-DM are 5-15 times more likely to develop T2DM than are people with normal glucose values^[1,4]. The risk of people with IGT and/or IFG developing T2DM is not uniform. An analysis of several prospective studies showed that the incidence rates of developing T2DM in people with IGT ranged from 35.8 to 87.3 per 1000 person-years^[5]. Environmental exposures, sedentary lifestyle, and high calorie, high-fat diets correlate with the development of metabolic syndrome including obesity and insulin resistance. All of these factors influence the rate of progression of Pre-DM. Progression to T2DM among those with Pre-DM is not inevitable and also is variable in terms of development of complications related to T2DM. People with Pre-DM who lose weight and engage in moderate physical activity can prevent or delay T2DM and may even return their blood glucose levels to normal^[6]. Similar to the prevalence of Pre-DM and T2DM in the United States, estimates suggest that more than 31 million people in the United States are affected by kidney disease, with less than 500000 (< 0.2%) having kidney failure treated by dialysis or transplantation. T2DM is an important independent risk factor for kidney disease in the United States and almost half of all new cases of End Stage Renal Disease (ESRD) are due to diabetic nephropathy. A tremendous amount of work has been done to better understand diabetic nephropathy and the risk of progression to ESRD and much of the work in renoprotection has focused on this population.

The etiological origins of T2DM are complex. A data-mining approach, which analyzed over 12 million Medline records to identify factors associated with the pathology of T2DM, identified epigenetic changes as among the most important causal factors in the pathogenesis of T2DM^[7]. The epigenome is increasingly gaining acceptance as playing an important role in diabetes and obesity, and the role of both nutritional status and endocrine disruptors would appear to be major factors in these conditions^[8-10]. Initial observations indicating a role for environmental cues in establishing epigenetic patterns came from studies of the agouti mouse model with offspring suffering from obesity, hyperinsulinaemia and diabetes^[11]. In human studies it has been shown that trans-generational effects of nutrition may be passed on to future generations. In a study of historical records from Överkalix, Sweden, the grandsons of men who were well-nourished prior to puberty had an increased risk of developing T2DM^[12].

A general defect in DNA methylation in T2DM is suggested by the observation that S-adenosylmethionine (SAM), the main physiologic donor of methyl groups, is decreased in erythrocytes of diabetic patients. In addition, decreased erythrocyte concentrations of SAM and other alterations were found to be associated with disease progression^[13]. Methylation plays an important role in regulating gene expression, most likely including the expression of those genes essential for the strict maintenance of normal blood glucose levels. Expression

patterns that develop in response to changes in diet or in response to environmental factors are likely to become locked by DNA methylation early in development^[14]. Methylation of DNA on specific cytosine residues in CpG islands, especially in promoter regions, leads to DNA hypermethylation, which generally is associated with lowering gene expression, while removal of methyl groups, leading to DNA hypomethylation, is generally associated with increasing gene expression. Methylation patterns have been suggested to be involved in the propagation of insulin resistance in insulin target tissues and, being a reversible modification, might also confer the adaptability of metabolism to loss of body weight.

In this longitudinal study, we used archived biological samples to examine the methylation patterns in DNA obtained from subjects at the time they were classified as Pre-DM and were also later obtained from the same subjects after they had transitioned to T2DM. All subjects in this cohort eventually developed diabetic nephropathy. This allowed for a longitudinal comparison of changes associated with transitioning from Pre-DM to T2DM. Our aims were two-fold: first, to obtain a global comparison of hyper- and hypomethylation patterns between the Pre-DM and T2DM states and analysis of these differences in terms of altered metabolic pathways; and second, to examine for methylation changes at the T2DM stage that might suggest early markers to future development of diabetic complications, specifically diabetic nephropathy.

MATERIALS AND METHODS

The study protocol was approved by the Human Subject Research Review Committee of the University of New Mexico Health Sciences Center. Genome-wide screening for DNA methylation was carried out with the Infinium 27K methylation array (Illumina Infinium® HumanMethylation27 BeadChip, Illumina, San Diego, CA). Quantitative measurements of DNA methylation were determined for 27578 CpG dinucleotide spanning 14495 genes. This methodology combines bisulfite conversion of genomic DNA and whole-genome amplification with direct, array-based capture and enzymatic scoring of the CpG loci. Allele-specific single-base extension of the oligos on the BeadChip, using the captured DNA as a template, incorporates detectable labels on the BeadChip and determines the methylation profile for the sample. One microgram of DNA was treated with sodium bisulfite using the Zymo EZ DNA Methylation Kit to convert un-methylated cytosines to uracil, while methylated cytosines remain unchanged. The DNA was purified and quantified in preparation for whole genome amplification, followed by fragmentation and ethanol precipitation. The DNA was re-suspended in hybridization buffer and applied to the bead chip array for an overnight incubation. Following hybridization, the arrays were washed to eliminate un-hybridized and non-specifically hybridized DNA. The samples then underwent single base extension and staining followed by more

washing. The arrays were allowed to dry and then scanned using the Illumina iScan system. Analysis of the scanned results is achieved using Illumina's GenomeStudio software in conjunction with the GenomeStudio methylation module. GenomeStudio Software is a modular analysis tool for genotyping, gene expression, and methylation applications. The Methylation Module allows users to combine Infinium methylation assay data with mRNA data, enabling convergence of data across gene expression and epigenetic analyses.

To evaluate the genes identified by the Infinium methylation assay for groupings that may identify metabolic pathways, the data were analyzed with ingenuity pathway analysis (IPA) (Ingenuity Systems, Redwood City, CA). Differentially expressed transcripts satisfying the statistical conditions were exported to IPA. This software determines the top canonical pathways by using the ratio of the number of genes in a given pathway that meet cutoff criteria divided by the total number of genes that constitute that pathway. The significance of a pathway for the data set reflects the likelihood that the pathway is associated with the dataset by random chance. The methylation assay results were analyzed and scored for significance of hyper and hypomethylation compared to controls. Output (Beta) was used in computations creating a *P*-value from a Diff score; $\text{DiffScore} = 10 * \text{sgn}(\text{Beta}_{\text{Condition}} - \text{Beta}_{\text{Reference}}) * \log_{10} p$. Level of significance related to DiffScore are as follows; *P*-value of 0.05, DiffScore = ± 13 ; For a *P*-value of 0.01, DiffScore = ± 22 ; For a *P*-value of 0.001, DiffScore = ± 33 .

This procedure was carried out using DNA isolated from 11 Pre-DM non-Hispanic white male subjects when they were diagnosed with Pre-DM and repeated after transitioning to T2DM, and from two reference subjects used to establish a baseline. The Pre-DM samples and the T2DM samples were normalized to this baseline in order to determine expression changes that occur over the extended time period between Pre-DM and T2DM. The cohort was limited to 11 non-Hispanic white males to minimize confounding variables of ethnicity and gender. Blood leukocyte samples were taken at each stage and phenotype for clinical parameters and anthropomorphic measurements. Every patient's transition from Pre-DM to T2DM involved a higher body mass index (BMI), weight gain as well as higher levels of blood glucose and HbA1c. Clinical standards set by the American Diabetes Association were used to classify the subjects: fasting plasma glucose levels of 99 mg/dL or below are considered normal; plasma glucose levels between 100 to 125 mg/dL indicate Pre-DM; and plasma glucose levels of 126 mg/dL and higher indicate T2DM. Subjects with a normal plasma glucose but elevated HbA1c (5.7%-6.0%) were also classified as Pre-DM.

RESULTS

The mean age at Pre-DM diagnosis was 40.27 ± 5.46 ,

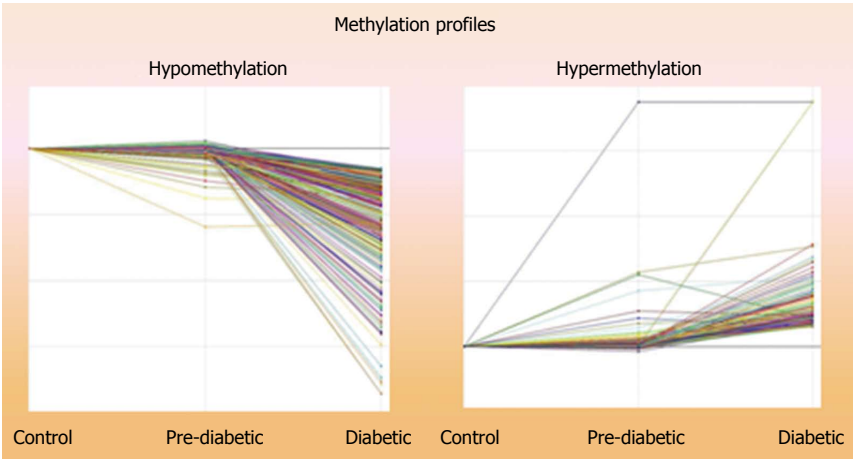


Figure 1 Global methylation profile depicting a total of 868 genes. Total of 694 were hypomethylated, 174 were hypermethylated.

Table 1 The top associated networks from ingenuity pathway analysis
Carbohydrate metabolism, small molecule biochemistry, cell signaling
Lipid metabolism, small molecule biochemistry, drug metabolism
Inflammatory response, cardiovascular system development and function, lymphoid tissue structure and development
Cellular function and maintenance, inflammatory response, cell-to-cell signaling and interaction
Cellular movement, hematological system development and function, immune cell trafficking

the mean transition time was 7.09 ± 1.97 years, and the mean age at T2DM diagnosis was 47.36 ± 5.97 years of age. In the Pre-DM state the BMI = 32.2 ± 6.9 , HbA1c = 5.5 ± 0.31 , glucose = 99.1 ± 15.9 (mg/dL), and weight = 226.6 ± 68.9 (lbs). In the diabetic state, the BMI = 37.2 ± 6.9 , HbA1c = 9.6 ± 2.15 , glucose = 225.9 ± 78.8 (mg/dL), and weight = 261.4 ± 65.1 (lbs).

Global changes in methylation patterns

Comparisons of the epigenetic profiles of methylated CpG loci in DNA from 11 non-Hispanic white male revealed that 694 CpG sites were consistently hypomethylated and 174 were hypermethylated in the DNA obtained at the time of transition to T2DM compared to the DNA obtained at Pre-DM (Figure 1). Analysis of the genes with IPA identified numerous putative genes associated with carbohydrate and lipid metabolism, inflammation, immune cell function and cell signaling, suggesting increased activities in these pathways at the T2DM state compared to the Pre-DM state. The five Top Associated Networks identified in the Ingenuity Pathway Analysis are summarized in Table 1.

The Top Biological Functions identified by IPA and the number of genes involved are summarized in Table 2. These include numerous genes that suggest changes that may be directly associated with or are early markers for diseases and disorders, molecular and cellular function and physiological system development and function, possibly related to the Pre-DM state

Table 2 The top biological functions identified by ingenuity pathway analysis and the number of genes involved	# of genes
Diseases and disorders	
Inflammatory disease	218
Inflammatory response	202
Immunological disease	195
Respiratory disease	126
Hematological disease	112
Molecular and cellular functions	
Cellular growth and proliferation	230
Cell death	224
Cell-to-cell signaling and interaction	188
Cellular development	152
Physiological system development and function	
Hematological system development/function	214
Immune cell trafficking	137
Hematopoiesis	126
Tissue morphology	105
Cell-mediated immune response	85

transitioning to the T2DM state and related nephropathy.

Genes associated with kidney disease

The entire ensemble of genes was evaluated by literature search to identify key candidate genes involved in kidney disease. Sixteen genes were selected: *SLC22A12*, Transient Receptor Potential Melastatin subtype 6 (*TRPM6*), aquaporin 9 (*AQP9*), *HP*, *HPR*, *ABCC2*, alanine-glyoxylate aminotransferase (*AGXT*), *UGT2A3*, *HAL*, *HYAL2*, *SLC13A1*, *SERPINF1*, *CD22*, *SIGLEC5*, *NEU4*, and *NOX1*. These genes may be directly related to risk factors or biomarkers that signify kidney damage progression or vulnerability. Hypomethylation is seen in all sixteen of the genes. The methylation patterns are shown in Figure 2.

A total of 92 genes were hypomethylated and 54 were hypermethylated in all subjects. Analysis of the sub-group of sixteen genes (Figure 2) associated with kidney disease for genes which were hypomethylated in all subjects identified six genes. The data for these six genes are shown in Figure 3. These genes and their

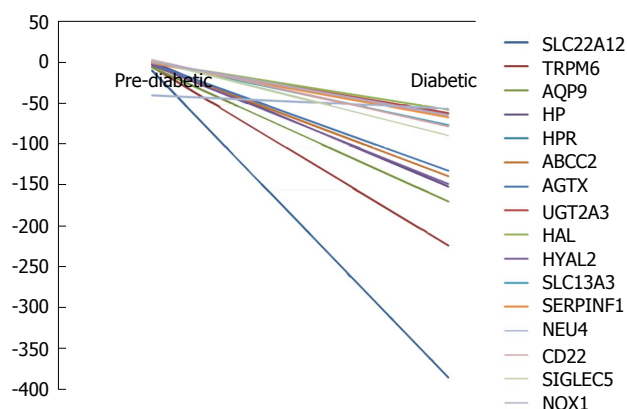


Figure 2 A total of 92 genes were hypomethylated and 54 were hypermethylated in all subjects using Illumina bead studio. Significantly hypomethylated genes were compared to putative genes identified in literature review forming of a sub-group of sixteen genes related to kidney disease. These sixteen genes are represented here as cumulative average DiffScore deviations from control comparisons. Scores of ± 13 P -value of 0.05, ± 22 P -value of 0.01.

associated products or functions are: *SLC22A12* (a urate transporter on the proximal tubule); *TRPM6* (a cation channel in the kidney); *AQP9* (an aquaporin); *HP* (haptoglobin, which binds plasma hemoglobin); *AGXT* (which is involved in oxalic acid secretion); *HYAL2* (a hyaluronidase).

DISCUSSION

This study demonstrated that there are a large number of methylation changes in the progression of Pre-DM to T2DM in a homogeneous longitudinal cohort of white males of which IPA of the associated genes identified numerous cellular pathways that potentially can be altered, leading to development and/or prediction of diabetes-related complications. Of particular interest, this study identified six genes that may be associated with/or predict the development of diabetic nephropathy. These six genes were hypomethylated in all subjects in the progression from Pre-DM to T2DM. The sample size was limited by the longitudinal observation of nearly a decade time period (approximately 7 years) required for the mean transition time from Pre-DM to T2DM. Although this sample size is small, the study identified a limited set of markers in this cohort that were hypomethylated in all subjects. Longitudinal studies similar to the present study but with larger numbers of subjects are especially difficult owing to the rare availability of DNA samples. However, the results from this study will aid in the design of future studies. For example, DNA from a range of subjects with diabetic nephropathy can be analyzed to confirm whether these changes in expression are observed in other ethnic groups during transition from Pre-DM to T2DM and also to compare with subjects who are resistant to developing T2DM related nephropathy.

Below is a brief discussion of the six genes

SLC22A12: Uric acid, which is the metabolic end

product of purine metabolism in humans, has protective antioxidant properties but can also be pro-oxidant. Urate, the ionized form of uric acid, scavenges potentially harmful radicals. Defective renal handling of urate is a frequent pathophysiologic factor in hyperuricemia. In response to genetic or environmental factors, such as diet, hyperuricemia may cause gout, nephrolithiasis, hypertension, and vascular disease. However, hypouricemia may also have pathological consequences. Humans have higher serum uric acid levels compared to other mammalian species; this is the result of genetic silencing of hepatic uricase, an enzyme that metabolizes uric acid into allantoin. Uric acid homeostasis is maintained by balance between production, intestinal secretion, and renal excretion. The kidney is important in the regulation of circulating uric acid levels through control of re-absorption of filtered urate and through uric acid excretion. In humans, urate transporters URAT1, MRP4, OAT1, and OAT3 play central roles in homeostasis. *SLC22A12*, a member of the organic anion transport family, encodes for the protein URAT1, which is a kidney-specific urate transporter that transports urate across the apical membrane of the proximal tubule various mutations in *SLC22A12* have been associated with renal disease. Given the importance of urate homeostasis, and the critical role of URAT1 activity in determining whether urate absorption vs secretion is balanced, epigenetic hypomethylation may be a determinant in the activity of URAT1^[15-21] (Figure 3).

TRPM6: TRPM6 is a member of the Transient Receptor Potential superfamily of cation channels, which are widely expressed and function in the regulation of absorption and secretion of cations. Many TRPs are expressed in kidney along the nephron. These channels are involved in hereditary as well as acquired kidney disorders. Increased expression of TRPM6 transporters is associated with diabetes mellitus and kidney damage in experimental animal models^[22,23]. TRPM6 channels are primarily located in the renal distal convoluted, the main site of active transcellular $\text{Ca}(2+)$ and $\text{Mg}(2+)$ transport in the kidney. The channels are regulated by many factors and hormones to maintain systemic concentrations of $\text{Ca}(2+)$ and $\text{Mg}(2+)$. Loss-of-function mutations in TRPM6 are a molecular cause of hypomagnesemia with secondary hypocalcemia. TRPM6 may be viewed as the gatekeeper of the body's Mg^{2+} balance although, even in the distal convolutions, multiple proteins involved in Mg^{2+} transport have been identified (TRPM6, proEGF, and FXYD2 which is the Na^+/K^+ -ATPase gamma-subunit). Drug treatment, acid-base status, and several hormones have been shown to regulate TRPM6 expression^[24-27].

AQP9: AQP are integral membrane channels for the transfer of water, and in some cases, small solutes across the membrane. Aquaporins are conserved in bacteria, plants, and animals. There are more than 10

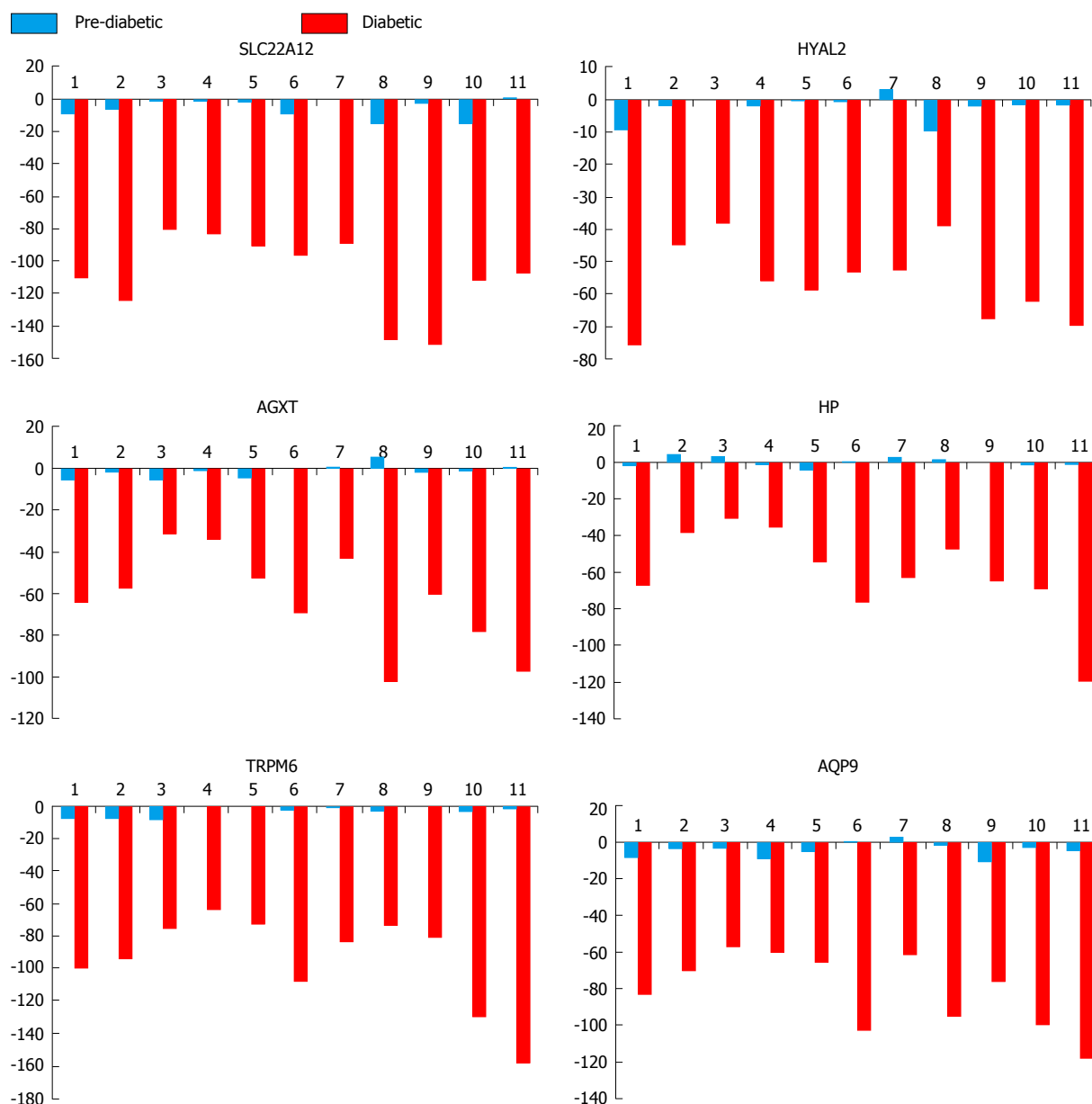


Figure 3 Hypo-methylated kidney disease associated gene loci. Six putative genes related to kidney disease were hypomethylated across patients (numbered 1-11) compared to controls ($n = 2$). Red bars indicate diabetic time point and blue bars representing prediabetic time point. Data represented as DiffScore with ± 13 equating to P -value 0.05, ± 22 equating to P -value of 0.01, and ± 33 equating to P -value of 0.001. SLC22A12 (a urate transporter on the proximal tubule); TRPM6 (a cation channel in the kidney); AQP9 (an aquaporin); HP (haptoglobin, which binds plasma hemoglobin); AGXT (alanine-glyoxylate aminotransferase which is involved in oxalic acid secretion); HYAL2 (a hyaluronidase).

isoforms of AQP. Several of the mammalian aquaporins (e.g., AQP1, AQP2, AQP4, and AQP5) are selective for the passage of water; others also transport glycerol (e.g., AQP3 and AQP8) and even larger solutes (AQP9). The human aquaporins, AQP3, AQP7, AQP8 and AQP9 are also permeable to ammonia. AQP9 is an aquaporin which stimulates urea transport and allows passage of a wide variety of non-charged solutes. AQP9 is expressed in numerous tissues and is especially abundant in liver. AQP9 as well as other AQPs also are expressed in kidney. The ammonia-transporting AQPs, including AQP9, supplement the ammonia transport of the Rhesus proteins; AQP9 also supplements the urea transporters. AQP9 can also transport arsenic trioxide^[28-32]. Given

the wide distribution of the AQPs, including AQP9, it is unclear whether epigenetic hypomethylation of AQP9 in diabetes would contribute to development of diabetic nephropathy. One possibility is that the special properties of AQP9 in urea transport may require highly controlled expression in tissues, such as kidney, which have important roles in urea transport.

HP: The *HP* gene encodes for haptoglobin (Hp) which functions to bind free plasma hemoglobin, thereby helping to prevent loss of iron through the kidney and protecting the kidneys from damage by hemoglobin. Iron status is influenced by environmental and genetic factors. The genetic polymorphism of Hp has been

shown to affect iron turnover. Hp captures hemoglobin in plasma to allow hepatic recycling of heme iron, which helps to prevent kidney damage during hemolysis. Hp acts as an anti-oxidant by binding hemoglobin. Two common alleles for Hp (1 and 2) produce three common Hp genotypes: Hp1-1, Hp2-1, and Hp2-2. The protein encoded by Hp1-1 provides superior antioxidant protection compared with that encoded by Hp2-2. Hp genotype is an independent risk factor for complications; individuals with Hp2-2 are more likely to develop nephropathy, retinopathy, and cardiovascular disease as compared with those with Hp2-1 or Hp1-1. In diabetic patients, urinary Hp levels and genotype predict renal functional decline. Aged animals are especially sensitive to the nephrotoxicity of hemoglobin. Hp synthesis is primarily a function of liver where Hp up regulation is a major stress response. However, in acute kidney injury, Hp synthesis in the proximal tubules is a major stress response^[33-39].

AGXT: The AGXT gene codes for the peroxisomal enzyme AGXT, which converts glyoxylate into glycine using L-alanine as the amino-group donor. Mutations in the AGXT are responsible for primary hyperoxaluria type 1 (PH1), which is a rare disease characterized by excessive hepatic oxalate production. When AGXT activity is absent, glyoxylate is converted to oxalate. Oxalate forms insoluble calcium salts that accumulate in the kidney. PH1 patients are at risk for recurrent deposition of calcium oxalate in the renal pelvis/urinary tract, deposition of calcium oxalate in the renal parenchyma, or ESRD. The PH1 is mostly due to single point mutations on the AGXT gene; more than 150 so far been identified^[40-42]. The epigenetic hypomethylation of AGXT (Figure 3), where hypomethylation generally is associated with enhanced expression, would seem counter to a role for AGXT where PH1 is associated with diminished activity. However, in a recent cluster analysis of microarray expression data for genes associated with T2DM and nephropathy, AGXT was identified as one of the more highly expressed genes^[43].

HYAL2: Hyaluronidases degrade hyaluronan, one of the major glycosaminoglycans of the extracellular matrix. The human genome contains six hyaluronidase-like genes. HYAL2 and HYAL1 are the major mammalian hyaluronidases in somatic tissues. They work together to degrade high molecular weight hyaluronan to tetrasaccharides. Initially large hyaluronan fragments (20 kD) are generated at the cell surface from digestion by the glycosylphosphatidyl-inositol-anchored HYAL2. These fragments are internalized and further digested by HYAL1. Alterations in hyaluronan have been reported in numerous renal diseases. The accumulation of hyaluronan in the renal cortex is observed in inflammatory renal diseases. In addition, the large fragments of hyaluronan produced by HYAL2 display inflammatory effects *in vitro* and may contribute to immune renal

injury. Increased activity of renal hyaluronidase occurs in streptozotocin-induced diabetic rats; this activity increases in multiple areas of the kidney during the progression of diabetic nephropathy^[44-47].

There are a number of limitations to this study. The sample size was small, reflecting the challenges in obtaining DNA samples from subjects at the Pre-DM and T2DM stages of patients who eventually developed diabetic nephropathy. The six selected kidney disease-associated gene based on literature evaluation, which were hypomethylated in all of the subjects, suggests but does not prove that the expression levels of these genes were up regulated during the progression to T2DM. In addition, the hypomethylation of these genes does not predict the interval of time before the development of nephropathy. Nevertheless, the fact that all of the subjects exhibited hypomethylation of these genes raises the question whether these changes might be predictive of diabetic nephropathy.

COMMENTS

Background

Type 2 diabetes mellitus (T2DM) affects more than 29 million in United States and about 79 million adults have pre-diabetes mellitus (Pre-DM). Environmental exposures, sedentary lifestyle, and high calorie, high-fat diets correlate with the development of metabolic syndrome including obesity and insulin resistance. All of these factors influence the rate of progression of Pre-DM. Recent studies suggest that gene-environment interactions relevant for T2DM are at least partly regulated by epigenomic mechanisms.

Research frontiers

The epigenome is increasingly gaining acceptance as playing an important role in diabetes and obesity, and the role of both nutritional status and endocrine disruptors would appear to be major factors in these conditions. A general defect in DNA methylation in T2DM is suggested by the observation that S-adenosylmethionine (SAM), the main physiologic donor of methyl groups, is decreased in erythrocytes of diabetic patients. In addition, decreased erythrocyte concentrations of SAM and other alterations were found to be associated with disease progression.

Innovations and breakthroughs

The study demonstrated that there are a large number of methylation changes in the progression of Pre-DM to T2DM. The study results revealed that 694 CpG sites were consistently hypomethylated and 174 were hypermethylated in the DNA obtained at the time of transition to T2DM compared to the DNA obtained at Pre-DM. The putative genes identified are associated with carbohydrate and lipid metabolism, inflammation, immune cell function and cell signaling, suggesting increased activities in these pathways at the T2DM state compared to the Pre-DM state. The authors further observed methylation changes in six candidate genes in all patients at the T2DM stage with nephropathy suggesting future development of diabetic complications.

Applications

Characterizing the epigenomic components that may regulate the transcriptional potential of a cell and contribute to the etiology, severity and progression of Pre-DM to T2DM and to complications including kidney disease will provide novel insights into disease pathogenesis and therapeutic approaches. This knowledge will enhance our ability to investigate, diagnose and ameliorate T2DM and kidney disease with a significant epigenomic component.

Peer-review

It is an interesting prospective study, analyzing DNA methylation profiling in

11 pre diabetic and 2 control individuals. In addition hypomethylation may be associated to difference genes in the nephropathy progression.

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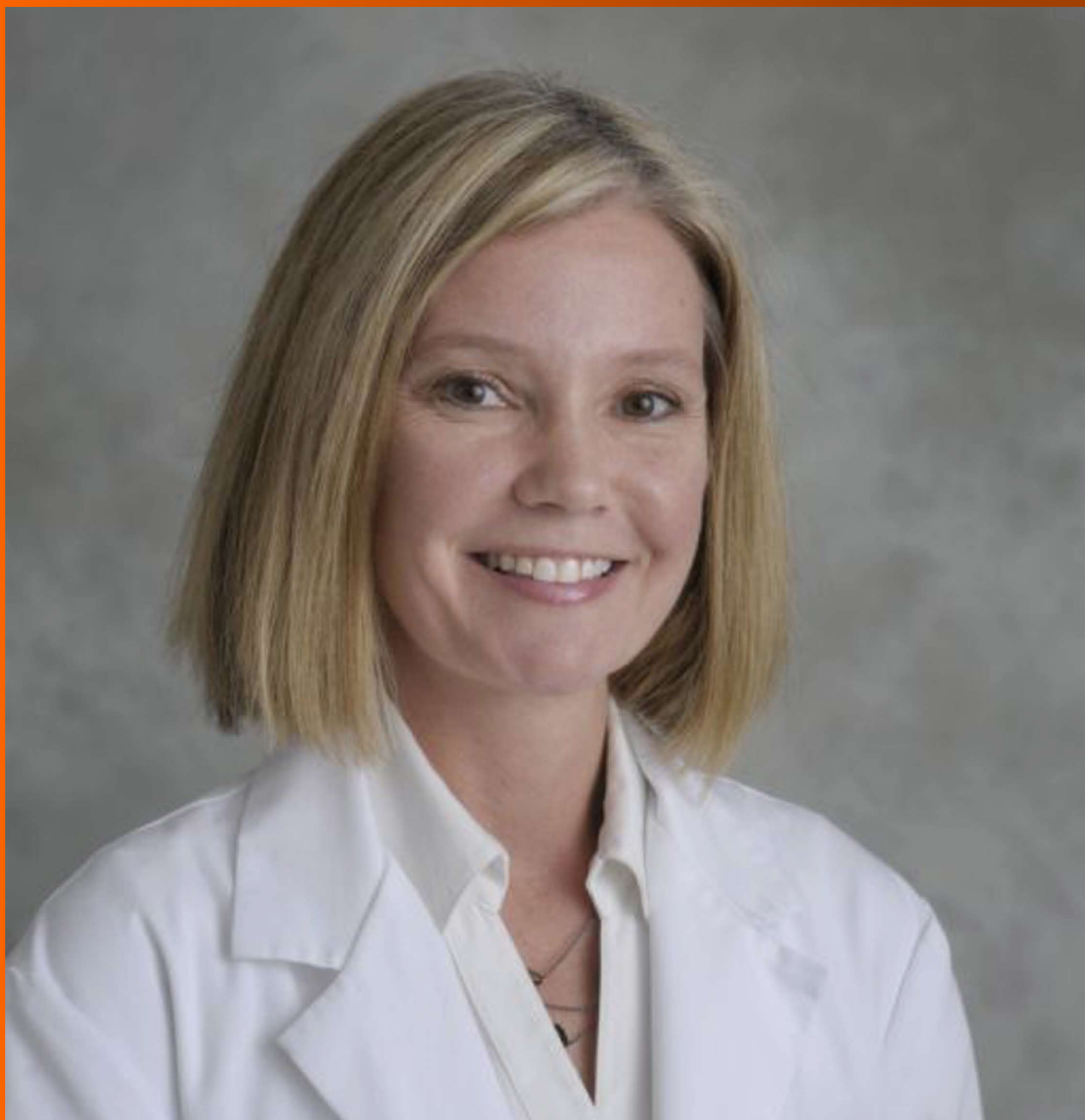
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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

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Telephone: +1-925-223-8242

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Effect of proton pump inhibitors on glycemic control in patients with diabetes

Kohzo Takebayashi, Toshihiko Inukai

Kohzo Takebayashi, Toshihiko Inukai, Department of Internal Medicine, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama 343-8555, Japan

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Correspondence to: Kohzo Takebayashi, MD, Department of Internal Medicine, Dokkyo Medical University Koshigaya Hospital, 2-1-50 Minamikoshigaya, Koshigaya, Saitama 343-8555, Japan. takeb@gmail.plala.or.jp
 Telephone: +81-48-9651111
 Fax: +81-48-9651127

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Abstract

Gastrin is a linear peptide hormone which is secreted mostly in the stomach pyloric antrum G cells. Although the main role of this hormone is the promotion of the secretion of gastric acid from the stomach parietal cells, gastrin can also behave as a growth factor and

stimulate gastric cell proliferation. It is also reported that gastrin promotes β cell neogenesis in the pancreatic ductal complex, modest pancreatic β cell replication, and improvement of glucose tolerance in animal models, in which the remodeling of pancreatic tissues is promoted. These findings suggest the possibility that gastrin has the potential to promote an increase of β cell mass in pancreas, and therefore that gastrin may improve glucose tolerance. Proton pump inhibitors (PPIs) are widely used clinically for the therapy of gastro-esophageal reflux disease, gastritis due to excess stomach acid, and gastric ulcers. PPIs indirectly elevate serum gastrin levels *via* a negative feedback effect. Recent evidence has revealed the beneficial effect of PPIs on glycemic control especially in patients with type 2 diabetes mellitus (T2DM), probably *via* the elevation of the levels of serum gastrin, although the detailed mechanism remains unclear. In addition, the beneficial effects of a combination therapy of gastrin or a PPI with a glucagon-like peptide-1 receptor agonist on glycemic control in animal models have been demonstrated. Although PPIs may be possible candidates for a new approach in the therapy of diabetes, a prospective, long-term, randomized, double-blind, placebo-controlled study is needed to establish the effect of PPIs on glycemic control in a large number of patients with T2DM.

Key words: Gastrin; Proton pump inhibitors; Glycemic control; Type 2 diabetes

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Core tip: Recently, it is reported that gastrin may improve glucose tolerance mainly by the promotion of pancreatic β cell neogenesis. Proton pump inhibitors (PPIs) are widely used clinically for the treatment such as gastric ulcers, and it is known that PPIs indirectly elevate serum gastrin levels. Recent evidence has showed the beneficial effect of PPIs on glycemic control especially in patients with type 2 diabetes, probably

via the elevation of serum gastrin levels. Therefore, PPIs may have the potential to be candidates for a new approach in the treatment of diabetes.

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INTRODUCTION

Gastrin is a linear peptide hormone which is secreted mostly in the stomach pyloric antrum G cells, in which high biologically active gastrin (gastrin-17 and gastrin-34) is formed^[1,2]. The secretion of gastrin is stimulated by various factors, such as considerable distension of the stomach^[3], vagal stimulation^[3,4], the presence of food (especially protein, peptides, and amino acids) in the stomach^[4-6], and high pH levels in the stomach cavity^[5,7]. Gastrin is released into the bloodstream. The main role of this hormone is the stimulation of secretion of gastric acid from the stomach parietal cells. The gastrin receptor, cholecystokinin B (CCK-B) receptor, binds to gastrin and to cholecystokinin with a similar high affinity^[8]. Gastrin can directly promote the secretion of gastric acid by binding to CCK-B receptor on parietal cells^[9,10]. However, the expression of this receptor is also found on enterochromaffin-like cells, and the binding of CCK-B receptor to gastrin on these cells promotes the secretion of the histamine resulting in subsequent promotion of the release of gastric acids by parietal cells, which may be the central mechanism of gastrin-stimulated acid secretion^[6,9-12]. Importantly, gastrin is also able to behave as a growth factor and stimulate gastric cell proliferation^[6,13]. It is reported that gastrin promotes β cell neogenesis in pancreatic ductal complex^[14], modest pancreatic β cell replication^[15], and improvement of glucose tolerance^[15] in animal models in which the remodeling of pancreatic tissues is promoted. These findings suggest the possibility that gastrin has a potential promoting effect for the increase in the pancreatic β cell mass. Therefore, gastrin improves glucose tolerance, and these effects appear to occur especially during adult pancreatic tissue remodeling but not in the normal tissue state.

Proton pump inhibitors (PPIs) are widely used clinically for the therapy of gastro-esophageal reflux disease, gastritis due to excess stomach acid, and gastric ulcers^[16]. PPIs can be orally administrated as an inactive form, which enters the bloodstream from the intestine, reaches the gastric parietal cells, and is activated by crossing the cell membrane into the intracellular compartment. After converting to the active form in the unique parietal cell environment, PPIs irreversibly block the proton pump and can strongly reduce the secretion

of gastric acid promoted by either gastrin, acetylcholine, or histamine. It is well known that PPIs indirectly elevate serum gastrin levels *via* a negative feedback effect^[17-22]. Interestingly, in type 2 diabetes mellitus (T2DM) animal models, it has been reported that PPIs improved glycemic control, probably *via* possible effects on augmenting both serum levels of gastrin and β cell mass^[23]. Although some clinical studies showed negative results on glycemic control by PPIs in patients with T2DM^[24,25], most studies have demonstrated a significant improvement of glycemic control by PPI administration to these patients^[26-32]. Therefore, these agents appear to have the possibility of being a new approach for the therapy of diabetes.

BASIC STUDIES ON THE EFFECT OF GASTRIN ON THE INCREASE IN β CELL MASS

Gastrin and the CCK-B receptor are transiently expressed in fetal tissues of pancreas under period of islet neogenesis^[33-35], but no expression is observed in both adult pancreatic β cells^[36,37] and the exocrine pancreas^[34,38-40]. It has been reported that in a rat model in which the splenic portion of the pancreas is ligated (an animal model for remodeling of pancreas tissue), transdifferentiation of acinar to ductal cells is promoted, and a ductal complex consisting of a mixture transdifferentiated acinar and ductal cells is formed^[41-44]. A similar ductal complex appeared to emerge in 95% of the pancreatectomized rats (an animal model for diabetes in which pancreatic remodeling is promoted)^[15]. Although the CCK-B receptor is not expressed in adult β cells even if the pancreatic tissue is undergoing remodeling, the ductal complex shows characteristics of fetal pancreatic ductal cells in addition to those in adult, including the CCK-B receptor expression^[34]. So, it appears that gastrin is able to enhance the process of β cell neogenesis, that was already induced during the remodeling state, *via* the CCK-B receptor followed by budding from the ductal complex^[14,41]. In general, gastrin does not affect β cell replication probably because of a lack of the CCK-B receptor on β cells^[14], but there is a report suggesting that, in 95% of the pancreatectomized rats, gastrin treatment not only increased β cells neogenesis from ductal cells but also caused both a modest increase in replication and a decrease in apoptosis in β cells with the resultant improvement of glucose tolerance. The detailed mechanism for these activities remains unclear^[15]. The replication of β cells is also reported in gastrinoma patients^[45] although only β cell islets located near the gastrinomas exhibited β cell turnover despite the fact that serum levels of gastrin were elevated to the degrees to induce clinically apparent gastrointestinal symptoms. Thus, it is possible that other hormones were also involved. On other hand, the synergistic effect of other hormones, such as transforming growth factor- α ^[46], epidermal

growth factor^[47], and glucagon-like peptide-1 (GLP-1)^[48], with gastrin has also been demonstrated. For example, GLP-1 induces both β cell replication with mitogens and neogenesis of β cell from ductal cells^[49]. In combination with GLP-1, gastrin appears to enhance β cell neogenesis even when it is added in animal models, such as either *db/db* mice (a model of T2DM)^[50] or non-obese diabetic (NOD) mice [a model of type 1 diabetes mellitus (T1DM)]^[48], although, in these models, pancreatic remodeling is not necessarily occurring. In addition, an effect on regulating the autoimmune response against pancreatic β cells by combination therapy was also reported in the NOD mice model^[48]. Taken together, these effects of gastrin suggest that this hormone may possess a potential protective effect for the progression of diabetes, especially in combination with other hormones, such as GLP-1.

THE EFFECT OF PPIs ON GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES: RESULTS OF CLINICAL STUDIES

Despite the possible effects of gastrin on both increasing β cell mass and improving glycemic control, gastrin treatment has not been used with the patients with T2DM mainly because of the difficulty with oral administration and the suggested side effects on the stomach. On the other hand, there are many publications describing the effects of PPIs on glycemic control in patients with T2DM.

Mefford *et al.*^[26] reported that a significant difference was obtained in HbA1c in patients with T2DM taking PPIs (7.0% of HbA1c, $n = 65$) vs those not taking PPIs (7.6% of HbA1c, $n = 282$, $P = 0.002$). Similarly, Boj-Carceller *et al.*^[27] reported that HbA1c was significantly different in T2DM patients who received PPIs ($6.7\% \pm 1.0\%$, $n = 54$) compared with those who did not received PPIs ($7.3\% \pm 1.4\%$, $n = 43$, $P = 0.018$). When these patients were assigned to two groups by the treatment of diabetes, those taking insulin and concurrent PPIs had better glycemic control, compared with those taking insulin but not PPI (-0.8% reduction, $P = 0.022$). In a very recent study, Barchetta *et al.*^[28], showed that the significantly different HbA1c and FPG levels were found in the T2DM patients with PPIs for longer than 2 years ($n = 245$) compared with those who did not take PPIs ($n = 303$) ($7.1\% \pm 1.07\%$ with PPIs vs $7.4\% \pm 1.4\%$ without PPIs for HbA1c, $P = 0.011$; 127 ± 36.9 mg/dL with PPIs vs 147.6 ± 49.6 mg/dL without PPIs for FPG, $P < 0.001$, respectively). The increase of the differences was observed in patients treated with insulin and in those treated with combination of PPIs and GLP-1 based therapy^[28]. The results of these cross-sectional studies suggest the significant association between treatment with PPIs and the improved glycemic control in patients with T2DM.

On the other hand, in a study using a retrospective analysis, patients were assigned to 2 groups: 21 patients who had taken esomeprazole (a PPI) for 11.3 ± 3 mo and 21 control subjects^[29]. Although there was a tendency for a decline in HbA1c in the patients treated with this PPI, it was not statistically significant (8.6% to 7.9%, $P = 0.054$), while in a subgroup with HbA1c $> 9\%$, the reduction was statistically significant (9.7% to 8.5%, $n = 11$, $P = 0.004$). No change in HbA1c was found in the entire control group and in a subgroup with HbA1c $> 9.0\%$ in control group (9.2% to 9.0%, $P = 0.455$; 10.3% to 10.0%, $P = 0.287$, respectively). Furthermore, Crouch *et al.*^[30] investigated 71 individuals with T2DM who were not taking insulin. The mean HbA1c was 7.11% during periods taking either prescription or over-the-counter PPIs, vs 7.7% during periods not taking PPIs (a significant difference, $P = 0.001$). Although there was no significant difference in mean HbA1c in a metformin monotherapy (6.81 treated with PPIs vs 7.10% treated without PPIs, $P = 0.25$), mean HbA1c was significantly lower in a concomitant therapy including metformin and/or sulfonylurea and/or glitazone (7.26 treated with PPIs vs 7.80 treated without PPIs, $n = 27$, $P = 0.002$). However, in another recent retrospective study of T2DM patients with relatively low levels of HbA1c, treatment with PPIs for ≥ 2 mo (mean duration: 180 d, $n = 43$) did not significantly change HbA1c levels ($6.86\% \pm 1.10\%$ to $6.77\% \pm 1.07\%$). Metformin monotherapy did not change HbA1c compared with a combination therapy including metformin and a therapy in antidiabetic agents not including metformin^[24]. Furthermore, 3 recent prospective randomized, double-blind, placebo-controlled studies using PPIs in small number of T2DM patients showed conflicting results with its effect on glycemic control. Singh *et al.*^[31] investigated the effect of a 12-wk pantoprazole (a PPI) therapy regimen on glycemic control in patients with T2DM^[31]. Thirty one eligible patients were randomly assigned to take either pantoprazole ($n = 16$) or placebo ($n = 15$). Pantoprazole (40 mg twice daily) significantly increased both plasma levels of gastrin (54.4 ± 14.9 to 75.6 ± 15.1 pg/mL, $P < 0.001$) and those of insulin (10.5 ± 4.0 to 13.9 ± 4.5 μ U/mL, $P < 0.001$) and improved the function of β cell as calculated by the homeostasis model assessment- β (HOMA- β). HbA1c significantly decreased with pantoprazole therapy ($7.60\% \pm 1.17\%$ to $6.80\% \pm 1.16\%$, $P < 0.001$). The decrease of HbA1c was positively associated with a significant elevation in both gastrin and insulin levels. González-Ortiz *et al.*^[32] investigated the effect of pantoprazole (40 mg once daily for 45 d) on secretion of insulin in 14 drug naive patients with T2DM. Significant increases in both the late insulin phase (215 ± 127 to 308 ± 151 pmol/L, $P = 0.028$) and total insulin secretion (174 ± 94 to 265 ± 135 pmol/L, $P = 0.028$), and significant decreases in HbA1c levels (7.5% to 6.6%, $P = 0.018$) were found with pantoprazole administration ($n =$

7), while there was no significant changes in these parameters in patients treated with placebo ($n = 7$). On the other hand, Hove *et al.*^[25] investigated the effect of esomeprazole on glycemic control in 41 T2DM patients using either dietary control or treatment with oral anti-diabetic agents. These patients were randomly assigned to take either add-on esomeprazole (40 mg daily, $n = 20$) or placebo ($n = 21$) during 12 wk^[25]. In the esomeprazole group, the area under the curve (AUC) for insulin did not change, while the AUC for the placebo group significantly decreased. Esomeprazole treatment caused a nine-fold elevation in the AUC for gastrin. Contrary to the expectation, HbA1c increased from $7.0\% \pm 0.6\%$ to $7.3\% \pm 0.8\%$ ($P < 0.05$) in the esomeprazole group and from $7.0\% \pm 0.6\%$ to $7.4\% \pm 0.8\%$ ($P < 0.05$) in the placebo group with no significant difference in change between both treatments (unadjusted, $P = 0.297$). These clinical findings from all of these studies are summarized in Table 1. Based on the published data to date, the degrees of the reduction of HbA1c by PPIs therapy in the studies with positive results appears to be approximately 0.6%-0.9%. This is somewhat milder or similar compared with those by recent available anti-diabetic drugs such as dipeptidyl peptidase-4 (DPP-4) inhibitors^[51] or sodium-glucose co-transporter 2 inhibitors^[52]. This suggests that the effect of PPI for glycemic control is probably moderate and that therefore PPI may have the potential for clinical benefit on glycemic control in patients with T2DM.

THE USE OF PPIs FOR THE TREATMENT OF TYPE 2 DIABETES: INTERPRETATION OF THE RESULTS AND POSSIBLE MECHANISMS OF GLYCEMIC CONTROL

As shown in the previous section, it appears that PPIs generally have a beneficial effect on glycemic control for T2DM patients with some studies showing no effect. The results of the different studies do not appear to be dependent on the type of PPI used. Based on the results of most clinical studies in which glycemic control was improved^[26-32], it appears that the actual basal levels of HbA1c may be important for the PPIs to show the apparent glucose-lowering effect because PPIs significantly decreased HbA1c level only when the basal HbA1c level was high in 1 retrospective study^[29]. In addition, the patients in most of the studies with negative results had a tendency to be under good glycemic control (approximate 7.0% of HbA1c)^[24,25], compared with those studies that showed positive results^[26-32]. In addition, treatment with PPIs and HbA1c levels were independent from possible confounders in a multivariate regression analysis in 1 study^[28], suggesting the importance of baseline HbA1c levels for the glucose lowering effect of PPIs. Next, if the possible effect of PPIs on glycemic control is based on

the mechanism of increase of β cell mass, treatment with PPIs for a longer period may be more effective in providing the full effect on glycemic control compared with that observed in most of the previous studies. However, in fact, the mechanism of the clinical effect of PPIs on glycemic control largely remains unclear. Because gastrin does not affect β cell neogenesis from the adult pancreatic ductal cells under a non-remodeling state as previously described^[14,15], it is not apparent whether the elevation of circulating gastrin levels induced by PPIs can really promote the increase of the mass of β cell in patients with T2DM, in whom pancreatic remodeling is not necessarily occurring. Nonetheless, elevated serum gastrin levels could affect the β cell mass in animal models of T2DM although the mechanism is not fully apparent. PPI mono therapy improved glycemic control with the increase in both plasma insulin and β cells mass in *Psammomys obesus*, an animal model of T2DM^[23]. In this study, a significant effect was obtained only when the PPI was used at a very high dose (lansoprazole 10-15 mg/kg); gastrin was elevated nine-fold at this dose. Since vonoprazan (a new generation PPI: potassium-competitive acid blocker) is more effective for inhibition of secretion of gastric acid and increases serum levels of gastrin (approximate six- to seven-fold with 10-40 mg of vonoprazan) compared with that of the existing PPIs^[53], it would be interesting to investigate in a future study whether this agent is also more effective on glycemic control. However, it is important to note that such elevation of serum gastrin levels by PPIs is not always needed to exhibit the clinically apparent glucose-lowering effect in T2DM patients because, in the study by Singh *et al.*^[31], in which positive results were obtained, the increase of gastrin by a PPI (pantoprazole) was only approximately 1.5-fold^[31], which was accompanied with an increase of insulin. These findings suggest the possibility that mechanisms other than the increase of β cell mass are also involved. One possible mechanism involves a gastrin-stimulated increase in insulin secretion by pancreatic β cells. It has been reported that because the secretion of the endogenous gastrin for the oral glucose tolerance test (OGTT) in healthy subjects is very small, it is unlikely that gastrin strongly promotes insulin secretion under this condition. However, an ordinary protein-rich meal (but not glucose-rich) largely increases both circulating gastrin and insulin levels^[2]. Therefore, gastrin appears to significantly stimulate secretion of insulin during and after a meal, this may partially explain the effect of PPIs on glycemic control. Another mechanism may involve the interaction of gastrin with other gastric hormones, such as ghrelin, which is reported to have an important role in energy homeostasis and appetite regulation. There is a report showing that ghrelin was down-regulated in primary gastric cells during gastrin-stimulation, and that ghrelin and gastrin levels had a significant negative correlation in humans. For example, a long-term 3-fold increase of

Table 1 Studies showing glucose-lowering effect of proton pump inhibitors in patients with type 2 diabetes

Mefford <i>et al</i> ^[26]	<p>Outcome measures: HbA1c levels in patients with type 2 diabetes taking PPIs (<i>n</i> = 65) <i>vs</i> those not taking PPIs (<i>n</i> = 282) was evaluated in cross-sectional design</p> <p>Key findings: There was a significant difference in HbA1c in patients taking PPIs <i>vs</i> those not taking PPIs (7.0% <i>vs</i> 7.6%, <i>P</i> = 0.002)</p> <p>Safety information: No information is described</p>
Boj-Carceller <i>et al</i> ^[27]	<p>Outcome measures: HbA1c levels in patients with type 2 diabetes taking PPIs (<i>n</i> = 54) <i>vs</i> those not taking PPIs (<i>n</i> = 43) was evaluated in cross-sectional design</p> <p>Key findings: HbA1c was significantly lower in type 2 diabetic patients who take PPIs compared with those not taking PPIs (6.7% ± 1.0% <i>vs</i> 7.3% ± 1.4%, <i>P</i> = 0.018)</p> <p>Safety information: No information is described</p>
Barchetta <i>et al</i> ^[28]	<p>Outcome measures: HbA1c and FPG levels in patients with type 2 diabetes taking PPIs for longer than 2 yr (<i>n</i> = 245) <i>vs</i> those not taking PPIs (<i>n</i> = 303) was evaluated in cross-sectional design</p> <p>Key findings: Patients with PPIs had significantly lower HbA1c (7.1% ± 1.07% <i>vs</i> 7.4% ± 1.4%, <i>P</i> = 0.011) and FPG (127 ± 36.9 mg/dL <i>vs</i> 147.6 ± 49.6 mg/dL, <i>P</i> < 0.001) levels than those who did not take PPIs</p> <p>Safety information: No information is described</p>
Hove <i>et al</i> ^[29]	<p>Outcome measures: HbA1c levels were retrospectively evaluated in patients with type 2 diabetes. Patients were assigned to 2 groups: 21 patients who had taken esomeprazole (a PPI) for 11.3 ± 3 mo and 21 control subjects</p> <p>Key findings: There was a tendency for a decline in HbA1c in the patients treated with this PPI (8.6% to 7.9%, <i>P</i> = 0.054). In a subgroup with HbA1c > 9% (<i>n</i> = 11), the reduction was statistically significant (9.7% to 8.5%, <i>P</i> = 0.004). No change in HbA1c was observed in the control group (9.2% to 9.9%, <i>P</i> = 0.455)</p> <p>Safety information: No information is described</p>
Han <i>et al</i> ^[24]	<p>Outcome measures: HbA1c was retrospectively evaluated in type 2 diabetic patients treated with PPIs for ≥ 2 mo (mean duration: 180 d, <i>n</i> = 43)</p> <p>Key findings: There was no significant change in HbA1c levels (6.86% ± 1.10% to 6.77% ± 1.07%; <i>P</i> = 0.406)</p> <p>Safety information: No information is described</p>
Crouch <i>et al</i> ^[30]	<p>Outcome measures: 71 individuals with type 2 diabetes who were not taking insulin was retrospectively investigated for the change of HbA1c</p> <p>Key findings: The mean HbA1c was 7.11% during periods with either prescription or over-the-counter PPIs, <i>vs</i> 7.7% during periods without PPIs (a significant difference; <i>P</i> = 0.001)</p> <p>Safety information: No information is described</p>
Singh <i>et al</i> ^[31]	<p>Outcome measures: The effect of a 12-wk pantoprazole (40 mg twice daily) therapy regimen on HbA1c, FPG, serum insulin, serum gastrin levels was prospectively measured in patients with type 2 diabetes in randomized double-blind, placebo-controlled study design. Thirty one eligible patients were randomly assigned to receive either pantoprazole (<i>n</i> = 16) or placebo (<i>n</i> = 15)</p> <p>Key findings: HbA1c and FPG significantly decreased with pantoprazole therapy (7.60% ± 1.17% to 6.80% ± 1.16%, <i>P</i> < 0.001 for HbA1c and 126.3 ± 10.3 to 109.2 ± 13.0 mg/dL, <i>P</i> = 0.017 for FPG), and the differences were significant between the two groups (<i>P</i> = 0.004 for HbA1c, <i>P</i> = 0.019 for FPG). Pantoprazole significantly increased both plasma gastrin (<i>P</i> < 0.001) and insulin levels (<i>P</i> < 0.001)</p> <p>Safety information: Nine patients reported adverse events as nausea, vomiting, headache and myalgia, which were similar and mild in the both groups. None of the patients had hypoglycemia</p>
González-Ortiz <i>et al</i> ^[32]	<p>Outcome measures: The effect of pantoprazole (40 mg once daily for 45 d) on insulin secretion in 14 drug naive patients with type 2 diabetes was prospectively investigated in a randomized, double-blind, placebo-controlled study design. Insulin secretion evaluated by hyperglycemic and hyperinsulinemic clamp technique, HbA1c, FPG and serum lipids were measured</p> <p>Key findings: Significant increases in total insulin secretion (<i>P</i> = 0.028), and significant decreases in HbA1c levels (7.5% to 6.6%; <i>P</i> = 0.018) but not FPG levels (<i>P</i> = 0.236) were found with pantoprazole therapy (<i>n</i> = 7), while there was no significant changes in these parameters in patients treated with placebo (<i>n</i> = 7). There were no significant changes in serum lipids in both groups</p> <p>Safety information: Two patients had mild headache (one in each group)</p>
Hove <i>et al</i> ^[25]	<p>Outcome measures: The effect of esomeprazole on glycemic control in 41 type 2 diabetic patients using either dietary control or therapy by anti-diabetic agents was prospectively examined in a randomized double-blind placebo-controlled 2 × 2 factorial study. These patients were randomly assigned to receive either add-on esomeprazole (40 mg daily, <i>n</i> = 20) or placebo (<i>n</i> = 21) for 12 wk. Insulin secretion, HbA1c levels and cardiovascular risk factors were evaluated</p> <p>Key findings: In the esomeprazole-treated group, the AUC (area under the curve) for insulin did not change (<i>P</i> = 0.838), while the AUC for the placebo group significantly decreased (<i>P</i> = 0.002). HbA1c increased from 7.0% ± 0.6% to 7.3% ± 0.8% (<i>P</i> < 0.05) in the esomeprazole-treated group and from 7.0% ± 0.6% to 7.4% ± 0.8% (<i>P</i> < 0.05) in the placebo group (no significant difference in change between both treatments; unadjusted, <i>P</i> = 0.297). The differences in cardiovascular risk factors were not significant between the two groups</p> <p>Safety information: Flatulence in 2 patients and diarrhea in 1 patient was reported in lansoprazole group, and flatulence in 2 patients and intermittent diarrhea in 1 patient was reported in placebo group</p>
Takebayashi <i>et al</i> ^[72]	<p>Outcome measures: The effect of alogliptin and lansoprazole (<i>n</i> = 46) combination therapy compared with alogliptin therapy without lansoprazole (<i>n</i> = 43) on glycemic control was investigated in a randomized open-label study. After 3 mo of treatment, the changes in HbA1c, FPG, serum gastrin were evaluated</p> <p>Key findings: A significant decrease in both HbA1c and FPG (respective 7.6% ± 0.6% to 6.8% ± 0.7%, <i>P</i> < 0.0001, 52.0 ± 35.6 to 127.3 ± 27.4 mg/dL, <i>P</i> < 0.0001 in the combination therapy group, and respective 7.7% ± 0.5% to 6.7% ± 0.5%, <i>P</i> < 0.0001, 153.6 ± 34.4 to 128.5 ± 26.6 mg/dL, <i>P</i> = 0.0001 in the alogliptin therapy group) was obtained. There were no significant differences in changes in HbA1c, FPG (<i>P</i> = 0.2945, <i>P</i> = 0.1901, respectively) and significant elevation in change in gastrin (approximate twofold, <i>P</i> = 0.0004) before and after therapy between the combination and the alogliptin mono therapy group</p> <p>Safety information: In alogliptin group, 1 patient discontinued the drug due to epi-gastric pain. In the combination group, 1 patient withdrew due to a mild cerebral infarction, and 1 patient noticed occasional hypoglycemic symptoms</p>

PPIs: Proton pump inhibitors.

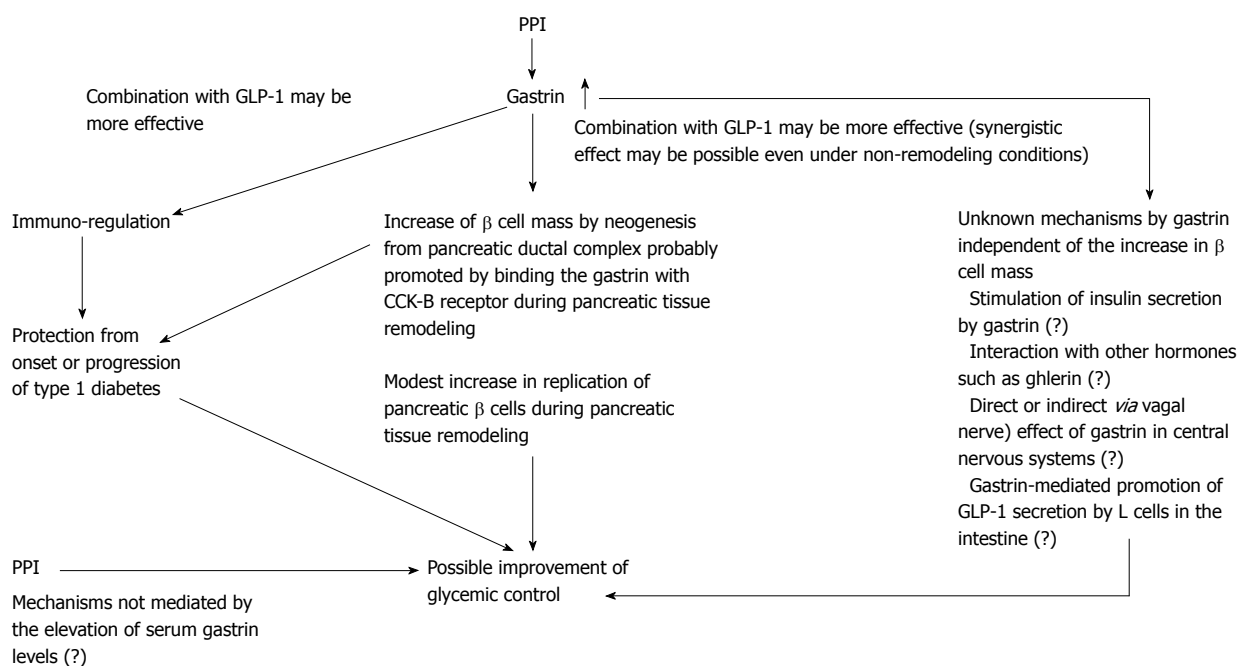


Figure 1 The possible mechanisms of proton pump inhibitors on the improvement of glycemic control. PPIs indirectly elevate serum gastrin levels. Gastrin promotes an increase in β cell mass by neogenesis of the β cells from the pancreatic ductal complex probably promoted by binding the gastrin with CCK-B receptor during pancreatic remodeling. In addition, a modest increase in the replication of pancreatic β cells during pancreatic remodeling is also reported although the mechanisms are not apparent because of the lack of a CCK-B receptor on β cells. Gastrin can enhance the effect of GLP-1 on β cell neogenesis from ductal cells. A synergistic effect may occur even under non-remodeling conditions in the pancreas. These mechanisms appear to contribute to the improvement of glycemic control in both type 1 and type 2 diabetes. Furthermore, a combination of GLP-1 and gastrin may protect from the onset or progression of type 1 diabetes by an immunoregulatory effect. Other possible gastrin-mediated mechanisms independent of the β cell mass increase may include stimulation of insulin secretion, interaction with other hormones such as ghrelin, direct or indirect (*via* vagal nerve) effects in the central nervous systems, and promotion of GLP-1 secretion by L cells in the intestine. Finally, it may be possible that PPIs affect glycemic control by unknown mechanisms independent of the elevation of serum gastrin levels. PPIs: Proton pump inhibitors; CCK-B: Cholecystokinin-B; GLP-1: Glucagon like-peptide-1.

gastrin in autoimmune gastritis significantly repressed ghrelin secretion^[54]. These findings suggest the possibility that the increase of gastrin levels is associated with less appetite and improvement of glycemic control *via* the decreased ghrelin levels although there is as yet no clinical evidence. Furthermore, it is known that the CCK-B receptor exists in the brain, especially in the hypothalamic area^[8,55]. Intracerebroventricular injection of gastrin decreases food intake, while inactivation of CCK-B receptor in mice changes the regulation of food-intake and body weight, and results in obesity^[56]. Despite the limitation of gastrin diffusion into the brain due to the blood brain barrier (BBB)^[57], there are reports suggesting that either peptide or peptide fragments might penetrate into the brain because of the lack of a BBB in the circumventricular organs^[58], and that intravenous gastrin administration activated neurons in several portions of brain^[59]. In addition, it is reported that gastrin in circulation is able to stimulate the area postrema neurons that express the CCK-B receptor and project to the nucleus of the solitary tract (NTS)^[60]. Mouse brain stem NTS-proopiomelanocortin neurons are associated with feeding-induced satiety^[61]. Therefore, we speculate that it might be possible that increased serum gastrin that is regulated by PPIs directly inhibits appetite *via* the central nervous system, although it may be possible that gastrin also

acts indirectly brain stem *via* the vagal nerve^[60]. In addition, a recent study revealed that gastrin stimulates GLP-1 secretion in L cells in the intestine^[62]. This can explain the possible effect of PPIs on glycemic control at least in part. Finally, it may also be important to consider whether PPIs potentially have a beneficial effect on glycemic control *via* unknown mechanism independent of gastrin. Taken together, the mechanisms of the possible PPI effects on glycemic control largely remain unclear, and multiple mechanisms appear to be involved. These possible mechanisms are described in Figure 1.

When treating patients, it is important to consider the potentially deleterious effects of PPIs on glycemic control, which may be more serious than the possible beneficial effect and which may modify the results. It is known that diabetes occasionally occurs with gastroesophageal reflux disease (GERD)^[63,64]. Because PPIs largely improve GERD clinical symptoms, it may be possible that the appetite of the patients with GERD is improved even if the elevation of gastrin levels by PPIs influences circulating ghrelin levels as previously described. These patients can thus potentially have worse glycemic control. In addition, it is reported that PPIs can induce dysbiosis^[65], which is connected with metabolic syndrome. Therefore, we speculate that PPIs can worsen glycemic control in this manner as well.

THE EFFECT OF COMBINATIONAL THERAPY OF PPIs (OR GASTRIN) WITH DPP-4 INHIBITORS (OR A GLP-1 RECEPTOR AGONIST) ON GLYCEMIC CONTROL IN TYPE 1 AND TYPE 2 DIABETES IN BOTH ANIMAL AND CLINICAL STUDIES

Recent evidence suggests the greater potential beneficial effect of a combination therapy of various hormones over that of a mono hormone therapy^[66]. As described in the previous section, gastrin enhances the effect of GLP-1 on β cell neogenesis, and this combination therapy more effectively improved hyperglycemia than mono therapy by each hormone in NOD mice^[48]. This result is also supported in the same animal model by combination therapy with DPP-4 inhibitors, which block degradation of GLP-1 by DPP-4 resulting in the elevation of serum active GLP-1 levels, and PPIs^[67]. Furthermore, Patel *et al*^[68], showed that combination therapy with exendin-4 (a GLP-1 receptor agonist) and omeprazole (a PPI) had better glycemic control compared with mono therapy with these drugs in *db/db* mice. Recently, Hao *et al*^[69] examined the effects of short periods of lansoprazole, sitagliptin (a DPP-4 inhibitor), and these concomitant therapy on glycemic control in mice with diet-induced obesity (DIO) and in healthy human subjects. In the DIO mice, lansoprazole therapy significantly improved glucose levels and increased both circulating insulin and C peptide levels than treatment in vehicles. Furthermore, concomitant treatment with lansoprazole and sitagliptin decreased glucose levels with higher levels in C-peptide and insulin compared to that with sitagliptin-treated mice. In a human study, the concomitant use (sitagliptin 100 mg daily and lansoprazole 30 mg daily) for 6 d resulted in significant decrease of glucose levels and increase of insulin levels in an OGTT vs the control, lansoprazole-, and sitagliptin-treated groups. Taken together, the results of these studies suggest the possibility that combination therapy with a GLP-1 receptor agonist (or DPP-4 inhibitors) and gastrin (or a PPI) may provide a more beneficial effect for glycemic control than each mono therapy. In addition, in *db/db* mice, a GLP-1-gastrin dual receptor agonist has showed a more continued regulatory effect of glucose with a significant increase in β -cell mass in pancreatic tissue than that of monotherapy in liraglutide (a GLP-1 receptor agonist)^[70]. However, the results of recent randomized, prospective studies evaluating the combination therapy with DPP-4 inhibitors and PPIs in patients with T1DM and T2DM were basically negative. Griffin *et al*^[71] reported the results of a randomized, placebo-controlled, multicenter, phase 2 trial (REPAIR-T1D) on the effect of concomitant use with sitagliptin and lansoprazole in patients with recent-onset T1DM. Patients aged 11-36 years, diagnosed with T1DM within

the past 6 mo, were recruited and were randomized (2:1) to take oral sitagliptin with lansoprazole or placebo for 12 mo. At 12 mo, the 2 h C peptide AUC was similar between the combination ($n = 40$) and placebo ($n = 18$) groups. HbA1c levels were mainly constant throughout the study period for both groups (no significant difference). HbA1c adjusted by insulin-dose was also similar (no significant difference) for both groups. Although these overall results were negative, this study is still ongoing with reassessments at both 18 and 24 mo. In T2DM, we investigated the effect of alogliptin (a DPP-4 inhibitor) and lansoprazole ($n = 46$) combination therapy compared with alogliptin therapy without a PPI ($n = 43$) on glycemic control in a randomized open-label study^[72] (Table 1). At 3 mo after the initiation of the therapy, the changes in HbA1c, FPG, HOMA- β , HOMA-insulin resistance (IR) and serum gastrin were evaluated. A significant decrease in both HbA1c ($7.6\% \pm 0.6\%$ to $6.8\% \pm 0.7\%$, $P < 0.001$ in the combination therapy group, and $7.7\% \pm 0.5\%$ to $6.7\% \pm 0.5\%$, $P < 0.001$ in the alogliptin therapy group) and FPG (152.0 ± 35.6 to 127.3 ± 27.4 mg/dL, $P < 0.001$ in the combination therapy group, and 153.6 ± 34.4 to 128.5 ± 26.6 mg/dL, $P = 0.001$ in the alogliptin therapy group), and a significant increase in HOMA- β were noted in both groups. However, significant differences were not obtained in the changes in HbA1c, FPG, and HOMA- β by therapy between the combination and the alogliptin mono therapy group ($P = 0.2945$, $P = 1901$, $P = 0.3042$, respectively). The levels of serum gastrin in the concomitant group was significantly elevated compared with those in the alogliptin mono therapy group ($P = 0.0004$). With the combination therapy, the serum gastrin levels increased approximately two-fold. Apart from the issue of the period of the administration, one of the possible reasons for these negative results may be due to the use of DPP-4 inhibitors rather than a GLP-1 receptor agonist with the PPI. The elevation of GLP-1 levels by DPP-4 inhibitors is relatively small compared with that observed with the GLP-1 receptor agonist. Therefore, despite the reports with the positive results on glycemic control using a combination of a PPI and DPP-4 inhibitors^[67,69], the effect may be small when compared to that observed with the combination of a PPI and a GLP-1 receptor agonist. The clinical data on the combination therapy of a PPI and a GLP-1 receptor agonist in patients with T1DM and T2DM are not available yet, but this therapy appears to be an attractive one, and future studies are warranted to confirm the effect of this combination therapy.

CONCLUSION

Although PPI therapy is attractive as a new approach for the therapy of diabetes (especially T2DM), the clinical effect on glycemic control of this drug is not yet fully established. The mechanisms of the clinical effect of PPIs on glycemic control are also not fully elucidated. A prospective, long term, randomized, double-blind,

placebo-controlled study on PPIs in a larger number of the T2DM patients is warranted to confirm the effect of PPIs on glycemic control, especially in patients with relatively poor glycemic control. The combination therapy of a PPI with a GLP-1 receptor agonist (rather than DPP-4 inhibitors) may improve glycemic control in both T1DM and T2DM. A clinical study with a large number of patients is needed to establish the potential efficacy. At present, the clinicians' concerns are whether the patients can have better glycemic control when PPIs are used for GERD or gastric ulcers in patients with T2DM, because the use of PPIs is not yet allowed for T2DM treatment in every country. If the treatment is for a long-term period, it is also important to consider the possible harmful effects of PPIs, including bone fracture^[73] and small intestine bacterial overgrowth^[74].

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Dysglycemia after renal transplantation: Definition, pathogenesis, outcomes and implications for management

David Langsford, Karen Dwyer

David Langsford, Karen Dwyer, Department of Nephrology, St Vincent's Hospital Melbourne, Fitzroy 3065, Australia

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Correspondence to: Dr. Karen Dwyer, Department of Nephrology, St Vincent's Hospital Melbourne, 59 Victoria Parade, Fitzroy 3065, Australia. karen.dwyer@svhm.org.au
 Telephone: +61-3-92883112
 Fax: +61-3-92313151

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Abstract

New-onset diabetes after transplantation (NODAT) is major complication following renal transplantation. It commonly develops within 3-6 mo post-transplantation. The development of NODAT is associated with significant increase in risk of major cardiovascular events and cardiovascular death. Other dysglycemic states, such as impaired glucose tolerance are also associated

with increasing risk of cardiovascular events. The pathogenesis of these dysglycemic states is complex. Older recipient age is a consistent major risk factor and the impact of calcineurin inhibitors and glucocorticoids has been well described. Glucocorticoids likely cause insulin resistance and calcineurin inhibitors likely cause β -cell toxicity. The impact of transplantation in incretin hormones remains to be clarified. The oral glucose tolerance test remains the best diagnostic test but other tests may be validated as screening tests. Possibly, NODAT can be prevented by administering insulin early in patients identified as high risk for NODAT. Once NODAT has been diagnosed altering immunosuppression may be acceptable, but creates the difficulty of balancing immunological with metabolic risk. With regard to hypoglycemic use, metformin may be the best option. Further research is needed to better understand the pathogenesis, identify high risk patients and to improve management options given the significant increased risk of major cardiovascular events and death.

Key words: Management; Epidemiology; Pathogenesis; Renal transplantation; Diabetes

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Core tip: New-onset diabetes after transplantation (NODAT) carries a significant cardiovascular burden. Its pathogenesis is multifactorial and includes modifiable factors. New insights into glucose and insulin homeostasis may lead to improved ability to identify high risk patients and to the development of management strategies that do not require alteration in immunosuppression, whilst simultaneously reducing the risk of NODAT.

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INTRODUCTION

Dysglycemia post renal transplantation, encompassing new onset diabetes after transplant (NODAT), impaired fasting glucose (IGF) and impaired glucose tolerance (IGT), is a challenging clinical problem. However, despite more than two decades of research the pathogenesis of post-transplant dysglycemia is incompletely understood and a consensus on approach to screening, diagnosis and management is lacking. This review will outline the issues of defining the clinically important states, detecting and predicting their development, the progress that has been made in understanding their pathogenesis and relationship to described risk factors (particularly immunosuppression therapies) and the implications for management and further research into this significant post-transplant complication.

DEFINITION

There have been several changes in the definition of dysglycemia post transplantation over time. Initially referred to as diabetes after renal transplantation, this name failed to capture the important distinction of those who were diabetic pre-transplant from those who developed diabetes after transplant. The term post-transplant diabetes mellitus (PTDM) also failed to clearly distinguish between the two states. The most common term currently used is new-onset diabetes after transplant (NODAT); however, this too fails to capture those with new onset IGT, which is also associated with poorer outcomes (see below). Some have proposed the term "transplant associated hyperglycemia"^[1], which captures the impact of dysglycemia, as opposed to the worst category of dysglycemia alone (diabetes), however it does not make a distinction between those who came to transplant with a dysglycemic state and those who developed it after transplantation.

Prior to 2003 the most common criteria used for the diagnosis of post-transplant diabetes was use of hypoglycemic agents. However, this is reliant upon clinician awareness of the results of appropriately timed and collected glucose testing and remains an insensitive marker of NODAT. With enhanced understanding of the pathophysiology of post-transplant dysglycemia and its clinical significance a more sensitive and clinical useful definition is needed. In 2003 an international expert panel devised a consensus document^[2] that adopted the World Health Organisation/American Diabetes Association (WHO/ADA) guidelines for the testing and defining of dysglycemic states post-transplant [fasting blood glucose level (F BGL) ≥ 7.0 mol/L; 2-h BGL ≥ 11.1 mmol/L], based on the definitions used for the

general population. However, whilst there is consensus on the interpretation of blood glucose levels, there is no consensus on who to test, when to test and which test to use. Table 1 shows the wide range of tests used and timing of these in studies that have reported NODAT outcomes: F BGL, random blood glucose level (R BGL), 2-h 75 g oral glucose tolerance test (oGTT), HbA1c at 10 wk, 3 mo, 6 mo, 1 year and use of hypoglycemic agents at 30 d. Furthermore, there is little recognition in the literature of the importance of reporting and understanding the significance of dysglycemic states other than NODAT such as IGT or IFG. Few studies report incident rates and/or outcomes of such dysglycemic states. As a result, drawing conclusions based on research in this area has unavoidable caveats, which can only be addressed by large multi-centred well designed trials with post-transplant dysglycemia as the primary outcome.

EPIDEMIOLOGY

One of the confounders in any study of NODAT is the rate of pre-transplant unrecognised dysglycemia. Table 2 shows the rates of unrecognised dysglycemia in patients on the transplant waiting list. Bergrem *et al.*^[30] investigated 889 Norwegian transplant wait listed candidates who were not clinically suspected to have diabetes. The majority of patients (62%) were not on dialysis and only 12% were on glucocorticoids. All patients underwent an oGTT. Using WHO/ADA diagnostic criteria, 330 (37.1%) patients were found to have dysglycemia, in addition to which, 72 (8.1%) were found to have diabetes. Importantly, of those patients found to be diabetic on oGTT, only 22% were identified by F BGL testing alone. Further receiver operating curve (ROC) analysis demonstrated that using a cut-off of 92 mg/dL (5.1 mmol/L) for F BGL testing as the threshold for initiating an oGTT detected 90% of the diabetic patients, requiring 53% of the wait listed patients to be tested.

It is interesting to note that not all patients with dysglycemia pre-transplant develop persistent post-transplant dysglycemia (IGF, IGT or NODAT). Caillard *et al.*^[31] screened 243 patients at time of wait listing with oGTT and found 37 (15.2%) dysglycemic patients and eight (3.3%) newly diagnosed diabetic patients. The time from pre-transplant oGTT to transplantation was not documented; however, 50% of the dysglycemic patients developed NODAT, 23% remained dysglycemic and 14% become normoglycemic post transplantation. In 26% of those diagnosed with NODAT, this abnormality could only be detected by oGTT. A Japanese study in which patients with no known history of diabetes were administered an oGTT two weeks before receipt of a living donor transplant, found that 30.4% were dysglycemic with an additional 4.0% found to be diabetic^[32]. Hornum *et al.*^[33] found 33% dysglycemia rate pre-transplant ($n = 57$) and over 12-mo follow up the pre-transplant dysglycemia was not associated with the development of NODAT. Interestingly, they too

Table 1 Selection of studies that reported rates of new-onset diabetes after transplantation or other dysglycemic states

Ref.	Criteria	n	Rates
Cosio <i>et al</i> ^[5]	Use of medications, F BGL	490	13% at 1 yr 33% dysglycemic
Hjeltnes <i>et al</i> ^[4]	Use of medications, F BGL, oGTT	201	20% at 3 mo
Vincenti <i>et al</i> ^[5]	oGTT	682	30% at 6 mo dysglycemic
Delgado <i>et al</i> ^[6]	oGTT, F BGL	374	6.7% at 4.1 yr 25.1% dysglycemic
Ramesh Prasad <i>et al</i> ^[7]	F BGL or R BGL	151	20.5%
Luan <i>et al</i> ^[8]	oGTT	203	11.8% at 10 wk 47.8% dysglycemic
Bayer <i>et al</i> ^[9]	Use of medications, F BGL, R BGL	640	31.4% at 1 yr
Bergrem <i>et al</i> ^[10]	Use of medications, F BGL, R BGL	301	13% at 10 wk
Valderhaug <i>et al</i> ^[11]	oGTT	1410	17% at 10 wk 38% dysglycemic
Ciancio <i>et al</i> ^[12]	Use of medications	150	15%-22% at 4 yr
Israni <i>et al</i> ^[13]	Medications, F BGL	1840	13% at 5 yr
Wauters <i>et al</i> ^[14]	Use of medications, F BGL	1146	14.1% at 1 mo, 11.1% at 4 mo, 13.4% at 1 yr 27%, 34.3% and 29.8% dysglycemic
Chan <i>et al</i> ^[15]	oGTT	292	24% at 6 mo
Vacher-Coponat <i>et al</i> ^[16]	Use of medications	289	16.8%-18.8% at 3 yr
Tillman <i>et al</i> ^[17]	oGTT	200	5% at 39 mo 30.5% dysglycemic
Bonet <i>et al</i> ^[18]	F BGL, R BGL, oGTT	138	13% at 6 mo
Cole <i>et al</i> ^[19]	Use of medications, F BGL, oGTT	49	4% at 6 mo
Nagaraja <i>et al</i> ^[20]	Use of medications, F BGL	118	21% at 3 mo, 37% at 1 yr
First <i>et al</i> ^[21]	Use of medications, F BGL, HbA1c	634	17.8%-36.5% at 1 yr
Nagaraja <i>et al</i> ^[22]	oGTT	76	13% at 5 yr, 24% at 11 yr 42% and 61% dysglycemic
Tokodai <i>et al</i> ^[23]	Use of medications, F BGL, R BGL	145	11.7% at 1 yr
Viecelli <i>et al</i> ^[24]	oGTT	83	17% at 3 mo, 15% at 15 mo 31% and 21% dysglycemic
Weng <i>et al</i> ^[25]	Use of medications, F BGL, R BGL	166	29.5%
Schweer <i>et al</i> ^[26]	R BGL, HbA1c	526	16.7%
Prasad <i>et al</i> ^[27]	oGTT	439	20% at 3 mo 33% dysglycemic
Silva <i>et al</i> ^[28]	HbA1c	638	21.3%-41.1% at 4 yr
Lv <i>et al</i> ^[29]	F BGL	428	20.3% at 5.7 yr

Definitions diabetes: F BGL ≥ 7.0 mmol/L (126 mg/dL) or ≥ 11.1 mmol/L (200 mg/dL) on oGTT or R BGL ≥ 11.1 mmol/L (200 mg/dL) plus symptoms. Other dysglycemic states. IFG: ADA criteria 5.6-6.9 mmol/L (100-125 mg/dL); WHO criteria 6.1-6.9 mmol/L (100-125 mg/dL); IGT: oGTT 7.8-11.0 mmol/L (140-199 mg/dL). F BGL: Fasting blood glucose level; R BGL: Random blood glucose level; oGTT: 2-h oral glucose tolerance test.

documented a small group of pre-transplant diabetic patients in whom the diabetic state remitted post-transplant.

The case finding described by table two highlights key differences in glucose homeostasis between end stage kidney disease (ESKD) uremic patients and the general population. Approximately 70% of general population patients can be diagnosed as diabetic *via* a F BGL^[34], as compared to 22% in the Norwegian transplant wait listed cohort. Moreover, the incidence of new diagnosis of diabetes in wait listed patients on dialysis is approximately 5%-6% per year^[33,35] (when using oGTT diagnostic criteria), compared with approximately 0.7%-1.3% per year in the general population^[36]. These figures ought to give the reader cause to be cautious with regard to the interpretation of rates of post transplantation dysglycemia and diabetes. This is particularly the case when reviewing retrospective data, in which often only a pre-transplant F BGL is available and the time from glucose testing to transplantation may extend for many months. It

may be that the denominator in the quoted rates of NODAT includes patients who were not normoglycemic at time of transplantation. This assessment is further complicated by the possibility that dysglycemia pre-transplant may not be a sufficient factor for dysglycemia post-transplant state (see below).

Further complicating the interpretation of incident rates of dysglycemia post-transplant is the spontaneous remission and normalisation of blood glucose levels observed in some patients. For example, early dysglycemia, such as in the period of hospitalisation post-transplant, is common and occurs in 75%-90% of patients within the first week^[37-39]. Luan *et al*^[8] in a prospective study of 203 non-diabetic patients showed the mean day 3 F BGL to be 124-134 mg/dL (6.9-7.4 mmol/L). Such dysglycemia should not be dismissed as due entirely to peri-operative factors, as some data suggests that day 7 F BGL may be predictive of NODAT at 1 year^[40]. A recent clinical study measured continuous capillary blood glucose levels for the first 4 d post-transplant in 43 patients. There was a considerable

Table 2 The rates of unrecognized dysglycemia in patients on the transplant waiting list

Ref.	Unrecognised on waiting list - diabetes	Unrecognised on waiting list - dysglycemia
Ramesh Prasad <i>et al</i> ^[7]	-	15%
Hornum <i>et al</i> ^[33]	-	33%
Bergrem <i>et al</i> ^[30]	8.1%	45.2%
Iida <i>et al</i> ^[32]	4%	30.4%
Caillard <i>et al</i> ^[31]	3.3%	15.2%
Bonet <i>et al</i> ^[18]	< 0.1%	8.9%

burden on hyperglycemia with 43% having blood glucose above 7.7 mmol/L for more than 12 h per day. The incidence of NODAT at 72 mo was 18.6% and the authors suggested that the day 1 capillary blood glucose may identify those at risk^[41]. Moreover, one study found that only 4% of patients normoglycemic early post-transplant later developed NODAT^[42] and a normal oGTT within the first week has been shown to have a NPV of 97.6% for later NODAT development^[43]. However, it is important to note that not all patients with early hyperglycemia develop permanent dysglycemic states, as there is a considerable degree of transience and variation in dysglycemic states^[33]. For example, a Chinese study, employing F BGL for NODAT found an incident rate of 20.32% after a mean follow up of 5.65 years in patients who survived more than one year post transplantation. Of these, 65.5% developed NODAT within 1 year and 17.2% had transient NODAT^[29]. Furthermore, such transience likely occurs within the first 3-6 mo. In an international trial comparing standard and reduced dose tacrolimus (Tac) the cumulative incidence at 6 mo of NODAT was 30.3%; however, the incidence in each group was lower at 6 mo compared to 3 mo (23.9% vs 28.4% and 13.2% vs 15.2%)^[15].

Notwithstanding the notable degree of transient dysglycemia, persistent NODAT often develops within 3 to 6 mo following renal transplantation. A mean time to diagnosis of 4.3 mo has been reported^[44]. This may help to determine the optimal time of testing. Using oGTT testing at 10 wk post transplantation, Valderhaug *et al*^[11,45] reported an incidence of NODAT of 14%-17%. Most studies find that NODAT develops early and this is confirmed by analyses of large data sets. For instance, an analysis of the organ procurement and transplantation network (OPTN) registry data has found a cumulative incidence of NODAT of only 16.2% at 3 years (registry data is limited by the nature of reporting of outcomes), the majority had developed within the first year post transplantation^[46]. Similar results have been reported in a United States cohort of 640 patients with a mean F BGL of less than 100 mg/dL (5.6 mmol/L) at time of transplantation. NODAT occurred in 31.4% of patients over 1 year, the majority of which had occurred within the first 6 mo (26.4% of total population by 6 mo). By 5 years post transplantation, 46.3% of previously believed to be non-diabetic patients had a diagnosis of NODAT^[9].

With regard to any dysglycemia (IGT/IFG or NODAT), a moderate sized ($n = 203$) prospective study of the risk of developing dysglycemia post transplantation, documented a rate of 47.8% when tested at 10 wk with an oGTT and applying WHO/ADA diagnostic criteria^[8]. Retrospective data has found rates of 39.7% who remained normoglycemic throughout the first year post-transplant^[47]. A study specifically designed to determine the rates of pre-diabetic dysglycemia found 30.5% of patients met accepted criteria using an oGTT at a median of 39 mo post-transplant^[17]. Similarly, in a large international study designed to determine the differences in diabetogenesis of cyclosporin (CsA) and Tac, at 6 mo post-transplant only 300 out of 587 patients (51.1%) remained normoglycemic^[5]; however, the criteria for definition of NODAT was need for medications at greater than 30 d. A cross sectional study of multiple Spanish centres found a rate of dysglycemia of 31.8% at almost 4 years post-transplant, the majority detected by oGTT^[6]. It is interesting to note that 58.8% of the dysglycemic patients had a simultaneous normal F BGL.

The above discussions reveal notable limitations when quoting rates of post-transplant dysglycemic states or NODAT alone. Whilst there is consensus with regard to blood glucose cut-off values, it is unclear which test should be employed and at which time post-transplant. Furthermore, the witnessed remission of some pre-transplant dysglycemia to normoglycemia post-transplant^[19,37] (although this has not been commonly documented), further complicates analyses of rates of new-onset post-transplant dysglycemia.

RISK FACTORS

Multiple risk factors have been associated with the development of NODAT (Table 3) many of which are not modifiable. The most consistently found risk factor is advancing age appreciated since the recognition of NODAT in the early period of use of CsA^[79]. Increasing age has been found to be a risk factor in small and large retrospectively analysed and prospectively collected data sets, including registry datasets in which the prevalence of NODAT may have been underestimated^[8,13,17,26,46,49,52-54]. Male gender, family history of diabetes and APCKD are documented as risk factors, but not consistently^[46,49,54-57,61,62]. With regard to genetic risk multiple polymorphisms, including mitochondrial, have been described as contributing risk to the development of NODAT^[53,54,63-67]. A closer analysis of genetic polymorphisms and their associated risk is beyond the scope of this review.

Transplant related factors: Calcineurin inhibitors

Potentially modifiable risk factors can be divided into transplant specific and generic. Of the generic, increasing body mass index (BMI) is associated with increased incidence of NODAT when categorised into intervals of 5 with < 20 as a reference, with increased

Table 3 Modifiable and non-modifiable risk factors associated with new-onset diabetes after transplantation or dysglycemic state

Variable	Ref.	Comment
ATG-divided dose	Stevens <i>et al</i> ^[48]	Increased dysglycemia compared to single dose in patients treated with Tac and sirolimus
African American	Kasiske <i>et al</i> ^[49]	OR = 1.68
	Shah <i>et al</i> ^[50]	RR = 1.38
	Johnston <i>et al</i> ^[51]	HR = 1.56
	Bayer <i>et al</i> ^[9]	HR = 1.35
Age	Kasiske <i>et al</i> ^[49]	Strong independent risk factor RR: 1.9-2.6
	Cole <i>et al</i> ^[52]	27707 registry patients OR: 1.33 If > 60 yr
	Ghisdal <i>et al</i> ^[53]	OR 1.03 of NODAT for each 6 mo of age
	Luan <i>et al</i> ^[8]	Increasing age associated with dysglycemia and new onset metabolic syndrome
	Luan <i>et al</i> ^[46]	Analysis of 25837 registry patients, increase in NODAT in each categorised group compared to reference 18-34 years old
	Israni <i>et al</i> ^[13]	HR: 1.33 of NODAT at 60 mo
	Tillmann <i>et al</i> ^[17]	Increase in dysglycemia at mean of 56 M post-transplant; RR of 1.28 for each 5 yr
	McCaughan <i>et al</i> ^[54]	OR 1.4 per decade in 427 Northern Irish patients
	Schweer <i>et al</i> ^[26]	NODAT 56.1 yr <i>vs</i> 47.9 yr; <i>P</i> < 0.01
APCKD	de Mattos <i>et al</i> ^[55]	Increased 1 yr incidence in a matched cohort
	Hamer <i>et al</i> ^[56]	Multivariate analysis OR 2.4
	Johnston <i>et al</i> ^[51]	No increase found in 21564 USRDS patients
	Luan <i>et al</i> ^[46]	Multivariate analysis OR: 1.17
	Ruderman <i>et al</i> ^[57]	No increased risk found
Basiliximab	Aasebø <i>et al</i> ^[58]	Basiliximab (<i>n</i> = 134) <i>vs</i> no induction historical control; increased dysglycemic state <i>P</i> = 0.017
	Prasad <i>et al</i> ^[27]	In living recipients who elected to receive basiliximab OR 2.34 for NODAT at 3 mo
BMI	Kasiske <i>et al</i> ^[49]	Increased BMI, NODAT RR: 1.7
	Cole <i>et al</i> ^[52]	Multivariate analysis OR 1.76 for NODAT
	Luan <i>et al</i> ^[46]	Analysis of 25837 registry patients. increase in NODAT in each categorised group of BMI compared to reference < 20
	Israni <i>et al</i> ^[13]	BMI ≥ 30, HR 1.69 for NODAT at 60 mo
CMV	Hjelmsaeth <i>et al</i> ^[59]	Asymptomatic infection OR: 4.0 for NODAT at 10 wk
CNI -	Chan <i>et al</i> ^[15]	NODAT 17% <i>vs</i> 31%, low dose <i>vs</i> standard dose Tac
Higher levels	Cole <i>et al</i> ^[19]	Single arm study of 49 patients with a 4% 6 mo incidence of NODAT. Early glucocorticoid reduction and low dose CsA
	Suszyński <i>et al</i> ^[60]	Higher Tac levels (plus sirolimus) compared to lower Tac (plus sirolimus) or CsA/MMF higher rates of NODAT with 10 yr FU
CNI -	Vincenti <i>et al</i> ^[5]	RCT. Dysglycemia at 6 mo higher in Tac/MMF <i>vs</i> CsA/MMF: <i>P</i> = 0.05
Tac <i>vs</i> CsA	Cole <i>et al</i> ^[52]	27707 registry patients OR 1.51 for NODAT
	Luan <i>et al</i> ^[46]	Analysis of 25837 registry patients. Increase in NODAT OR: 1.24
	Vacher-Coponat <i>et al</i> ^[116]	No difference in CsA/Aza <i>vs</i> Tac/MMF in RCT (<i>n</i> = 289)
	Cotovio <i>et al</i> ^[44]	Retrospective multivariate analysis higher Tac not CsA levels associated with NODAT
Family history of diabetes	Bora <i>et al</i> ^[61]	Recipients from living related donors
	Santos <i>et al</i> ^[62]	Retrospective (<i>n</i> = 303). RR: 3.6 for NODAT
Gender	Kasiske <i>et al</i> ^[49]	Greater risk in males in registry patients
	McCaughan <i>et al</i> ^[54]	OR 2.2 for male gender in 427 Northern Irish patients
Genetic polymorphisms	Ghisdal <i>et al</i> ^[53]	rs7903146 polymorphism of TCF7L2 OR 1.6 of NODAT at 6 mol/L, but not associated with IGT
	Ghisdal <i>et al</i> ^[63]	Summarises known associations
	Kurzwaski <i>et al</i> ^[64]	Polish Caucasian patients. Increasing SNPs associated with increased risk, OR = 1.37
	Yao <i>et al</i> ^[65]	Fok1 vitamin D polymorphism associated with NODAT OR 11.8 <i>P</i> = 0.012
	McCaughan <i>et al</i> ^[54]	7 SNPs involved with β-cell apoptosis associated with NODAT
	Nicoletto <i>et al</i> ^[66]	Adiponectin gene polymorphism associated with NODAT
	Tavira <i>et al</i> ^[67]	Mitochondrial haplogroup H associated with NODAT in Tac treated patients
Glucocorticoids	Boots <i>et al</i> ^[68]	Early glucocorticoid withdrawal associated with reduced NODAT incidence in the first year
	Ghisdal <i>et al</i> ^[53]	OR 2.78 of NODAT at 6 mol/L if AR treated with glucocorticoids
	Luan <i>et al</i> ^[46]	Analysis of 25837 registry patients. OR 1.42 for NODAT if discharged on maintenance.
	Rizzari <i>et al</i> ^[69]	Glucocorticoid only induction associated with increase in NODAT OR: 1.31
	Cole <i>et al</i> ^[19]	Significant reduction in NODAT compared with historical control when glucocorticoids rapidly tapered
	Schweer <i>et al</i> ^[26]	Single arm study of 49 patients with a 4% 6 mo incidence of NODAT. Early glucocorticoid reduction and low dose CsA
HCV +	Kasiske <i>et al</i> ^[49]	Pulse glucocorticoid for BPAR associated with increasing NODAT incidence
	Cole <i>et al</i> ^[52]	HCV+, NODAT RR: 1.3
	Johnston <i>et al</i> ^[51]	27707 registry patients OR for NODAT 1.82
	Baid-Agrawal <i>et al</i> ^[70]	21564 USRDS registry patients, HR: 1.7 for NODAT
	Luan <i>et al</i> ^[46]	14 HCV+ 24 HCV- patients. HCV+ increased insulin resistance; <i>P</i> = 0.008
	Lv <i>et al</i> ^[29]	Analysis of 25837 registry patients. Increase in NODAT OR: 1.43
	Prasad <i>et al</i> ^[27]	Cohort of 428 Chinese patients. NODAT associated with HCV at mean 5.6 yr follow up, OR = 2.72
		439 Indian patients, OR = 6.37

Hyper-parathyroidism post transplant	Ivarsson <i>et al</i> ^[71]	PTH > 13.8 pmol/L associated with NODAT at 1 yr, OR = 4.25
Impaired glycemic state pre-transplant	Ramesh Prasad <i>et al</i> ^[7] Bora <i>et al</i> ^[61] Hornum <i>et al</i> ^[33] Cotovio <i>et al</i> ^[44] Garg <i>et al</i> ^[72]	Higher within the normal range random BSL associated with NODAT IGT at time of transplant associated with NODAT IGT NOT predictive of NODAT Higher fasting BGL associated with NODAT 1 mol/L lower Mg associated with dysglycemia; no association with 1M CNI trough level
Magnesium post-transplant		
Magnesium pre-transplant	Augusto <i>et al</i> ^[73]	Lower magnesium immediately pre-transplant associated with NODAT; $P < 0.02$
Metabolic syndrome post-transplant	Israni <i>et al</i> ^[13] Luan <i>et al</i> ^[8] Nagaraja <i>et al</i> ^[22] Bayer <i>et al</i> ^[9]	MS in first 6-12 mo associated with NODAT by 60 mo, HR = 3.46 10 W dysglycemia associated with MS Development of MS predicts progressive dysglycemia HR: 1.34 for NODAT at 1 yr
Metabolic syndrome pre-transplant		
Sirolimus	Teutonico <i>et al</i> ^[74] Ekberg <i>et al</i> ^[75] Johnston <i>et al</i> ^[51] Guerra <i>et al</i> ^[76] Gyurus <i>et al</i> ^[77] Veroux <i>et al</i> ^[78] Suszynski <i>et al</i> ^[60]	No improvement when changing from CNI to sirolimus Low dose sirolimus may confer less risk than low dose Tac 20124 registry patients. Compared to CsA + MMF/AZA: Sirolimus + CsA HR 1.61; Sirolimus + Tac HR 1.66; Sirolimus + MMF/AZA HR 1.36 RCT ($n = 150$) Tac/sirolimus <i>vs</i> Tac/MMF <i>vs</i> CsA/sirolimus. No difference in NODAT Retrospective ($n = 514$). Sirolimus HR 3.5 for NODAT over 10 yr 21 NODAT converted to sirolimus, 80% remission of NODAT on basis of F BGL Increased risk with high dose Tac/low dose sirolimus combination

F BGL: Fasting blood glucose level; oGTT: 2-h oral glucose tolerance test; NODAT: New-onset diabetes after transplantation; ATG: Antithymocyte globulin; USRDS: United States Renal Data System; BMI: Body mass index; CMV: Cytomegalovirus; CNI: Calcineurin inhibitors; Tac: Tacrolimus; MMF: Mycophenolate mofetil.

risk in the higher categories of BMI^[47]. The most significant transplant specific modifiable risk factors are immunosuppressive medications specifically the use of calcineurin inhibitors (CNI - Tac and CsA) and glucocorticoids. The diabetogenic impact of CsA has been described since the early 1980s^[79-82]. The introduction of Tac into clinical practice was associated with less acute rejection and improved graft function but at the expense of a greater incidence of NODAT^[83]. The diabetes incidence after renal transplantation trial was first large randomised study ($n = 682$; not diabetic at baseline $n = 567$) designed primarily to investigate the increase risk posed by Tac use instead of CsA. The primary endpoint was a 6-mo composite endpoint of dysglycemia (NODAT or IFG) based on oGTT administered at 90 and 180 d. They found 6-mo cumulative incidence of 33.6% in Tac treated patients and 26% in CsA treated patients ($P = 0.046$). Furthermore, more patients required hypoglycemic treatment in the Tac treated group ($P = 0.005$) and more patients in the CsA treated group who were not treated with hypoglycemic agents had an improvement in their glycemic state by 6 mo ($P = 0.067$)^[5]. This, however, was in the era of high trough Tac targets of approximately 10-15 in the first 3 mo.

Noting that over time target drug levels have decreased, the use of therapeutic drug monitoring may assist in the management of prevention of rejection and complications of immunosuppression. There is some evidence that dysglycemic states are related the degree of CNI exposure. For example, Chan *et al*^[15] randomised 292 patients to low dose Tac (trough level 5-9 for first 3 mo then trough level 3-6 following 3 mo) or standard dose (trough level 10-15 for first 3 mo then trough level 8-12 following 3 mo). All patients received basiliximab,

similar doses of MMF and glucocorticoids over the follow up period of 6 mo. Those in the low dose Tac group had significantly less NODAT incidence over 6 mo of follow up, with a tendency towards lower incidence rate of treated diabetes^[15]. Similarly the dose response effect with respect to NODAT risk has also been described with the use of CsA with less dysglycemia post-transplant in those treated with low dose CsA (C2 600-800)^[19]. Sub-analyses of data from larger trials, such as Efficacy Limiting Toxicity Elimination-SYMPHONY, have also suggested a dose-dependent relationship. SYMPHONY found significantly higher rates of NODAT in the low-dose Tac group, compared with low-dose CsA, low-dose sirolimus or standard dose CsA without induction agent ($P = 0.02$)^[75]. Given the issues with choice of diagnostic test it is not surprising that when analysed according to F BGL there were no significant differences between the groups^[84].

As age is commonly identified as a risk factor in univariate analysis, it is important to know if older age interacts with other risk factors. In a multivariate analysis of OPTN data there is a clear increase in risk with increasing age when grouped into age groups using 18-34 years old as a reference group^[46]. Amongst the other identified risk factors use of Tac increased risk of NODAT. An analysis of the OPTN registry data compared rates of acute rejection and rates of NODAT and their impacts of graft survival. The rates of acute rejection were less in the older Tac treated patients, but the rates of NODAT were greater in the same older Tac treated group^[51]. The authors comment that targeted and individualised use of immunosuppression based on the patient's risk profile may help to ameliorate worse outcomes. Part of this may be to reconsider the use of CNI, in particular Tac, in the older recipient in whom the

development of NODAT may precipitate morbidity and mortality. However, as outlined below, other strategies may be safer and more effective.

Transplant related factors: Glucocorticoids

Oral glucocorticoids form the backbone of many immunosuppressive regimens and the diabetogenic potential of these agents is well documented. The development of diabetes is related to the cumulative exposure to glucocorticoids. The data available on glucocorticoid withdrawal, glucocorticoid free or rapid glucocorticoid tapering suggests an incidence rate of 1%-22% over a 1-5 year follow-up period^[12,19,26,46,60,69,85] which compares with rates of 15%-35% in regimens without glucocorticoid maintenance (Table 1). However, not all analyses find a benefit in glucocorticoids avoidance. For example, a meta-analysis of higher quality trials in which patients had glucocorticoid withdrawn within 14 d post-transplant and were treated with CNI/MMF did not find a reduction in NODAT^[86]. However, the largest randomised placebo-controlled trial ($n = 386$) of early glucocorticoid withdrawal within 7 d of transplantation found no difference in the rate of NODAT, although fewer of the NODAT patients required insulin therapy in the early glucocorticoid withdrawal arm^[83]. Furthermore, a matched cohort analysis of glucocorticoid free and maintenance therapy with glucocorticoid ($n = 190$ in each group) there were no differences in renal specific outcomes or any differences between F BGL or use of hypoglycemic agents. It is noteworthy that there was significantly more use of Tac and basiliximab in the glucocorticoid free group^[85]. Nonetheless, many other studies do find an advantage to glucocorticoid avoidance. Analysis of United States Renal Data System (USRDS) data found that patients discharged on a glucocorticoid containing regimen had an OR of 1.42 for NODAT compared to those discharged on a glucocorticoid free regimen^[46]. These results must be interpreted with caution, as it is not possible to capture the cumulative glucocorticoid exposure in the USRDS database. One small ($n = 62$) randomised prospective study in which glucocorticoids were ceased in one group by day 10 found a significant decrease in the incidence of NODAT when defined as used of hypoglycemic agents^[68]. A more recent pilot study ($n = 48$) of thymoglobulin induction, MMF, low dose CsA and rapid glucocorticoid reduction in low immunological risk patients found that this protocol resulted in 42 of 48 patients being normoglycemic at 6 mo^[19]. A larger single centre population ($n = 1291$) retrospectively analysed in which NODAT was defined as need for hypoglycemic agents found an incidence rate of only 2%-4% in the first year post transplantation in patients treated with glucocorticoid withdrawal after day 5 post-operative in combination with thymoglobulin induction, CNI plus sirolimus or MMF^[69]. This was a significant improvement compared to a non-matched historical control group who received a glucocorticoid containing maintenance regimen. Despite the theoretical

benefits of glucocorticoid withdrawal the studies referenced above demonstrate conflicting results^[87]. The impact of glucocorticoid exposure on the development of NODAT may be answered by a current trial in which patients of low immunological risk will be randomised to one arm including thymoglobulin induction and glucocorticoid free CNI/MMF maintenance or basiliximab induction and ongoing glucocorticoid exposure^[88].

The development of dysglycemia subsequent to the diagnosis and treatment of acute rejection may also disclose the risk of dysglycemia created by glucocorticoid exposure. A single centre review of 526 transplant recipients had a NODAT incidence of 16.7% when defined using ADA/WHO criteria for assessing random blood glucose or HbA1c. They found that there was a greater incidence of acute rejection in patients who developed NODAT and that intensified treatment with glucocorticoid and possible conversion to Tac was associated with increased risk of NODAT on multivariate analysis. However, the analysis did not treat rejection as a time varying co-variate^[26].

Transplant related factors: Sirolimus

Sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, is an immunosuppressive agent used in conjunction with, or instead of, calcineurin inhibitors. Clinical data suggests that sirolimus use is not without risk for the development of NODAT^[77]. Analysis of USRDS of 2598 patients recorded as having received sirolimus, found that the combination of sirolimus with a CNI created a higher HR for cumulative 1yr incidence of NODAT compared to CNI with mycophenolate/azathioprine (MMF/AZA) or sirolimus with MMF/AZA. A sub-group multivariate analysis of USRDS data of 16861 patients known to have remained on the same immunosuppressant regimen patients treated with the combination of sirolimus and a CNI remained at increased risk of 1 year NODAT^[51]. In one study of non-NODAT renal transplant recipients who were switched from CNI to sirolimus there were no improvements noted in the glycemic state of the patients when studied robustly with oGTT. Indeed higher sirolimus levels in the absence of CNI may have increased the risk of NODAT^[74].

However, just as with the data on CNI and glucocorticoids there are inconsistent findings in the literature on sirolimus. A recent large ($n = 440$) prospectively randomised trial found that higher dose Tac, but not high or standard dose sirolimus contributed to the NODAT^[60]. A further example is a recent study of patients randomised to tacrolimus/mycophenolate, Tac/sirolimus or CsA/sirolimus. The median follow up was 8 years and the quoted cumulative incidence of NODAT was 19%-32%, with no significant differences between the groups based on the use of hypoglycemic agents^[76]. Lastly, as with CNI, it is likely that there is an important interaction between modifiable and non-modifiable risk factors. For example, a multivariate

analysis has found that older age and higher sirolimus trough levels were associated with increased hazard for NODAT^[77], once again suggesting that drug level targets in older recipients could be reviewed, for both effect and toxicity.

Transplant related factors: Other medications

Calcineurin inhibitors and glucocorticoids are the most well studied drugs in terms of impact upon glycemic control. There is no data on the contribution of MMF or AZA to the development of dysglycemia. In the transplant literature, there does not appear to be a signal that these drugs may be implicated. Recently, there has been interest in the possibility that basiliximab, a widely used induction agent particularly in the lower immunological risk patients, may be implicated in contributing to dysglycemia; although this is based on two data sets, neither of which were prospective or randomized^[27,58]. There is also little data on the contribution of thymoglobulin to the development of NODAT. A study of single dose vs divided dose antithymocyte globulin (ATG) induction analysed dysglycemia as a secondary outcome. In this study, fasting blood sugar levels after 1 mo to 6 mo were significantly lower ($P = 0.02$) in patients who received single dose ATG induction^[48].

PATHOGENESIS

The pathogenesis of dysglycemia post transplantation is complex and is widely assumed to be closely aligned to the pathogenesis of type 2 diabetes mellitus. However, this assumption underestimates that the impact of end stage renal failure and dialysis on glucose homeostasis. There is also little known about the histological changes in the graft over time when exposed to persistent NODAT. Small case series have found *de novo* diabetic nephropathy within 5-10 years of diagnosis of NODAT^[89,90].

Changes in both insulin resistance and insulin secretion can be shown to underlie the development of the dysglycemia post transplantation. These changes are however dynamic and sometimes transient, particularly in the early post-transplant period. Lastly, the role of changes in incretin hormones remains to be elucidated, as does the impact of the severity of chronic kidney disease (CKD) pre- and post-transplant on insulin metabolism and resistance.

Pre-transplant factors

The dynamic nature of dysglycemic states has been documented by Hornum *et al.*^[33]. They followed 57 patients from pre- to 12 mo post-transplant. Importantly, none were diabetic on an oGTT pre-transplant, however only 67% were normoglycemic. At 3 mo only 46% were normoglycemic and this increased to 56% by 12 mo. Pre-transplant, patients were compared with uremic controls. The transplanted patients were significantly younger (39 vs 47 years old) with shorter period of

time on dialysis (24 mo vs 45 mo); however, they did not differ in terms of measure of glycemic state. These measures included F BGL, oGTT and then specific validated measures of insulin resistance and secretion. It is noteworthy that both the uremic controls and transplant patients had a worse glycemic state than a small group of healthy controls - despite normal F BGL [5.1 mmol/L (all ESKD) vs 5.0 mmol/L]. The normal F BGL would suggest that hepatic gluconeogenesis was not impaired by the ESKD state; however, the ESKD patients had oGTT results of 7.4-7.5 mmol/L (vs 5.4 mmol/L) and this seemed to be accounted for by increased peripheral insulin resistance. Interestingly, the increased resistance in ESKD patients was matched by increased insulin secretion compared to healthy controls (although not statistically significant). This may have been expected for two reasons. Firstly, ESKD patients will have reduced renal clearance of insulin^[91]. Secondly, as insulin resistance and insulin secretion are described as being related in a hyperbolic fashion^[92], such that changes in one parameter would be expected to drive compensator changes in the other parameter. Whilst there is evidence in these cohorts of compensatory increase in insulin secretion, it can be postulated that it was insufficient as the ESKD patients had markedly higher oGTT results and 33% were found to have IGT. At 12 mo, 14% of patients had developed NODAT and this was associated with increased insulin resistance and increased insulin secretion, which nonetheless, appeared not to be sufficient to maintain normoglycemia. The development of NODAT was not associated with pre-transplant IGT. However, those who developed dysglycemia tended to be older and have a higher pre-transplant BMI, which may co-vary (although not significant in multivariate analysis) with the noted increased pre-transplant insulin resistance and, again, higher compensatory pre-transplant insulin secretion.

Insulin resistance

Increasingly, understanding the factors responsible for insulin resistance and decreasing insulin secretion is being recognised as important for determining modifiable and treatable causes of NODAT. An increase in insulin resistance would be consistent with exposure to glucocorticoids. Glucocorticoids are believed to impair peripheral glucose uptake, impair hepatic glycogen synthesis and enhance gluconeogenesis. At higher doses they may induce β -cell apoptosis^[93]. Furthermore, it has been proposed the diabetogenic risk is not restricted to higher dose of glucocorticoid but also occurs with chronic exposure to low doses^[94]. In addition to duration and dose of glucocorticoid, older age and higher BMI also predispose to the development of diabetes in those receiving glucocorticoid treatment^[95]. Perhaps it is less well recognised that CKD and uremia may also contribute to insulin resistance. It may be that the relief from uremia, but the nonetheless persistent state of CKD post-transplant contributes to the dynamic nature of post-

transplant dysglycemia. It may also be that whilst clearly the biological stress of transplantation and exposure to diabetogenic medications is crucial in the pathogenesis, the persistence of CKD in certain older and perhaps genetically predisposed patients forms a background milieu upon which the dysglycemia can develop. There has been renewed interest in the contribution of uremia or CKD to insulin resistance and the various mechanisms are beyond the scope of this article. However, when reading literature on post-transplant dysglycemia it is important to remember that transplant patients have had periods of severe CKD/ESKD requiring dialysis and, for the most part, remain a CKD patient^[96,97]. One study of 27 diabetic and 35 non-diabetic ESKD patients using a homeostatic model assessment-insulin resistance model to assess insulin resistance found increased insulin resistance in the diabetic patients. The non-diabetic patients with increased insulin resistance had elevated C-peptide levels, indicating a compensatory response maintaining non-diabetic state^[98].

Other factors that may increase insulin resistance post-transplant include hepatitis C virus (HCV) and metabolic syndrome. Two studies have found that HCV-positive patients have increased insulin resistance compared to non-HCV transplant patients. One of these studies found a compensatory increase in insulin secretion^[99] and one did not find such compensation^[70]. On the other hand, CMV, the other recognised diabetogenic virus, seems to be associated with impaired insulin secretion; although, the exact mechanism is not well studied^[59]. Whilst metabolic syndrome has been described in the general population to be associated with insulin resistance, there is a paucity of data considering metabolic syndrome and insulin resistance in transplant recipients. A recent retrospective review of 76 patients with a mean 11.1 years post-transplant follow up found that even when adjusted for age, the presence of metabolic syndrome was associated with increased risk progression of dysglycemia^[22]. In a larger cohort of patients ($n = 640$), the presence of metabolic syndrome pre-transplant remained a significant risk factor for developing NODAT even when adjusted for age^[9]; however, there is no data available on insulin resistance in any significant cohort of transplant recipients who develop metabolic syndrome and NODAT.

Insulin secretion

It seems likely that as modifiable risk factors are altered, importantly including immunosuppressive agents, that the weights of forcing factors of NODAT will also be altered. As such, studies that repeatedly measure insulin indices throughout the post-transplant period, in particular in the higher risk first year post-transplant, are particularly valuable. Nagaraja *et al.*^[22] has recently described insulin indices pre- and 3 and 12 mo post-transplant in non-diabetic patients ($n = 118$) as defined by F BGL less than 7.0 mmol/L pre-transplant. The patients defined as NODAT had increased insulin

resistance at 3 and 12 mo, although less resistance at 12 mo when compared to 3 mo. By 12 mo, insulin secretion had fallen in patients with NODAT; however, despite the fall in insulin resistance the levels of secretion failed to be compensatory, suggesting that even in the face of falling doses of glucocorticoid and improving peripheral insulin sensitivity, impaired insulin secretion increasingly threatens normoglycemia^[20,100]. This data is supported by previous studies in which oGTT was used for diagnosis^[101,102]. Nam *et al.*^[102] first demonstrated impairment in insulin secretion as a necessary component in the pathogenesis. They followed 144 patients pre- and post-transplant and noted that higher, although normal, oGTT results pre-transplant were associated with increased risk of dysglycemia post-transplant. They also noted that those who developed post-transplant dysglycemia 9-12 mo post-transplant had significantly lower insulin secretion in the face of improved insulin resistance. A long term study found similar results when using oGTT at 10 wk and 6 years post-transplant. Patients who were dysglycemic at 10 wk and became normoglycemic had improvement in insulin resistance and a non-significant impairment of insulin secretion, thus retaining a compensatory response. On the other hand, those who remained diabetic or became diabetic over the follow-up period had a non-significant deterioration in insulin resistance and a significant fall in insulin secretion^[103].

The mechanism of impairment in insulin secretion post-transplant is thought to be related to CNI use. The mechanism of action is believed to be the impairment of pancreatic cell function due to the binding of CNI to calcineurin. Calcineurin is a systolic phosphatase that has two targets in the β -cell: the nuclear factor of activated T cells and cyclic-AMP-responsive element-binding protein transcriptional co-activator. In mice models, normal β -cell function has been shown to be dependent upon calcineurin^[104]. Calcineurin may be important for the proper response to hyperglycemia and incretin activation. Human islet cells when treated with Tac increased β -cell apoptosis, possibly mediated by the above calcineurin targets and ameliorated by the administration of incretin analogues^[105,106].

Incretins

Finally, there is no data on the impact of immunosuppression in renal transplant patients on incretin hormones. It is interesting to note that in healthy volunteers the administration of glucocorticoids in the setting of being sedentary and on a high calorie diet (not unlike the initial period of time post-transplant) have impaired responses to incretin hormones^[107]. In dialysis dependent patients, those with IGT have been shown to have a reduced incretin effect^[108], and even normoglycemic dialysis dependent patients have reduced insulin secretion with increased incretin secretion suggesting that uremia or CKD impacts upon the proper β -cell stimulation and response^[109]. However,

Table 4 Risk of mortality, cardiovascular events and graft loss associated with new-onset diabetes after transplantation or dysglycemic state

	Mortality	CV event/death	Graft loss	Ref.
Diabetes at	3 mo: 37% at 8 yr (HR = 2.1) 10 wk: 34% at 6.7 yr (HR = 2.0) 1 yr: 44% at 11 yr (HR = 2.2)	20% (death) at 8 yr (HR = 3.5)		Hjelmsaeth <i>et al</i> ^[4] Valderhaug <i>et al</i> ^[11] Nagaraja <i>et al</i> ^[20]
Dysglycemia at	10 wk: 29% at 6.7 yr (HR = 1.78) each 1 mmol/L oGTT: 5% risk increase 4 mo: 0.5 mmol/L increase F BGL: 4% risk increase 12 mo: 0.5 mmol/L increase F BGL: 15% risk increase	Death HR: 2.72 Events increased with increased F BGL 1 mmol/L oGTT: 6% risk increase in death 12 mo: 0.5 mmol/L increase F BGL: 11% risk increase for event	3 mo: RR 3.6 at 6 yr	Cosio <i>et al</i> ^[3] Valderhaug <i>et al</i> ^[11] Wauters <i>et al</i> ^[14] Wojtusciszyn <i>et al</i> ^[41]

F BGL: Fasting blood glucose level; oGTT: 2-h oral glucose tolerance test.

the dynamics of incretin hormones are yet to be described in the post-transplant setting.

OUTCOMES

There is an urgent need to develop a consensus on the best test to detect and how to manage dysglycemic states post-transplant, as there is a direct correlation with the presence of dysglycemic states and mortality predominantly from cardiovascular causes (Table 4)^[3,4,11,14,20]. An analysis of the USRDS database in which NODAT was defined according to Medicare claims analysed 27707 patients with data available greater than 1 year and not diabetic pre-transplant. Death censored graft loss was more likely in those who suffered acute rejection when compared to those who developed NODAT. Conversely, those who developed NODAT had a higher hazard ratio of death with a functioning graft compared to those with episodes of acute rejection (1.41 and 1.15 respectively) compared to patients with neither exposure^[51]. Analysis of earlier data from the same database found the development of NODAT associated with increased risk for acute myocardial infarction after a minimum 3 year follow up^[110]. Similarly, in an analysis on the International Collaborative Transplant Study database ($n = 39251$) with up to 10 years of follow up, Cox regression analysis of death with a functioning graft due to cardiovascular disease revealed an increased risk for NODAT (HR = 1.6, $P < 0.001$), which was greater than episodes of rejection within the first year (HR = 1.2, $P = 0.036$) but not as great as the risk associated with pre-transplant diabetes (HR = 2.5, $P < 0.001$)^[111].

The above datasets are large and their analyses robust, but what is needed are large prospective datasets with well-defined populations and sufficient duration of follow up. Smaller studies have found significant risk for mortality from the development of NODAT, but these findings have disappeared when adjusted for confounding factors. In one such study, major cardiac events occurred in 20% of persistent NODAT patients compared to 7% without NODAT and 21% with pre-transplant diabetes over a 8 year follow up^[4]. The outcomes of the largest prospectively followed well defined

population was described by Valderhaug *et al*^[112]. They followed 1410 patients for a mean of 6.7 years, of whom 55% were dysglycemic at 10 wk post-transplant of which 17% had NODAT. They reported a significant increase in the incidence of all cause mortality between the normoglycemic and dysglycemic groups, the rates being highest in those with NODAT. After adjusting for confounding traditional and transplant associated variables, the HR for all cause mortality was 1.54 was NODAT and 1.39 for IGT ($P < 0.05$). When analysed treating glucose as a continuous variable: on adjusted analysis, for every 1 mmol/L (18 mg/dL) increase in oGTT result there was a 5% increase risk in all cause mortality ($P < 0.05$). The main cause of death was cardiovascular disease, and those with NODAT by 10 wk were at significant increased risk on adjusted analysis (HR = 1.8 $P < 0.05$). For every 1mmol/L (18 mg/dL) increase in the oGTT result there was significant 6% increase risk in cardiovascular death ($P < 0.05$). Despite the findings of the continuous glucose analysis, other dysglycemic states were not associated with cardiovascular death. Further analysis of the same cohort found a graft failure rate of 28%, 60% of which was due to death. There was no association with death censored graft loss, but for every 1 mmol/L (18 mg/dL) increase in oGTT result there was a 3% increase risk in overall graft failure^[110]. This suggests similar conclusions as the large registry analyses described above: NODAT may not be associated with increased graft loss, but is associated with increased mortality.

In another large single centre prospectively followed group an increase in risk of all cause mortality and cardiovascular death according to the presence of NODAT at 1 year post-transplant was reported^[14]. The 12-mo rate of dysglycemia was 29.8% and NODAT 13.4%. Continuous analysis of the glucose levels revealed that for every 10 mg/dL (0.56 mmol/L) increase in F BGL there was an increase in all cause mortality censored at graft failure over a follow up period of 90.4 mo. At 12 mo, patients with IFG had a HR of 1.7 ($P = 0.009$) and those with NODAT a HR of 3.5 ($P < 0.0001$). Of note, in this study the patients on treatment for NODAT did not have a reduced mortality risk compared to the NODAT

patients not on treatment. Given the retrospective nature of the analysis it is not possible to conclude that treatment does not affect outcomes. However, such findings indicate the importance of well-defined prospectively followed transplant population analyses and potentially the need to identify early those patients at risk of dysglycemia so that directed interventions (be they aggressive glucose or metabolic risk factor control) may ameliorate the increased risk of mortality. Furthermore, such data highlights that in the transplant population clinicians do not have targets of glycemic control that can be achieved with treatment and are associated with improved outcomes. Even in the general population there is conflicting data concerning improved macrovascular outcomes achieved by treating to more intensive targets^[113,114]; however, in the transplant population, it remains unknown if meeting these same targets may improve outcomes.

SCREENING AND DIAGNOSIS

Use of oGTT remains the gold standard for diagnosis of NODAT or dysglycemia. This test, however, is not an easily completed screening test. Simple office or laboratory based tests that may be used to adequately screen for NODAT, particularly in high risk patients, include F BGL, 4 pm capillary blood glucose or HbA1c. All of these parameters have limitations. For example, a Spanish study of 374 non-diabetic pre-transplant patients found that normal F BGL in 59% of patients with an abnormal oGTT over the first 12 mo post-transplant^[6]. It is well known that changes in red cell viability, need for (due to for example, drug induced bone marrow suppression) and use of erythropoietin stimulating agents, administration of red cell transfusions and changes in hemoglobin will impact upon HbA1c levels. Notwithstanding this issue more readily encountered in ESKD, some small studies ($n = 71$) have shown concordance between oGTT and an HbA1c cut-off of 6.2% for the diagnosis of NODAT^[115]. It would be clinically more likely to find concordance between these tests after 2-3 mo post-transplant once there has been renal function recovery and the impact of uremia on erythropoiesis has resolved. However, analysis of a much larger cohort ($n = 1571$) found that using if HbA1c was used as a screening tool and oGTT as the gold standard test, then the cut-off should be 5.8%^[44]. More recently, when using a combined test of HbA1c $\geq 6.5\%$ and F BGL ≥ 7.0 a Norwegian group ($n = 1619$) have demonstrated a negative predictive value (NPV) of 97.4% for NODAT, using oGTT as gold standard test at 10 wk post transplantation^[116]. Notably, the combination of the two tests had very little additive value (NPV F BGL alone 94.2%) and the lower the HbA1c cut-off value made little difference in exclusion of NODAT (e.g., $\geq 5.5\%$ NPV 97.5 compared with $\geq 6.5\%$ NPV 93%). However, the positive predictive value of HbA1c $\geq 6.5\%$ or 6.2% or in combination with F BGL ≥ 7.0 mmol/L was poor (53.4%, 42.1%, 69.4% and 50.9%,

respectively). Thus, while HbA1c may be of use in screening for NODAT, current evidence does not support its use as a diagnostic test in transplant patients.

Determining the best test to use in transplant patients is complicated by the need to certain of the best time to administer the test. It has recently been shown that glucocorticoid administration in the morning leads to increased afternoon or evening blood glucose levels, at approximately 7-8 h after administration of glucocorticoid. Thus, reliance on F BGL may underestimate the incidence of dysglycemia. In fact, at six weeks post transplantation a 4 pm capillary blood glucose significantly outperformed oGTT, F BGL and HbA1c in detecting NODAT. Combining the tests done at 3 and 12 mo, the cumulative incidence of NODAT with oGTT was 14% and IGT 28%. Interestingly, using an HbA1c range of ≥ 5.7 and < 6.5 to detect IGT detected an incidence of 51%; but HbA1c did not perform as well as oGTT in detecting NODAT. Hence, the authors suggested using HbA1c as a screening test from 3 mo and using oGTT to determine the presence or absence of NODAT in patients detected to have dysglycemia by HbA1c. This strategy would avoid oGTT in 49% of patients and achieve a sensitivity of 94%^[117]. As yet, this data and strategy has not been replicated. Furthermore, the results of these studies suggest that the cut-offs that have been applied in the general population may not apply in CKD, ESKD or post-transplant patients. The question of the cut-off levels for any of the possible tests will only be settled by long-term large prospectively collected data sets which permit determination of the risk for poorer clinical outcomes associated with different cut-off points. Some of this data has already been described, but it is worth emphasising that only oGTT results have been shown to be associated with poorer outcomes when analysed categorically (as distinct from continuous data) and not F BGL^[11].

PREDICTING NODAT

If it is difficult to develop easy to administer diagnostic tests, it is even more challenging to develop to models that may predict the development of NODAT, based either on pre- or post- transplant data. There are very few studies able to draw conclusions about predicting NODAT using pre-transplant data. Post-transplant dysglycemia is dynamic phenomenon and there are multiple physiological changes post-transplant that may impact upon insulin and glucose handling. This is emphasised by the remarked upon cases of diabetic or dysglycemic pre-transplant patients resolving their dysglycemic state post-transplant. Hence, the pre-transplant prediction of those increasingly likely to have NODAT post-transplant is fraught with multiple difficult variables that need to be taken into account.

A range of pre-transplant variables has been described as predictors of NODAT. These include age, BMI, fasting and R BGL and metabolic syndrome. For example, one study of 139 non-diabetic patients

pre-transplant found that higher (albeit normal) pre-transplant R BGL were predictive ($P = 0.011$) of NODAT, although this data has not been replicated^[7]. A matched cohort retrospective analysis of 47 patients who developed NODAT found that a higher, albeit normal range, F BGL was associated with the development of NODAT on multivariate analysis^[44].

One reasonable sized study ($n = 640$) with a NODAT incidence at 1 year of 31.4% found an adjusted hazard for NODAT of 1.34 (1.00-1.79, $P = 0.047$) for pre-transplant metabolic syndrome. On multivariate analysis, only pre-transplant low HDL remained an independent predictor^[9]. Other groups have attempted to apply scores that are predictive of type 2 diabetes mellitus in the general population. A retrospectively analysed cohort of 191 patients in which 41 developed NODAT, two general population risk scores were found to have AUC-ROC of 0.756-0.807 for NODAT at 1 year, but the PPV for each test was poor (24.5%-31.2%). However, the authors point out that the NPV were high (92.5%-93.7%) perhaps allowing the identification of high risk patients^[118].

There is a small body of literature considering the development of predictive models that may be more unique to the transplant patient. Analyses in the general population of the patterns of oGTT results may be predictive of future type 2 diabetes^[119]; similar analyses in renal transplant patients may be useful. An analysis of a 5 time point oGTT conducted pre-transplant in 145 patients found that whilst F BGL did not predict NODAT, the AUC of the oGTT and the glucose concentrations at each time point post glucose load could be used to predict NODAT^[23]. Given the logistical difficulties in studying recipients of deceased donor organs, there is little data available that would enable us to reliably assess if pre-transplant markers for NODAT can be identified. For example, one study in which 120 transplanted patients were screened with oGTT pre-transplant found that pre-transplant IGT was significantly associated with NODAT; however, these patients were screened during the 3 mo prior to being waitlisted and there was no information provided regarding the time on the waiting list. This may introduce a potential bias in that some normoglycemic patients may have developed further dysglycemia pre-transplant^[31].

Chakkerla *et al.*^[120,121] have attempted to develop and validate a model of pre-transplant factors to predict the development of NODAT. On univariate analysis they described seven pre-transplant factors associated with increased risk of NODAT, which was defined by use of HbA1c, F BGL or requirement for treatment, including dietary changes. The seven factors were: age greater than 50 years old, use of maintenance glucocorticoids, use of gout therapies, BMI ≥ 30 , F BGL ≥ 5.6 mmol/L, fasting triglycerides ≥ 2.24 mmol/L and a family history of type diabetes. Insulin indices were not measured and pre-transplant oGTT were not done pre- or post-transplantation, potentially treating pre-transplant diabetic patients as normoglycemic. Complex statistical

methods, including bootstrapping were used. Within the limitations of this study, there were clear differences in the 1 year incidence of NODAT for those classified as low, moderate or high risk according to seven factor risk score. The results were similar in the initial and validation groups. In the higher risk group the incidence of NODAT was 44%-56% compared to the low risk group of 11%-13%. This was a first step in attempting to develop a risk score that may assist in identifying patients who could be targeted for trials of preventive therapies.

The analysis of the data from the 5 time point oGTT points towards the possibility of identifying higher risk patients by evaluating for impaired glucose and insulin regulation pre-transplant. A test that is helpful in this regard is known as the disposition index. This is a quantification of the hyperbolic balance between insulin secretion and insulin resistance. It can be measured either *via* oral or IV glucose loads and has been shown to be associated with increased risk for developing type 2 diabetes mellitus in the general population^[122,123]. There is little literature using the disposition index as a predictive pre-transplant marker. However, there are some small studies measuring insulin resistance and secretion pre-transplant and testing their relationship with NODAT. Various models that utilise data derived from oGTT or IV GTT measure insulin resistance. The homeostasis model (HOMA) is widely used, and has been validated in studies of the general population. Variations of HOMA can be used to estimate insulin resistance and secretion. There is conflicting data on whether pre-transplant insulin indices may be predictive and most studies are small^[124]. A study with the primary purpose of comparing Tac and CsA ($n = 150$) was used to retrospectively review the risk of NODAT from pre-, 3 and 12 mo indices of insulin resistance. Pre-transplant, there were no differences in insulin resistance or secretion found between those patients who developed NODAT at 3 or 12 mo^[20]. This is in contrast to an earlier study ($n = 57$) in which those patients more resistant at baseline (and older) had an increased odds of a dysglycemic state after 1 year follow up^[33]. However, as it appears increasingly more likely that falls in insulin secretion (and thus failing to compensate for insulin resistance) is crucial in the development of NODAT, it is interesting to note that measurements of insulin secretion in non-diabetic post-transplant patients can be used to predict the future development of NODAT^[98].

MANAGEMENT

The principles of management of post-transplant dysglycemia are: (1) Pre-transplant risk assessment and development of amelioration strategies; (2) Early detection and monitoring for transient or permanent dysglycemia; and (3) Appropriate therapies that may reduce the poorer outcomes in those in whom post-transplant dysglycemia develops. The issues surrounding risk assessment and detection have been discussed

above. Current advice for glucose targets during post-transplant hospitalization suggest maintaining glucose levels below diabetic range; *i.e.*, F BGL 4-7 mmol/L (72-126 mg/dL)^[125]. Following discharge, current guidelines recommend that patients be screened weekly for the first four weeks, and every 3 mo for the first year and yearly after the first year. Screening should also be commenced if there is commencement of, or substantial increase in dose of, CNI, mTOR inhibitor or glucocorticoids^[126]. There is no consensus on the best screening test to utilise; however, a combination of tests as discussed above would appear to be of greatest clinical use. This may involve weekly F BGL or 4 pm capillary blood glucose (although this is not currently part of guidelines). Detection of IFG would then prompt oGTT assessment^[125]. Perhaps use of HbA1c after the first 3 mo is warranted in stable patients. There are also few recommendations as to what the targets for blood glucose and HbA1c ought to be, as it is not known at what ranges there is substantial reduction in poorer outcomes. At present, guidelines give an ungraded suggestion to aim for an HbA1c of 7%-7.5% in United States^[126] and < 7% in Scandinavia^[125].

Adjusting immunosuppression

One approach to management is amelioration of risk. It remains difficult to identify patients at risk for dysglycemia with certainty; equally, it is challenging to know what may be done should they be identified. On the basis of data available concerning modifiable risks, physicians may wish to replace, minimise or withdraw one or more agents that form part of the maintenance immunosuppression; in particular CNI or glucocorticoids. For instance, perhaps older patients with a higher BMI and a worse (if still normal) pre-transplant oGTT may be judged to be at risk and as a result not exposed to maintenance glucocorticoids, or use of CsA in preference to Tac. This approach clearly needs to balance the immunological risk of reduced immunosuppressive exposure against the higher metabolic (and ultimately cardiovascular and infection) risk. Some authors have proposed protocols to assist in balancing the metabolic and rejection risk^[61]; however, there are no well validated methods for reliably making such assessments in a broad transplant population. In addition, the clinician is also faced with the complicated issue of applying risk assessments to individual patients with varying degrees of co-morbidities.

One potentially helpful immunosuppressive agent that has not been discussed above is belatacept. This co-stimulatory blockade agent, which remains available in for off-label use in many countries, can be used as part of a maintenance regimen in place of CNI, in combination with MMF and glucocorticoids. The BENEFIT and BENEFIT-EXT (extended criteria donors) trials have reported up to 5 year results, comparing belatacept with MMF and glucocorticoids with CsA, MMF and glucocorticoids. There is a concern that there

may be greater early acute rejection, however, over longer follow up there is no greater rejection rate. There has also been a concern about increased risk for EBV associated post transplant lymphoproliferative disease^[127-130]. With regard to NODAT, results from 1 year follow-up of BENEFIT and BENEFIT-EXT have been published. There was a significant reduction in the 1 year cumulative incidence of NODAT in the belatacept arm, with rates of NODAT in the CsA arm being comparable to that found in other studies. This was in conjunction with clinically significant reductions in blood pressure, cholesterol and triglycerides, suggesting it may have a role in management of patients at higher risk of poorer cardiovascular and metabolic outcomes^[131].

Lifestyle changes

Aside from altering immunosuppressive agents, other modifiable risk factors include reduced physical activity and poor diet. There is some data to suggest that low levels of physical activity post-transplant, particularly in patients whose appetite may now be improved, are at greater cardiovascular and all-cause mortality risk^[132]. Improved diets, increased physical activity and weight loss has also been shown to improve dysglycemia in renal transplant patients^[133]; however, this is not a well studied therapeutic approach.

Intensive and early glycemic control

As there are many obstacles to overcome should immunosuppression be tailored to meet metabolic and immunological risk, it may be that we require strategies to "rest" β -cells in patients without changing immunosuppression in those at higher risk of metabolic complications. Hecking *et al.*^[38] in a proof of concept trial ($n = 50$) randomised patients to (non-blinded) early basal insulin or standard therapy. NODAT was defined by oGTT or need for hypoglycemic agents at study visit. All patients received maintenance Tac, glucocorticoids and MMF. Patients were given isoprene insulin if their evening blood glucose was > 140 mg/dL (7.8 mmol/L) in the treatment group; the standard of care group received short acting insulin or oral agents if their blood glucose was 180-250 mg/dL (10-13.9 mmol/L), as directed by the treating clinician. All 25 patients in the treatment group received isoprene insulin on postoperative day 3, having had high evening blood glucose the day prior. By 12 mo, no patient in the treatment group required hypoglycemic agents compared to 8 in the control group. The majority of the patients in the treatment group did not receive any hypoglycemic agent after 120 d post-operative. All patients not on hypoglycemic agents had oGTT at 3, 6 and 12 mo. By 12 mo, 5 patients in the treatment group had NODAT on oGTT compared to 4 in the control group; thus, there was a reduction in NODAT from 12 to 5. More patients in the treatment group had IGT (8 vs 5); but, overall, more patients in the treatment group were normoglycemic (12 vs 8). Furthermore, consistent with the more

recent literature on insulin secretion as a significant contributor to the pathogenesis of post-transplant dysglycemia, measures of insulin resistance between the groups did not differ at 12 mo. There was, however, a significant difference in the insulinogenic index, an oGTT derived measure of β -cell function. There was also an improvement in the disposition index (although not significant). Together these results would indicate better or more preserved insulin secretion in those whose β -cells were “rested” at time of maximal stress. Should such results be achieved in a larger study population (perhaps of higher risk patients) who are studied for a longer period of time and found to have better metabolic and cardiovascular outcomes, then it may be that early basal insulin in those with elevated evening blood glucose may become a standard of care obviating any need to tailor immunosuppression.

Standard hypoglycemic agents

Nonetheless, currently patients receive care more like the standard care administered in Hecking *et al.*^[38]. If these patients then develop NODAT, they receive hypoglycemic agents. The choice of agent is mostly guided by opinion and knowledge of risks associated with administration of these agents in CKD. This is due to the paucity of trial data on use of hypoglycemic agents within this population. There is only one small study ($n = 48$) that compares potential therapies in which a DDP IV inhibitor, vildagliptin, was compared with pioglitazone or placebo in patients with IGT at more than 6 mo post renal transplantation. Both medications reduced oGTT blood glucose levels over 3 mo, with no differences between the treatment groups^[134]. As there is concern that thiazolidinediones may be associated with poorer cardiovascular outcomes, such medications may not be considered as first line therapy. The incretin analogues, remain the only other hypoglycemic agent studied in transplant patients. Vildagliptin has been studied as part of a randomised placebo controlled trial, in which patients with oGTT defined NODAT at least 6 mo post-transplant were recruited. Thirty-three patients were recruited, all of whom were on a similar maintenance regimen of CNI/MMF and glucocorticoids. The follow up period was short, however, vildagliptin did significantly reduce oGTT and HbA1c results at 3 mo with no hypoglycemic events^[135]. Caution should be used with vildagliptin in conjunction with ACE inhibition as there is an increased risk of angioedema (OR = 4.57), albeit on the basis of a small absolute risk^[136]. Another small study ($n = 19$) has shown that sitagliptin can significantly increase insulin secretion in patients known to have NODAT^[137]. Sitagliptin, saxagliptin and vildagliptin should be dose reduced in renal impairment, linagliptin is not renal excreted. It is unclear if incretin analogues are ameliorating an impact upon the incretin effect or assisting β -cell function in other ways. There is no data in the transplant population concerning the incretin effect. In healthy people administered glucocorticoids the incretin effect has been noted to be

impaired^[103]. In favour of incretin analogues, they do not tend to produce hypoglycemia or weight gain; but they have not been shown to reduced cardiovascular events, have been associated with pancreatitis and may theoretically increase cancer risk^[138].

The incretin analogues are not widely used in the transplant population, with use of sulfonylureas and more common. Metformin may not be favoured as it can contribute to gastrointestinal side effects, potentially exacerbating the same caused by MMF use. Moreover, there is no also no data on its use in transplant patients with GFR < 30 mL/min and risks of lactic acidosis. However, its lack of contribution to weight gain, its association with reduced cardiovascular events in non-transplant patients and its role as an insulin sensitiser rather than stimulating further insulin secretion from “stressed” β -cells, may make metformin more favoured than sulfonylureas^[139]. Sulfonylureas do not have the cardiovascular benefits and can contribute to weight gain. However, as long as dose adjusted to prevent hypoglycemia, their use is not associated with other serious adverse events. Nonetheless, it may be that some, if not most, transplant patients with develop dysglycemia have impaired β -cell function and that potentially a treatment strategy that induces more work from the β -cells may be counter-productive in terms of relieving dysglycemia and preventing worse cardiovascular outcomes^[140]. Problematically, the paucity of data on treatment (including treatment targeted at the underlying pathology) in this area of transplantation means it is not possible to make any firm recommendations on the choice of oral hypoglycemic agents.

CONCLUSION

In summary, dysglycemic states, not limited to NODAT, are associated with increased risk of mortality, principally as a result of cardiovascular disease. NODAT is better studied than other dysglycemic states. The natural history of dysglycemic states is not well characterized, apart from the recognition of transient dysglycemia and NODAT within the first 3-6 mo post transplantation. The majority of persistent NODAT develops within one over the first year post transplant. Whilst the diagnosis is made using the WHO/ADA criteria accepted in the general population, there is no consensus on which test should be employed, either for screening or diagnosis. At present, oGTT remains the most reliable diagnostic test in the post-transplant setting. However, predicting the development of NODAT remains challenging. Possibly, the small group of patients who remain normoglycemic within the first week post-transplant are at very low risk of developing NODAT. There are a few studies that may assist in developing tools for identifying those at high risk.

There are multiple risk factors, some of which are modifiable. The most consistently found risk factor is increasing age and there is a growing body of liter-

ature documenting the genetic risk factors. The most well described modifiable risk factor is the use of immunosuppressive agents, in particular CNI (Tac more than CsA) and glucocorticoids. These agents likely contribute to the development of NODAT *via* different mechanisms – glucocorticoids encouraging insulin resistance and CNI *via* β - cell failure. It seems that reduction in insulin secretion is more important in the pathogenesis than insulin resistance.

Any attempt to balance the metabolic and immunological risks by adjusting immunosuppression is complicated. It may be better to identify higher risk patients and utilise a preventive strategy, such as described by Hecking *et al.*^[38]. As evidence emerges of the importance of β -cell failure as a major contributor to NODAT, such as strategy appears promising. In the absence of prevention, the management of NODAT in order to prevent the poorer outcomes is important. However, it is not clear which agent is most likely to successfully treat NODAT and ameliorate the poorer outcomes. A number of options exist, and it may be that metformin is the best option if insulin is not required.

Finally, further research is needed on pathogenesis, identification of higher risk patients and development of preventive and safe treatment options. Such research needs to take into account the caveats that are identified with respect to previous research: confirming normoglycemia pre-transplant, using oGTT as the primary diagnostic test (although there may be a role for capillary blood glucose early post-transplant), using WHO/ADA to define clinical states, testing regularly to detect transient and permanent states and having adequate follow up to detect the development of permanent dysglycemic states that impact upon poorer clinical outcomes. It would be ideal if future research could also map the changes in insulin secretion, resistance and the incretin effect pre- and post- transplantation in an effort to better understand the pathogenesis and further delineate targeted prevention and treatment options.

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Magnesium and type 2 diabetes

Mario Barbagallo, Ligia J Dominguez

Mario Barbagallo, Ligia J Dominguez, Geriatric Unit, Department of Internal Medicine and Medical Specialties, University of Palermo, 90127 Palermo, Italy

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Correspondence to: Mario Barbagallo, MD, PhD, Geriatric Unit, Department of Internal Medicine and Medical Specialties, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy. mario.barbagallo@unipa.it
Telephone: +39-91-6552885
Fax: +39-91-6552952

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Abstract

Type 2 diabetes is frequently associated with both extracellular and intracellular magnesium (Mg) deficits. A chronic latent Mg deficit or an overt clinical hypomagnesemia is common in patients with type 2 diabetes, especially in those with poorly controlled glycemic profiles. Insulin

and glucose are important regulators of Mg metabolism. Intracellular Mg plays a key role in regulating insulin action, insulin-mediated-glucose-uptake and vascular tone. Reduced intracellular Mg concentrations result in a defective tyrosine-kinase activity, postreceptorial impairment in insulin action and worsening of insulin resistance in diabetic patients. A low Mg intake and an increased Mg urinary loss appear the most important mechanisms that may favor Mg depletion in patients with type 2 diabetes. Low dietary Mg intake has been related to the development of type 2 diabetes and metabolic syndrome. Benefits of Mg supplementation on metabolic profiles in diabetic patients have been found in most, but not all clinical studies and larger prospective studies are needed to support the potential role of dietary Mg supplementation as a possible public health strategy in diabetes risk. The aim of this review is to revise current evidence on the mechanisms of Mg deficiency in diabetes and on the possible role of Mg supplementation in the prevention and management of the disease.

Key words: Magnesium; Type 2 diabetes; Metabolic syndrome; Inflammation; Aging; Hypertension; Insulin resistance; Endothelium

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Core tip: Diabetes is frequently associated with Mg deficit. The fact that most but not all diabetic subjects have low magnesium (Mg) and that no large randomised controlled trial (RCT) has been specifically focused on subjects with Mg deficit, diagnosed with a reliable technique, may help explain discrepancies of the role of supplemental Mg on glycemic control, and the impact on diabetes risk in prospective epidemiological studies. Different baseline Mg, metabolic control, and age are other potential factors that may contribute. Future prospective RCTs are needed to support the potential role of dietary Mg supplementation as a possible public health strategy to reduce diabetes risk in the population.

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INTRODUCTION

Magnesium (Mg) is an electrolyte of chief physiological importance in the body, being the *most abundant* divalent intracellular cation in the cells, the second most abundant cellular ion next to potassium and the fourth cation in general in the human body^[1].

Type 2 diabetes mellitus (DM2) is often accompanied by alteration of Mg status. An increased prevalence of Mg deficits have been identified in DM2 patients, especially in those with poorly controlled glycemic profiles, with longer duration of the disease and with the presence of micro- and macrovascular chronic complications^[2-6].

Laboratory tests with a high sensitivity and specificity and easy to perform to allow an accurate clinical assessment of Mg status are missing. Patients are considered frankly hypomagnesemic with serum Mg concentrations ≤ 0.61 mmol/L or 1.5 mg/dL^[7-9]. Mg concentrations ≤ 0.75 mmol/L or 1.8 mg/dL may be considered as preclinical hypomagnesemia^[10,11].

Mg deficiency can be present without hypomagnesemia. However, hypomagnesemia, when present, is usually indicative of an important systemic Mg deficit. A depletion in intracellular and/or ionized plasma Mg can be found in individuals with normal total serum Mg^[12]. However, most of the studies in the literature have measured total serum Mg instead of the free, ionized (bioactive) or the intracellular Mg concentrations, which make it a challenge to correlate Mg deficits to diseases.

We have recently confirmed that diabetic older patients are more prone to hypomagnesemia; this condition being closely related to metabolic control as measured by glycated hemoglobin even after adjustment for relevant confounders. Ionized Mg may help to identify diabetic older adults with low concentrations of blood Mg that are not evident with the only measurement of total Mg^[12].

Intracellular free Mg levels are consistently reduced in subjects with DM2, when compared with nondiabetic subjects^[1,13,14]. Although the mechanism has not been fully elucidated, an alteration in the mechanism(s) of the Mg uptake in the cells, and/or a deficit of ATP, may help to understand the cellular Mg deficit observed in DM2^[15]. The relationship between intracellular Mg and ATP concentration is rather complex. The decrease in cellular ATP might partially explain the decrease in cellular Mg. Otherwise, a decrease in cellular ATP leads to a decreased binding of Mg to ATP in the formation of MgATP, which might increase the intracellular Mg concentration.

The aim of this review is to revise current evidence

on the mechanisms of Mg deficiency in DM2. The evidence on the role of Mg supplementation in the management of DM2 will also be discussed.

MECHANISMS OF MG DEFICIENCY IN DM2

Reduced Mg intake and/or augmented Mg urinary loss are among the most important causes of Mg deficits in DM2, while Mg absorption and retention seems to be maintained^[16-18].

A relationship between Mg levels in the plasma and the development of DM2 in the general population has been suggested^[19]. DM2 is frequently accompanied by renal calcium and Mg loss^[20,21], but the mechanism(s) of this wasting is still not completely elucidated^[22].

Both hyperglycemia and hyperinsulinemia may increase urinary Mg excretion. Urinary Mg excretion and fasting blood glucose have been found to be inversely related to serum Mg levels. Thus, hyperglycemia decreases Mg tubular reabsorption^[20]. A good metabolic control is associated with a reduction of the urinary Mg wasting^[3].

In streptozotocin-induced diabetic rats, Lee *et al.*^[22] found an increase in renal Mg transporters. The alteration was corrected by insulin administration. Insulin resistance and hyperinsulinemia may also affect Mg transport^[21].

MG AND INSULIN SENSITIVITY

Hypomagnesemia in DM2 is present only in severe (and generally long lasting) Mg deficits. A chronic latent Mg deficiency without alteration in serum total Mg is more commonly observed^[12]. These often undetected Mg insufficiencies have clinical importance, since Mg is a main co-factor in numerous enzymatic reactions (> 300 enzymatic reactions including all the enzymes of glycolysis). Mg also is deeply involved in the regulation of insulin signaling, in the phosphorylation of insulin receptor kinase, in the post receptorial action of insulin, and in insulin-mediated cellular glucose uptake^[17,23].

The clinical consequence of a chronic Mg deficit is post-receptorial insulin resistance and consequent reduced glucose utilization in the cells, worsening the reduced insulin sensitivity present in DM2^[18].

Another possible link between Mg deficiency and reduced insulin sensitivity is the presence of oxidative stress and/or inflammation. Thus, free radicals are often increased in DM2, hypertension, metabolic syndrome and aging, conditions also associated with Mg deficits^[24,25]. In particular, we demonstrated an age-dependent deficit of cellular Mg in persons aged 65 years and over, as well as in patients with essential hypertension or DM2, independently of age^[14,25].

Nevertheless, independently of the mechanisms of Mg deficits in DM2, metabolic syndrome, essential

hypertension and aging, it is apparent that this Mg deficiency may contribute to enhance the insulin resistance status of these conditions^[17,18]. Mg deficit could precede and cause post-receptorial resistance of insulin and alter glucose tolerance.

MG DEFICIENCY AND CARDIO-METABOLIC DISEASES

Mg deficiency may be also a factor implicated in DM2 complications. We found a relation between ionic changes and echocardiographic indices alterations^[26]. We observed an significant association of reduced cellular Mg with cardiac hypertrophy in DM2 patients^[26].

Cellular Mg measured *in vivo* in skeletal muscle and in the brain with ³¹P-NMR, was directly related to aortic distensibility^[27].

Reduced Mg levels were also associated with an increased prevalence of arrhythmias in DM2 obese subjects^[6], and with a more rapid decline of renal function. Thus, hypomagnesemia is currently considered an accurate predictor of progression of diabetic nephropathy^[28-30]. Mg deficits have also been associated with cognitive decline^[31], multimorbidity^[32] and aging^[25,33].

DIETARY MG DEFICIENCY MAY PREDISPOSE TO DM2

Dietary Mg deficiency may cause insulin resistance as shown by several studies both in humans and in experimental animals^[34-40]. In sheep, Mg-deficient diet caused a significant impairment of insulin-mediated glucose uptake^[35]. In rats, Mg supplements were able to postpone the onset of diabetes^[36]. In healthy women (without DM2), the higher was the intake of Mg, the lower were fasting levels of insulin^[37]. In young, nondiabetic African Americans, low dietary Mg was associated with insulin resistance and insulin responses to an oral glucose tolerance test^[38]. A low Mg diet in rats produced an increase in triglyceride and plasma glucose levels^[39]. In rats, a maternal restriction of dietary Mg was able to cause insulin resistance in pups^[40]. Suárez *et al.*^[41] suggested that the worsening of glucose metabolism induced by Mg dietary restriction in experimental rats is due to an impairment of both, insulin secretion and insulin action.

Deficiencies of Mg status including both hypomagnesemia and/or reduced dietary Mg intake have been linked to an enhanced risk to develop DM2 or glucose intolerance^[19,42-44]. Higher Mg intakes were conversely associated with a reduced incidence of DM2^[45].

Several studies have shown a clear association of Mg intake with DM2 and with cardio-metabolic syndrome, suggesting that a higher Mg consumption is related to a reduction of the incidence of these conditions. Two meta-analyses of prospective studies concluded that Mg intake is inversely associated with the onset of DM2^[46,47]. In addition, the development of the cardio-metabolic

syndrome has been linked to dietary Mg content^[34,48]. Hypomagnesemia itself in a 10-year follow-up study was associated with glucose tolerance impairment^[49]. Conversely, higher Mg intake was associated with increased insulin sensitivity^[50] and with decreased risk of incident DM2, with a decreased risk of 0.68 in the higher compared with the lower quintiles^[51,52].

Similar findings were obtained in the CARDIA study, during a 20-year follow-up, which also confirmed the reverse relationship of dietary Mg with inflammation markers^[53].

POSSIBLE USE OF MG SUPPLEMENTS IN THE MANAGEMENT OF DM2

The detection and correction of altered Mg status in diabetic patients is clinically appropriate, although many physicians tend to ignore Mg status. The increased risk of developing impaired glucose tolerance and/or frank DM2 in persons with dietary or serum Mg deficits have suggested a potential benefit of Mg supplements in patients with DM2 or in the presence of risk factors for DM2. Mg supplements have been proposed as a complementary tool for the prevention of DM2 and its metabolic control^[54,55]. Some benefits of Mg supplements on glycemic profiles have been found in most but not all studies.

Regrettably, results from clinical trials are still limited^[56]. Thus, the clinical evidence of a clear effect of Mg supplementation on metabolic indices in persons with DM2 are controversial. Some benefit has been found in several^[8,54,57,58], but not in all clinical studies^[59]. The hypothesis of a role of supplemental Mg in the control of DM2 still needs to be ascertained by large randomized clinical trials^[60,61]. Mg supplementation may improve glycemic concentrations in fasting and postprandial states, and insulin sensitivity. We found a significant relationship between the increase in serum and cellular Mg and insulin sensitivity^[62]. We also showed that Mg supplementation is able to improve an altered endothelial function in DM2 older adults^[63]. Barragán-Rodríguez *et al.*^[64] suggested a positive effect in the treatment of depression in older persons with DM2 and hypomagnesemia. Presumably, the main problem is that all RCTs were underpowered, partially through overestimation of the treatment effect. Differences may be related to the fact that most of the existing studies have included a small number of subjects, using different Mg doses and different Mg salts.

Several studies have linked high Mg content present in fiber with the positive action of whole grains to improve insulin sensitivity^[65-68]. Oral Mg supplements have been shown to improve fasting and postprandial glucose levels and insulin sensitivity in hypomagnesemic DM2 patients^[57], to improve insulin sensitivity in non-diabetic subjects with insulin resistance^[8], and to decrease C-reactive protein levels in hypomagnesemic patients with prediabetes^[69].

In summary, oral Mg supplements appear to be useful in persons with DM2 to restore Mg deficiencies, to improve insulin resistance, oxidative stress, and systemic inflammation.

The absence of large trials in DM2 patients specifically focusing on those with Mg deficit may help to explain the inconsistency between epidemiological (mainly positive) and clinical (mostly controversial) studies. Since most, but not all, DM2 patients have Mg deficiency, it would be useful to focus on those with deficit in order to correct it. Differences in Mg balance, glycemic control, and age are other potential factors that may help to explain the differences among the studies. Most studies used total serum Mg concentration instead of the free, ionized (bioactive) Mg concentration, which make it a challenge to correlate Mg deficiency to diseases.

Future prospective large RCTs would be important to support the possible inclusion of Mg supplements in the guidelines for the management of DM2.

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Vitamin paradox in obesity: Deficiency or excess?

Shi-Sheng Zhou, Da Li, Na-Na Chen, Yiming Zhou

Shi-Sheng Zhou, Institute of Basic Medical Sciences, Medical College, Dalian University, Dalian 116622, Liaoning Province, China

Da Li, Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China

Na-Na Chen, Department of Molecular Immunology, Graduate School of Medicine, Nagoya University, Nagoya 466-8550, Japan

Yiming Zhou, Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Institutes of Medicine, Harvard Medical School, Boston, MA 02115, United States

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Correspondence to: Shi-Sheng Zhou, MD, PhD, Professor, Institute of Basic Medical Sciences, Medical College, Dalian University, No.10 Xuefu Avenue, Dalian Economic and Technological Development Zone, Dalian 116622, Liaoning Province, China. zhouss@ymail.com
 Telephone: +86-411-87402740
 Fax: +86-411-87402053

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Abstract

Since synthetic vitamins were used to fortify food and as supplements in the late 1930s, vitamin intake has significantly increased. This has been accompanied by an increased prevalence of obesity, a condition associated with diabetes, hypertension, cardiovascular disease, asthma and cancer. Paradoxically, obesity is often associated with low levels of fasting serum vitamins, such as folate and vitamin D. Recent studies on folic acid fortification have revealed another paradoxical phenomenon: obesity exhibits low fasting serum but high erythrocyte folate concentrations, with high levels of serum folate oxidation products. High erythrocyte folate status is known to reflect long-term excess folic acid intake, while increased folate oxidation products suggest an increased folate degradation because obesity shows an increased activity of cytochrome P450 2E1, a monooxygenase enzyme that can use folic acid as a substrate. There is also evidence that obesity increases niacin degradation, manifested by increased activity/expression of niacin-degrading enzymes and high levels of niacin metabolites. Moreover, obesity most commonly occurs in those with a low excretory reserve capacity (*e.g.*, due to low birth weight/preterm birth) and/or a low sweat gland activity (black race and physical inactivity). These lines of evidence raise the possibility that low fasting serum vitamin status in obesity may be a compensatory response to chronic excess vitamin intake, rather than vitamin deficiency, and that obesity could be one of the manifestations of chronic vitamin poisoning. In this article, we discuss vitamin paradox in obesity from the perspective of vitamin homeostasis.

Key words: Obesity; Type 2 diabetes; Developmental

origin of disease; Folic acid; Vitamin D; Niacin; Oxidative stress; Insulin resistance; Vitamin fortification

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Core tip: Obesity rates have dramatically increased among the United States population, including children, since the 1980s. Considering the lag time between risk exposure and the development of child obesity, the risk must have been imposed on the whole United States population around the late 1970s. Although evidence suggests that the risk is high vitamin intake due to the update of vitamin fortification in 1974 and the implementation of the Infant Formula Act of 1980, why do obese individuals paradoxically show low levels of fasting serum vitamins? In this paper, we try to give an answer to this question based on the current understanding of vitamin homeostasis.

Zhou SS, Li D, Chen NN, Zhou Y. Vitamin paradox in obesity: Deficiency or excess? *World J Diabetes* 2015; 6(10): 1158-1167 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i10/1158.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i10.1158>

INTRODUCTION

Obesity, a global health problem, is associated with co-morbidities such as metabolic syndrome, diabetes, hypertension, asthma, nonalcoholic fatty liver disease, renal disease, cardiovascular disease and cancer, which are thought to be of developmental origin^[1]. Since the late 1930s, when synthetic vitamins, thiamin, riboflavin and niacin (nicotinic acid and nicotinamide), were used to fortify foods or as dietary supplements, the daily intake of vitamins of the United States population has significantly increased, especially after the update of mandatory fortification in 1974^[2] and the implementation of the Infant Formula Act of 1980 (without setting an upper limit for most vitamins)^[3]. In fact, the introduction of synthetic vitamins into the diet was followed by a dramatic increase in the prevalence of obesity among all age groups in the United States^[4,5]. Similar correlations between increased obesity and vitamin fortification were observed in other vitamin-fortified countries, such as Canada and Saudi Arabia^[2]. Over the past 20-30 years, China has also been experiencing a rapid growth in the rates of obesity^[6] after having shifted from a low to a high vitamin intake, due to a combination of increased intake of animal-derived foods (rich in vitamin B₁, B₂ and niacin)^[7] and mandatory flour fortification with these vitamins, which was introduced in China in the late 1980s and was been mandatorily implemented in 1994^[2]. Paradoxically, it is frequently reported that obesity and type 2 diabetes are associated with low levels of fasting serum vitamins, including vitamin B₁, D, and folate^[8-10]. Although

the mechanism of the paradox remains unclear, it is generally thought that the low vitamin status in obesity is due to inadequate intake.

Since 1998, enriched grain products in the United States have been fortified with folic acid to prevent neural tube defects. Recent studies on folic acid fortification show that obese individuals also show lower fasting serum folate concentrations, but, paradoxically, their red blood cell (RBC) folate concentrations and MeFox (5-methyltetrahydrofolate oxidation product) are significantly higher, when compared with nonobese individuals^[11,12]. Moreover, obesity is also found to be associated with increased activity of cytochrome P450 (CYP) 2E1, a monooxygenase enzyme that can use folic acid as a substrate^[13]. Folate content in RBC is known to reflect long-term average consumption and tissue stores because RBC only accumulates folate during erythropoiesis^[14], and increased serum MeFox suggests increased degradation of folic acid. Moreover, recent evidence shows that obesity is associated with high fasting serum N¹-methylnicotinamide without significant changes in nicotinamide levels^[15] and that plasma N¹-methylnicotinamide correlates with increased tissue expression of nicotinamide N-methyltransferase (NNMT, a major enzyme responsible for the degradation of nicotinamide to N¹-methylnicotinamide) and the degree of insulin resistance^[16]. Collectively, these observations raise the possibility that the vitamin paradox in obesity may involve vitamin excess rather than deficiency. After more than seven decades of practice of vitamin fortification and painful global experience of increasing prevalence of obesity and related diseases worldwide, it is time for us to examine the relationship between vitamin fortification and vitamin paradox from the perspective of vitamin homeostasis.

VITAMIN HOMEOSTASIS AND OXIDATIVE STRESS

Vitamins are essential micronutrients needed by the body in small amounts. Vitamin homeostasis is a balance between vitamin intake and clearance. A deficiency or excess may lead to deleterious effects. Since the introduction of synthetic vitamins into food, high vitamin intake is very common during a person's lifespan from conception through to old age^[2]. In this case, the removal of excess vitamins becomes particularly important in maintaining vitamin homeostasis. This depends on the efficiency of both excretory organs and drug-metabolizing enzymes.

Excretion of vitamins

The kidneys and sweat glands are the two major excretory organs responsible for the elimination of water-soluble vitamins, and the sebaceous glands excrete lipid-soluble vitamins in the sebum^[17]. The excretion of vitamins is positively related to their intake. Aging is known to be associated with decreasing function

of excretory organs^[18,19] and thus may reduce the clearance of vitamins. It is noteworthy that sweat excretion may be particularly important in eliminating excess water-soluble vitamins, because vitamins (e.g., folate^[20], nicotinic acid and nicotinamide^[2,21]) are barely excreted in the urine before degradation due to the reabsorption by the renal tubules, but they can be easily excreted in the sweat^[22-24]. The efficiency of sweat excretion is determined by several factors, including genetic background, intrauterine and early postnatal development, environmental temperature and physical activity. Compared with whites, blacks have a high sweating threshold, manifested by lower skin conductance (*i.e.*, low insensible perspiration)^[25] and sweating rates^[26] under the same ambient temperature condition, suggesting that blacks may have lower sweat excretion of vitamins than whites.

The formation of functional sweat glands begins at week 36 of gestation and completes within 10 wk of postnatal life^[27,28]. This process is affected not only by gestational age but also by the environmental temperature during the early postnatal period. As demonstrated in the literature, preterm birth is associated not only with a lower renal reserve capacity^[29] but also with a low sweating function^[30,31]. Low temperature may cause newborn hypothermia^[32], which may occur even in summer season^[32]. Reduced sweat gland function (*i.e.*, low skin conductance) has been found to be associated with a winter birth in schizophrenia^[33]. Therefore, preterm birth and newborn hypothermia may be associated with decreased vitamin clearance.

Ambient temperature and physical activity are two important factors affecting the excretion rates of sweat and sebum. For example, a decrease in temperature from 30 °C to 22 °C reduces insensible perspiration from about 700 mL/d to 380 mL/d in adults^[34], and a one-degree decrease in local skin temperature decreases the sebum excretion rate by 10%^[35]. There is evidence showing that the levels of plasma vitamin A and E are lower in summer than in winter^[36], and a similar seasonal variation is found in blood drug concentrations^[37]. Thus, it is conceivable that physical inactivity and winter or cold weather would decrease the tolerance to high vitamin intake.

On the other hand, it should be noted that excess sweat vitamin excretion may cause or worsen water-soluble-vitamin deficiency if there is poor vitamin intake. A good example may be pellagra, a niacin-deficiency disease that affects those who live in poverty without sufficient animal-source foods (rich in nicotinamide), with the symptoms occurring during the summer^[38], a season with the highest sweat excretion rates. However, over the past decades, both natural and artificial sources (*i.e.*, vitamin fortification and supplementation) of vitamins have significantly increased^[2], while sweat excretion has significantly decreased due to physical inactivity and the widespread use of air conditioning. These dietary and lifestyle changes may increase the

risk of excess accumulation of vitamins in the body, especially in those with reduced excretory capacity and/or activity.

Degradation of vitamins

Besides being directly excreted, vitamins also undergo degradation through phase I (including oxidation, reduction, and hydrolysis) and phase II metabolisms (e.g., sulfation, methylation and glutathione conjugation), which are catalysed by phase I and phase II drug-metabolizing enzymes, respectively. After phase I and/or phase II degradation, vitamins become more water-soluble and then can be more easily excreted from the body. Excess vitamins are degraded very rapidly. For example, cumulative administration of 2000 mg nicotinic acid [166 times the estimated average daily requirement (EAR)] in 13 h 10 min is found to only increase the levels of its metabolites in the plasma, without significantly changing plasma nicotinic acid concentrations^[39]. We found that, at 5 h after oral administration of 100 mg nicotinamide (8.3 times the EAR), plasma nicotinamide had returned to near baseline levels, while its metabolite *N*¹-methylnicotinamide remained at high levels^[24]. Thus, it is clear that a transient increase in vitamin intake may not change fasting vitamin levels.

Vitamins, xenobiotics, neurotransmitters and hormones share the same drug-metabolizing enzyme system, so they may interact with one another in their metabolism by inducing and competing for the enzymes^[3,40]. For example, CYP2E1, highly expressed in obesity and type 2 diabetes^[13], has more than 50 compounds, including some vitamins and ethanol^[41]. Thus, it is conceivable that alcohol may cause low fasting vitamin levels by induced CYP2E1.

Phase II metabolism of vitamins consumes detoxification resources, such as methyl-group donors, sulphate donors and glutathione, which are also necessary for the degradation of neurotransmitters and hormones. Therefore, excess vitamins can disturb the phase II metabolism of neurotransmitters and hormones by competing for the limited detoxification resources^[3]. Here, we take niacin methylation as an example to explain how excess vitamins affect metabolism of neurotransmitters and hormones. Methylation is a methyl-group transfer reaction from a methyl donor to a substrate, which is mediated by the methionine-homocysteine cycle. Methyl donors, including betaine and choline, are non-renewable resources in the body, while other components in the methylation system, including methionine, folate, vitamin B₁₂ and relevant enzymes, can be repeatedly used in the reaction system. Choline can be used as a methyl donor only after being converted to betaine in the liver and kidneys. According to the relationship of the components in the methylation reaction system shown in Figure 1, it is quite clear that an increase in the levels of substrates will mainly increase the demand for betaine. Since niacin is degraded mainly through

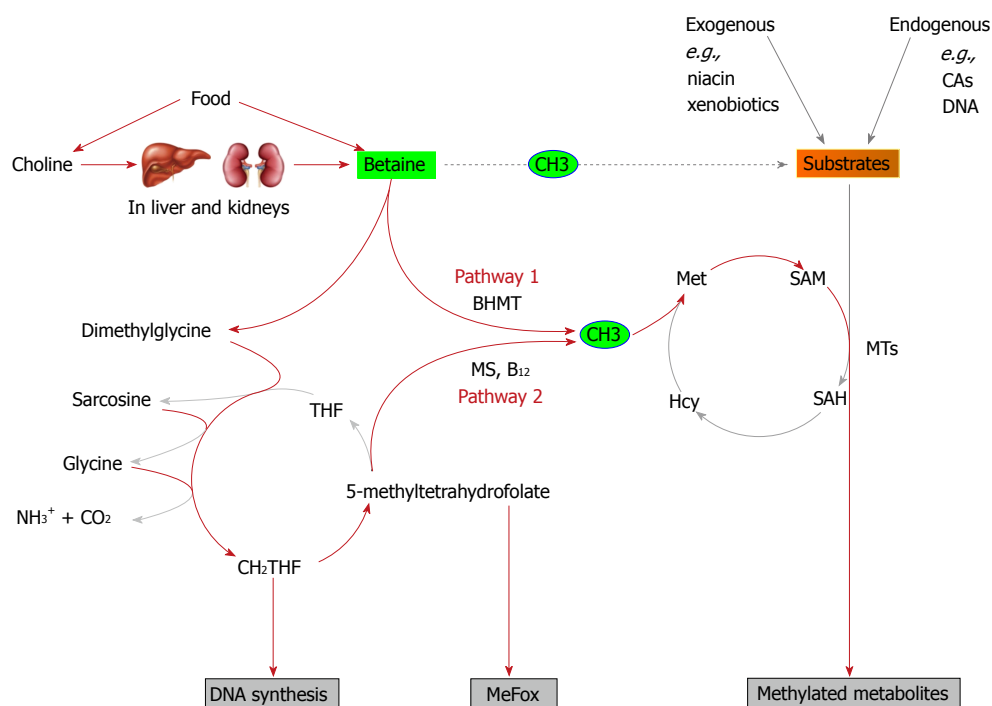


Figure 1 Relationship between methyl donors and mediators in the methylation of substrates. Methylation is a methyl-group transfer reaction from a methyl donor to a substrate, which is mediated by the methionine (Met) cycle. The deep red-arrow lines indicate the flow/transfer of methyl groups/one-carbon units from dietary sources to substrates. In this regard, methylation can be considered as a reaction between betaine and substrates (dashed line). An increase in the levels of substrates will increase the demand for betaine rather than for methylation mediators, e.g., folate and vitamin B₁₂ (B₁₂), because betaine is a non-renewable resource, while the mediators can be recycled if there is an adequate supply of methyl donors. Pathway 1: Betaine-dependent homocysteine (Hcy) remethylation; Pathway 2: Folate-dependent Hcy remethylation. BHMT: Betaine-homocysteine-methyltransferase; CAs: Catecholamines; CH₂-THF: 5,10-methylene tetrahydrofolate; CH₃: Methyl groups; MeFox: An oxidation product of 5-methyltetrahydrofolate; MS: Methionine synthase; MTs: Methyltransferases; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; THF: Tetrahydrofolate.

methylation, niacin fortification/supplementation (usually using its nicotinamide form) increases the demand for methyl groups on the one hand, and on the other hand, it can reduce the utilization of choline as a methyl donor by causing hepatic and renal oxidative injury, as demonstrated in a rat model^[42]. As a result, excess nicotinamide reduces the size of betaine pool and subsequently inhibits the methylation of endogenous substrates (e.g., catecholamines and DNA), leading to an increase in plasma norepinephrine levels^[43] and DNA hypomethylation, an important epigenetic alteration in human diseases^[42,44].

Relationship between vitamin excretion and degradation

There is close cooperation between the excretory system and the drug-metabolizing enzyme system in maintaining vitamin homeostasis. If the body's excretory capacity is too low to effectively eliminate excess vitamins, the activity/expression of the drug-metabolizing enzyme system will compensatorily increase due to induction by their substrates^[45]. Blacks have a lower sweat rate^[2], but have a higher drug/vitamin-metabolizing activity than whites^[46]. For example, compared with whites, blacks have a significantly higher catechol-*O*-methyltransferase (a phase II enzyme that converted norepinephrine to epinephrine)^[47] activity and norepinephrine clearance rate^[48] and, during exercise

stress, they show lower venous plasma norepinephrine and higher epinephrine^[49]. Blacks are prone to low fasting serum vitamin D and folate levels^[12,50] and need a higher vitamin D doses to achieve a desired serum 25-hydroxyvitamin D concentration^[51]. This suggests an increase in plasma vitamin clearance. Given that the levels of plasma and urinary vitamin metabolites are linked to vitamin intake and that vitamins can induce their own degrading enzymes, the findings that increased activity/expression of drug-metabolizing enzymes (e.g., CYP2E1^[13,52] and NNMT^[16]) and high levels of vitamin metabolites (e.g., MeFox^[12], *N*¹-methylnicotinamide^[15,16] and nicotinuric acid^[53]) can be considered as increased compensation for decreased vitamin excretion in response to high vitamin intake.

The degradation of vitamins is accompanied by the generation of reactive oxygen species (ROS). Although ROS at physiological levels functions as signalling molecules, at large levels they can induce cellular toxicity and insulin resistance. In our previous study, we found that co-administration of nicotinamide and glucose (like grain fortification with niacin) can induce insulin resistance due to excess ROS and subsequent reactive hypoglycaemia, demonstrating that vitamin-fortified grains can increase appetite^[2,5]. This may explain the sharp increase in prevalence of obesity in the United States after the levels of vitamin fortification

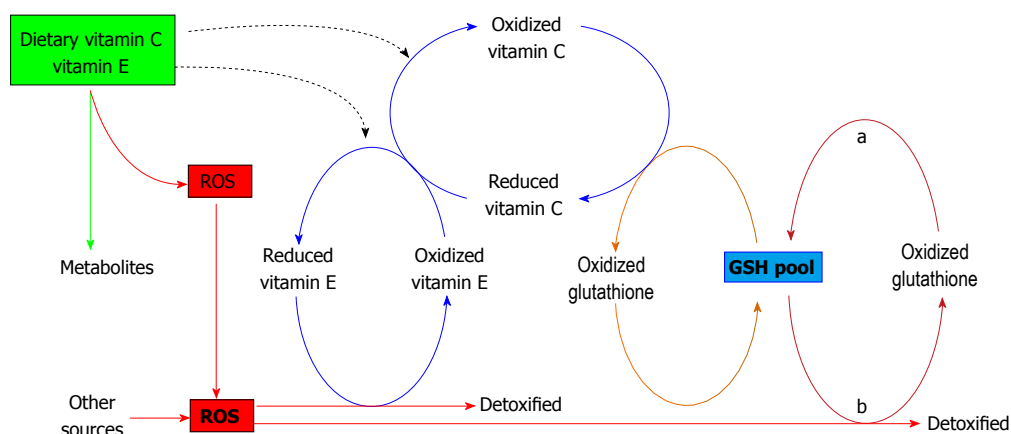


Figure 2 Glutathione-vitamin C-vitamin E interrelationship in the detoxification of reactive oxygen species. The endogenous glutathione antioxidant system maintains vitamin C and vitamin E recycling and actually determines the antioxidant effect of these vitamins. GSH: Reduced glutathione; a: Glutathione reductase; b: Glutathione peroxidase; ROS: Reactive oxygen species.

were increased in 1974^[4,5]. Because decreased sweat excretion may increase enzymatic vitamin degradation and thereby ROS generation, individuals with reduced excretory capacity are at increased risk of insulin resistance, obesity and related diseases when exposed to identical high-vitamin diets.

As shown in Figure 2, it is clear that although vitamin E and C can scavenge ROS, their antioxidant effect actually depends on the capacity of the endogenous glutathione antioxidant system, by which vitamin C and vitamin E recycling is maintained^[54]. Because the endogenous glutathione antioxidant system *per se* directly scavenges free radicals, high levels of supplementation of vitamin C and vitamin E are not only unnecessary but harmful due to increasing the burden of the glutathione antioxidant system. It is obvious that excess vitamin intake may provide an additional source of ROS. Thus, it is not surprising that some randomized clinical trials show that high-dosage vitamin E supplementation may increase, rather than decrease, cardiovascular events and all-cause mortality^[55].

FOLIC ACID FORTIFICATION-INDUCED PARADOX

Although mandatory vitamin fortification has been implemented since the early 1940s and updated in 1974, unfortunately it is hard to determine the relationship between vitamin fortification and the increased prevalence of obesity, mainly because of the lack of studies regarding the effects of vitamin fortification and excess vitamin degradation on the metabolism of the body. Fortunately, the effects of the mandatory folic acid fortification that was started in 1998 in the United States are closely monitored based on the data from National Health and Nutrition Examination Surveys (NHANES). This provides a valuable opportunity for us to understand the vitamin paradox in obesity. The major results of studies on folic acid fortification are summarized as

follows: (1) Blood folate concentrations in the United States population show first a sharp increase from pre- to postfortification (2.5 times for serum and 1.5 times for RBC folate) and then a decline over time (decreased by 17% for serum and 12% for RBC folate during 1999–2010)^[56]; (2) Unmetabolized folic acid was detected in nearly all serum samples measured, and serum unmetabolized folic acid concentrations > 1 nmol/L are associated with being older, non-Hispanic black, nonfasting (< 8 h), higher total folic acid intake (diet and supplements), and higher RBC folate concentrations^[57]; (3) Serum and RBC total folate concentrations, including MeFox (an oxidation product of folate), are high in older adults and individuals with low renal function^[12]; (4) Body mass index is associated negatively with serum unmetabolized folic acid and 5-methyltetrahydrofolate, but positively with serum MeFox and RBC folate concentrations^[12]; (5) Compared with non-Hispanic whites, non-Hispanic blacks have lower serum and RBC total folate concentrations^[12]; (6) In folic acid supplement users, it was found that non-Hispanic black users have lower serum 5-methyltetrahydrofolate concentrations than non-Hispanic-white users^[57]; and (7) Alcohol intake is negatively associated with serum unmetabolized folic acid, 5-methyltetrahydrofolate and MeFox, without significantly affecting RBC folate concentrations^[12].

Evidently, there are significant differences in response to folic acid fortification among the United States population. From the perspective of vitamin homeostasis, the differences may actually reflect differences in folic acid excretion and degradation. Because folic acid is not a natural form of folate, the detection of unmetabolized folic acid in fasting serum suggests a folic acid overload. This overload is more evident in individuals with low excretion capacity, including either low renal function or sweat excretion (in non-Hispanic blacks), or both (in older adults).

The decline in post-fortification serum and RBC folate concentration over time in the United States

population^[56], and the association between increased MeFox levels and decreased renal function^[12] suggests a compensatory increase in folic acid degradation. As mentioned above, blacks may have a higher drug-metabolizing activity to compensate for their reduced sweat excretion. This may account for the finding that non-Hispanic blacks have low serum and RBC total folate concentrations. The association between unmetabolized folic acid concentrations > 1 nmol/L and non-Hispanic blacks^[57] suggests that folic acid intake in this population may exceed their folic acid clearance capacity. Moreover, the low serum 5-methyltetrahydrofolate concentrations in non-Hispanic black users^[57] may suggest a lack of one-carbon donors (due to the increased drug-metabolizing activity in blacks), because the formation of 5-methyltetrahydrofolate consumes one-carbon donors (Figure 1).

Many obesity risk factors, such as being blacks^[11], having a low birth weight/preterm birth^[58], a winter (or cold weather) birth^[59,60], or physical inactivity^[61], are related to decreased sweat-gland function. This is also supported by the finding that an equivalent dose of folic acid (by body weight) caused a greater increase in serum folate in obese than non-obese individuals^[62]. Given that obesity is associated with folate-degrading enzyme CYP2E1^[13,52], the association of increased serum MeFox and RBC folate levels and low fasting serum folate levels in obesity may reflect a severe folic acid overload. From this point of view, the finding that the inverse association between body mass index and serum folate is no longer evident among folic acid supplement users in the United States^[63] can be considered as saturation of the compensatory capacity of the drug-metabolizing enzyme system in obesity.

Ethanol is known to induce drug-metabolizing enzymes^[64,65], including CYP2E1^[66]. This may explain the association between alcohol consumption and low fasting serum folate status. It should be pointed out that alcohol consumption-induced low fasting serum folate does not mean folate deficiency, because there is no significant decrease in RBC folate concentrations^[12].

Overall, four conclusions can be reached: (1) the current folic acid intake of Americans has exceeded their excretory capacity; (2) there is increased compensation for increased folic acid intake, especially in individuals with low excretion capacity; (3) further folic acid supplementation after fortification can saturate the drug metabolizing enzyme system; and (4) the production of MeFox suggests that excess folic acid may increase the consumption of one-carbon units (Figure 1) and provide a source of ROS.

MECHANISM BEHIND LOW VITAMIN D STATUS

There is also a paradox after vitamin D is used in fortification and as a supplement. Vitamin D, although considered a vitamin, can be produced in the skin by

sun exposure. Numerous studies have documented an association between low serum concentrations of 25-hydroxyvitamin D and many non-skeletal disorders. Many studies have examined the effect of vitamin D supplementation on the disorders^[67], including obesity^[68], diabetes^[69], hypertension^[70], dyslipidemia^[71], cardiovascular disease^[72], cancer^[73], depression^[74], and asthma^[75]. Unfortunately, most, if not all, of published meta-analyses have failed to show a significant benefit of vitamin D supplementation with or without calcium^[68-75]. It is likely that low fasting serum 25-hydroxyvitamin D status may be not the cause of these diseases.

The skin is a major determinant of 25-hydroxyvitamin D status. Besides synthesizing vitamin D, the skin also functions as a powerful excretory organ^[17]. Notably, the skin functions fluctuate with seasonal temperature fluctuation, with the highest activities in summer and lowest activities in winter. Thus, it is likely that decreased skin excretory function may be a cause of human diseases. In fact, although not directly focusing on the excretory function of the skin, many studies have suggested a direct link of between the levels of plasma compounds and skin excretory function. For example, sebum excretion decreases in winter^[76,77] and inhibition of sebum excretion increases the levels of blood triglycerides and cholesterol^[78]. Sweat-inhibiting factors (e.g., acute cold exposure^[79,80]) increases plasma norepinephrine levels. Decreased sweating function is found to be closely linked to diseases, for example, skin conductance non-response in schizophrenia and depression^[81], low skin conductance in hypertension^[82] and type 2 diabetes^[83], and the association between psoriasis and metabolic syndrome^[84]. Moreover, many well-known chronic disease risk factors, such as being of black origin, having a preterm birth or winter birth, or physical inactivity, are associated with decreased skin excretory function, as mentioned above. Taken together, it can be concluded that decreased skin excretory function may play a major role in diseases, and 25-hydroxyvitamin D status may be an indicator of skin excretory function.

Interestingly, there is a graded relationship between vitamin D status and body mass index^[85]. Sadiya *et al.*^[86] found that it is difficult to achieve target levels of 25-hydroxyvitamin D above 75 nmol/L in type 2 diabetic obese subjects with a relatively high daily dose of vitamin D₃. Recently, Didriksen *et al.*^[87] performed a 5-year intervention study with vitamin D₃ at a dose of 20000 IU (500 µg) per week vs placebo in subjects with impaired glucose tolerance and/or impaired fasting glucose, and they found that those given vitamin D₃ had significantly higher vitamin D concentration in their adipose tissue (about 6.5 times the placebo group), while their median serum 25-hydroxyvitamin D level only increased from the baseline of 61 to 99 nmol/L. This study clearly demonstrates that large amounts of vitamin D₃ are stored in adipose tissue after vitamin D₃ supplementation, and suggests that overweight and

obese subjects may store more vitamin D than normal-weight subjects because they have larger amounts of adipose tissue. Moreover, vitamin D is known to induce drug-metabolizing enzymes^[88]. Thus, it seems likely that the prevalence of low 25-hydroxyvitamin D status after the introduction of vitamin D fortification may share a similar mechanism to that of low folate status: increased degradation and storage in compensation for excess intake.

THE CLINICAL SIGNIFICANCE OF THE VITAMIN PARADOX

Understanding the vitamin paradox in obesity and related diseases is crucial in determining how to manage the low vitamin status in these diseases. From the above analysis, it is apparent that the vitamin paradox in obesity may be due to increased vitamin degradation and storage in compensation for decreased vitamin excretion. This condition will continue until drug-metabolizing enzymes are saturated by their substrates, in which high expression of vitamin-degrading enzymes and elevated vitamin-metabolite levels may serve as indicators. The vitamin paradox can be resolved by reducing vitamin intake and increasing sweat rates, rather than by giving vitamin supplementation. Indeed, a recent study shows that bariatric surgery (restricting food intake) and exercise are associated with a significant reduction in NNMT expression plasma MNA levels^[16]. This can be explained by decreased niacin intake and increased sweat excretion.

Excess vitamins have three major detrimental effects: (1) increasing ROS generation and subsequently leading to oxidative tissue damage and insulin resistance; (2) disturbing the degradation of neurotransmitters and hormones by competing for drug metabolizing enzymes and detoxification resources; and (3) causing epigenetic changes (*e.g.*, altered DNA methylation) by depleting the body's methyl-group pool^[2,89]. Thus, fortification-induced sustained excess vitamin intake may deplete the drug-metabolizing system (*e.g.*, manifested by high levels of unmetabolized vitamins) and the antioxidant system, and eventually cause a variety of metabolic disorders and oxidative tissue damage. This may play a causal role in the increased prevalence of obesity and related diseases, as hypothesized in our previous work^[2,4,5].

The association between high vitamin intake and chronic diseases can be considered as vitamin poisoning. Vitamin poisoning is dose dependent. For example, high-dosage vitamin E may increase cardiovascular events and all-cause mortality^[55]. Two recent large-scale randomized niacin trials (nicotinic acid, 1500-2000 mg/d) show that nicotinic acid has many adverse effects, including loss of glycaemic control among persons with diabetes, new-onset diabetes^[90,91] and increased risk of death, with borderline statistical significance ($P = 0.08$)^[90]. There are three factors that can increase

the risk of vitamin poisoning: (1) the function of excretory organs is too low to effectively remove excess vitamins from the body, for example, due to early-life malnutrition-induced renal insufficiency^[92]; (2) the amount of vitamin intake has exceeded the excretory capacity of individuals without any developmental defect, which may account for excess chronic diseases in blacks and those with physical inactivity; and (3) the combination of both (1) and (2), accounting for the high rates of chronic diseases in subjects born preterm after the implementation of vitamin fortification. Because the reserve capacity of excretory/detoxifying organs has been determined in early life, whether or not chronic diseases occur will depend on whether there are chemical overloads of the excretory/detoxifying organs in late life. This may be the mechanism of the origin of chronic diseases. Excess vitamin is a kind of chemical overload, accounting for the association between the prevalence of obesity and diabetes and increased B-vitamin intake^[4].

CONCLUSION

In summary, it can be concluded that the vitamin paradox in obesity may be a reflection of excess vitamin intake, rather than a vitamin deficiency. Given that there is a correlation between high vitamin intake and the increased prevalence of obesity, it can be assumed that obesity could be one of manifestations of chronic vitamin poisoning. Susceptible individuals to high vitamin intake are those with a low reserve capacity of excretory organs. Therefore, on an individual basis, prevention of obesity should focus on reducing their intake of vitamin-fortified foods, and for a country, more attention needs to be paid to the role of vitamin fortification and abuse in the increased prevalence of obesity and related diseases.

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Basic Study

Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats

María J Crespo, José Quidgley

María J Crespo, Departments of Physiology and Anesthesiology, University of Puerto Rico-School of Medicine, San Juan, PR 00936-5067, United States

María J Crespo, José Quidgley, Departments of Physiology, University of Puerto Rico-School of Medicine, San Juan, PR 00936-5067, United States

Author contributions: Crespo MJ and Quidgley J contributed equally to this work.

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Correspondence to: Dr. María J Crespo, Departments of Physiology and Anesthesiology, University of Puerto Rico-School of Medicine, GPO Box 365067, San Juan, PR 00936-5067,

United States. maria.crespo3@upr.edu
 Telephone: +1-787-7530120
 Fax: +1-787-7530120

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Abstract

AIM: To investigate if the effect of statins improving cardiovascular (CV) status of diabetics is drug-specific or class-dependent, and the underlying mechanisms involved.

METHODS: We compared the results of daily administration over a four-week period of a low dose (10 mg/kg per day) of atorvastatin (AV), simvastatin (SV), and pravastatin (PV) on cardiac performance in diabetic rats. Echocardiographic variables were tested, as well as systolic blood pressure (SBP), acetylcholine (ACh)-induced relaxation, plasma cholesterol levels, and perivascular fibrosis. Malondialdehyde (MDA) and 4-hydroxyalkenal (4-HAE), and endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) protein levels were also measured in cardiac and aortic homogenates.

RESULTS: In untreated diabetic rats, cholesterol levels were higher than in control rats (CT; $n = 8$, $P < 0.05$), and the low dose of statins used did not modify these levels. In diabetic rats, SBP was higher than in CT, and was significantly reduced by all three statins ($n = 10$, $P < 0.05$). Echocardiographic parameters (EF, SV, and COI) were all lower in untreated diabetic rats than in CT ($n = 10$, $P < 0.05$). These CV parameters were equally

improved by all three statins. The maximal relaxation (E_{Max}) induced by ACh in aortic ring from diabetic rats was also improved. Moreover, this relaxation was abolished by 1 mmol/L NG-nitro-L-arginine methyl ester, suggesting the involvement of a NO-dependent mechanism.

CONCLUSION: AV, SV, and PV are equally effective in improving CV performance in diabetic rats. All three statins decreased media thickness, perivascular fibrosis, and both MDA and 4-HAE in the aortas of diabetic rats, without affecting eNOS and iNOS protein levels. The observed hemodynamic benefits are cholesterol-independent. These benefits appear to be secondary to the improved endothelial function, and to the reduced vascular tone and remodeling that result from decreased oxidative stress.

Key words: Statins; Diabetes; Oxidative stress; Cardiac function; Perivascular fibrosis

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Core tip: Despite evidence that statins are useful therapeutic tools in treating diabetes, questions remain as to whether their effects are drug-specific or class-dependent, what mechanisms underlie these effects, and which statin is the most appropriate. We found that atorvastatin, simvastatin, and pravastatin are equally effective in improving cardiovascular performance in Type 1 diabetic rats, and that the observed benefits are likely to be secondary to the reduction of oxidative stress by these drugs.

Crespo MJ, Quidgley J. Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats. *World J Diabetes* 2015; 6(10): 1168-1178 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i10/1168.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i10.1168>

INTRODUCTION

Diabetes is a group of metabolic diseases primarily characterized by hyperglycemia resulting from defects in insulin production, action, or both. This condition has been associated with an increased risk of cardiovascular (CV) deterioration, which is the major cause of death in diabetic patients^[1-3]. CV complications include hypertension, ischemic heart disease, heart failure, and diabetic nephropathy. The etiology of cardiac abnormalities in diabetes has been linked to increased oxidative stress and endothelial dysfunction, although the precise mechanism for these complications remains elusive^[4-6].

The addition of statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, to standard antiglycemic therapies decreases CV complications in diabetic patients. The American

Diabetes Association's "Standards of Medical Care in Diabetes-2015"^[7], recommends the use of statins for all diabetics under 40 years of age with additional CV risk factors, or with overt CV disease. It further recommends that diabetics over the age of 40 take statins, regardless of the absence of CV risk factors. Indeed, in Type 2 diabetics without elevated cholesterol, the risk of suffering the first CV event is reduced by atorvastatin (AV)^[8]. The statin-induced improvement of cardiac function in normo-cholesterolemic patients suggests that these drugs have pleiotropic benefits that may be independent of their ability to lower cholesterol levels^[9,10]. The mechanisms underlying these beneficial effects may include improvement of endothelial function through increased systemic NO bioavailability^[11] and endothelial nitric oxide synthase (eNOS) expression^[12], or through reduced oxidative stress^[13,14].

Despite evidence that statins are useful therapeutic tools in diabetes, questions remain as to whether their effect is drug-specific or class-dependent, which statin is most appropriate, and what mechanisms underlie this effect. In the present study, we compared the effects of three different statins (AV, SV, and PV) on the CV profile of streptozotocin (STZ)-induced diabetic rats that did not receive insulin supplementation. This animal model of Type 1 diabetes is a validated model for the study of diabetic effects on the CV system. At four weeks following diabetic induction, the rats are hypertensive and have decreased cardiac output, stroke volume, and ejection fraction, when compared to age-matched controls (CT)^[14,15]. To evaluate and compare the effects of these statins on cardiac function, we measured stroke volume, ejection fraction, and cardiac output with echocardiography. The effects of statins on endothelial function, cholesterol level, and vascular remodeling were also evaluated. The results from this study may help to identify the most effective statin for improving the CV profile in diabetics.

MATERIALS AND METHODS

Experimental animal model

Four-week-old male Sprague-Dawley rats (120-125 g average weight) were acquired from Hilltop Lab Animals, Inc. (Scottsdale, PA). A total of 160 rats were divided into two groups, diabetic and CT, with each group containing 80 animals. Diabetes was induced by injecting intraperitoneally (IP) streptozotocin (STZ, 65 mg/kg) dissolved in 0.1 mol/L citrate buffer (pH 4.5) after an overnight fast. Diabetic induction was confirmed with positive blood glucose tests twenty-four hours after STZ injection, (Accu-Chek Simplicity, Roche, Indianapolis, IN). Glucose was weekly monitored. The rats did not receive insulin and the experiments were performed at 4 wk after induction of diabetes.

Drug administration

After diabetic induction, each rat was treated daily

with the selected drug (AV or SV or PV) over a four-week period. The statins were suspended in corn oil and administered by gavage at a dose of 10 mg/kg per day. The volumes of all administered drugs were adjusted weekly according to each animal's weight in order to ensure a constant dose. Untreated diabetic and CT groups received by gavage only corn oil, as a placebo. Statin doses were selected based on previous studies on diabetic rats, and from our laboratory^[16,17]. In order to obtain cholesterol level reductions similar to those attained in humans, a 50 mg/kg per day statin administration is needed in rats^[13]. Thus, a low dose of 10 mg/kg per day allowed us to assess the effect of statins on the CV system independently of the benefits derived from cholesterol reduction.

Echocardiographic evaluation

Serial transthoracic echocardiographic evaluations were performed using an ultrasound system with a 7.5 to 9.0 MHz transducer (Sonosite Inc. WA), after anesthesia (30 mg/kg BW, IP), following a previously described protocol^[17,18]. Image analysis was performed using Sitelink Image Manager (Sonosite Inc., Bothell, WA).

Noninvasive measurement of systolic blood pressure

Noninvasive systolic blood pressure (SBP) was evaluated using a RTBP-2000 system (Kent Scientific, Litchfield, CT), and analyzed with Lab View Program (National Instruments Co. Austin, TX) as previously described^[19].

Evaluation of acetylcholine-induced relaxation

To evaluate endothelium-dependent relaxation, aortic rings (5 mm) from the descending thoracic aorta were placed in Krebs' bicarbonate solution (composition in mmol/L: 118 NaCl, 2.5 CaCl₂, 5 KCl, 1.1 MgSO₄, 25 NaHCO₃, 1.2 KH₂PO₄ and 10 glucose, pH = 7.4). The rings were suspended horizontally with a resting tension of 2.0 g, and connected to a FT03C Grass transducer, following the protocol previously described by our laboratory^[19]. The effect of statins on acetylcholine (ACh)-induced relaxation was evaluated in rings pre-contracted with norepinephrine (NE, 1.0 µmol/L). Cumulative concentration-response curves (from 0.1 nmol/L to 10 µmol/L) for ACh were generated after equilibration. An additional dose response curve was then performed after a 45-min incubation period with L-NAME (1 mmol/L). For a particular ACh concentration, the relaxation was expressed as a percentage of the maximal contraction induced by 1.0 µmol/L of NE.

Cholesterol level determination

Blood samples from both untreated and treated diabetic rats and from CT were centrifuged (5000 rpm; 5 min; 4 °C to measure cholesterol concentration. Total cholesterol levels were quantified a cholesterol quantitation kit (Sigma-Aldrich, MAK043). A SpectraMax Microplate Reader (Molecular Devices, CA) was used to

measure sample absorbance at 570 nm. A calibration curve using cholesterol standards was used to quantify cholesterol levels.

Measurement of malondialdehyde and 4-hydroxyalkenals levels

The effect of statins on lipid peroxidation, a marker of oxidative stress, was evaluated following the previously described protocol^[20]. Malondialdehyde (MDA) and 4-hydroxyalkenals (4-HAE) levels were determined in cardiac and vascular homogenates at an absorbance of 586 nm.

Measurement of media thickness and perivascular fibrosis

Perivascular fibrosis and media thickness from the thoracic aorta from untreated and treated animals were determined to assess the effect of statin treatment. Tissues were stained with Azan-Mallory and Hematoxylin and Eosin (H and E) following the methodology previously described by our laboratory^[20]. Results (in µm) were normalized to body weight.

Western Blot for eNOS and inducible nitric oxide synthase

Western Blot studies were performed using a modified protocol described previously^[21]. Protein samples were separated by electrophoresis in a 6% SDS-PAGE gel. Proteins were transferred to a nitrocellulose membrane. Membranes were blocked with 5% Blotto for 1 h. Mouse monoclonal antibodies for eNOS (1:2000 for cardiac tissue, 1:3000 for aortic tissue; BD Biosciences, San Jose, CA), inducible nitric oxide synthase (iNOS) (1:500 for cardiac tissue, 1:750 for aortic tissue; BD Biosciences, San Jose, CA), were added to the membrane after dilution in Blotto, and incubated overnight at 4 °C. The nitrocellulose membranes were incubated with the secondary anti-mouse antibody coupled to Horseradish Peroxidase (HRP) (1:4000; Santa Cruz Biotechnology, Santa Cruz, CA). Before exposure and development, the membranes were incubated with Super Signal West Femto Maximum Sensitivity Substrate (Thermoscientific, Waltham, MA) to enhance the HRP signal derived from the secondary antibody. The Versadoc™ Imaging System and Quantity One Software (Bio-Rad Laboratories, CA) were used to develop and analyze the membranes. eNOS and iNOS levels were standardized by comparison with the β-actin housekeeping gene detected (1:4000; Sigma-Aldrich, St. Louis, MO).

Statistical analysis

All data are expressed as the mean ± SEM (GraphPad Software, Inc., San Diego, CA). Differences between experimental groups were analyzed using Student's *t* and ANOVA, followed by Student-Newman-Keuls test for posthoc analysis. Values were considered statistically significant at a *P* value less than 0.05.

Table 1 Blood glucose (mg/dL) in diabetic and control rats treated with statins

Condition	Day 0	Day 1	Day 7	Day 14	Day 28
CT none	131.25 ± 3.14	130.50 ± 1.51	126.78 ± 4.86	112.42 ± 4.55	133.88 ± 13.66
CT + AV	137.0 ± 5.86	123.33 ± 2.85	126.8 ± 2.2	128.40 ± 7.02	180.67 ± 52.21
CT + SV	143.13 ± 1.75	128.50 ± 3.10	126.80 ± 2.22	128.40 ± 7.02	152.88 ± 18.46
CT + PV	142.63 ± 5.79	127.13 ± 3.36	122.20 ± 4.79	120.80 ± 4.47	130.63 ± 4.06
Diabetic none	133.65 ± 3.51	445.41 ± 24.11 ^a	490.45 ± 34.34 ^a	530.09 ± 26.65 ^a	517.76 ± 18.11 ^a
Diabetic + AV	133.00 ± 3.30	473.82 ± 40.23 ^a	497.38 ± 47.68 ^a	485.38 ± 48.73 ^a	500.73 ± 32.65 ^a
Diabetic + SV	133.75 ± 2.70	413.19 ± 21.22 ^a	473.69 ± 27.39 ^a	483.23 ± 39.90 ^a	498.94 ± 30.62 ^a
Diabetic + PV	126.88 ± 2.08	430.44 ± 27.31 ^a	524.38 ± 19.92 ^a	564.85 ± 13.57 ^a	557.25 ± 12.92 ^a

^a*P* < 0.05 *vs* age-matched C. Values are means ± SEM. Rats were injected with STZ on day 0. Blood glucose for diabetic rats: *n* = average of 10 rats per group. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control; STZ: Streptozotocin.

Table 2 Body weight (g) of diabetic and control rats treated with statins

Condition	Day 0	Day 1	Day 7	Day 14	Day 28
CT none	179.74 ± 4.02	182.28 ± 4.15	242.78 ± 6.18	304.06 ± 13.26	391.34 ± 9.80
CT + AV	167.17 ± 4.38	172.97 ± 4.53	234.77 ± 5.27	287.67 ± 4.67	404.10 ± 12.46
CT + SV	181.89 ± 8.26	184.19 ± 8.12	248.40 ± 7.99	291.69 ± 6.93	394.14 ± 15.92
CT + PV	193.81 ± 8.25	198.85 ± 7.66	262.99 ± 9.03	301.19 ± 8.02	389.64 ± 11.48
Diabetic none	180.22 ± 5.99	172.53 ± 5.24	198.19 ± 7.41	211.94 ± 11.11 ^a	267.10 ± 27.98 ^a
Diabetic + AV	176.30 ± 3.10	159.86 ± 9.93	202.32 ± 5.13	235.45 ± 8.56 ^a	255.40 ± 17.09 ^a
Diabetic + SV	183.14 ± 4.68	178.24 ± 4.29	207.69 ± 6.81	230.13 ± 7.23 ^a	241.65 ± 12.73 ^a
Diabetic + PV	187.88 ± 5.62	182.58 ± 4.60	204.85 ± 7.18	243.69 ± 5.60 ^a	253.99 ± 11.82 ^a

^a*P* < 0.05 *vs* age-matched C. Values are means ± SEM. Rats were injected with STZ on day 0. Blood glucose for diabetic rats: *n* = average of 10 rats per group. STZ: Streptozotocin; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

Table 3 Total cholesterol levels in plasma from diabetic and control rats after four weeks of statin treatment (10 mg/kg per day)

Condition	Cholesterol (mg/dL)
CT none	156.01 ± 7.32
CT + AV	143.69 ± 14.21
CT + SV	169.86 ± 12.78
CT + PV	155.53 ± 7.08
Diabetic none	248.68 ± 15.78 ^a
Diabetic + AV	233.35 ± 18.44 ^a
Diabetic + SV	234.40 ± 12.11 ^a
Diabetic + PV	235.57 ± 18.20 ^a

Values shown are the means ± SEM of an average of 8 animals per group.

^a*P* < 0.05 *vs* age-matched treated and untreated CT. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

RESULTS

Blood glucose, body weight, and cholesterol levels are shown in Tables 1, 2 and 3. Twenty four-hours after diabetic induction, blood glucose levels were significantly higher in diabetic rats than in CT rats (445.41 ± 24.11 mg/dL *vs* 130.50 ± 1.51 mg/dL, respectively; *n* = 10, *P* < 0.05; Table 1). This difference was maintained throughout the course of the study and was not affected by the administration of any statin. Body weight increased in both diabetic and CT rats over the course of this study, although it was significantly lower in aged-matched diabetic rats (Table 2). This parameter also was not modified by any statin. Total cholesterol levels were significantly increased in diabetic

rats when compared to aged-matched CT (248.68 ± 15.78 mg/dL *vs* 156.01 ± 7.3 mg/dL; *n* = 8, *P* < 0.05; Table 3). At 10 mg/kg per day, once again, statins did not modify plasma cholesterol levels in either diabetic or CT rats (*n* = 8, *P* > 0.05).

In diabetic rats, stroke volume (Figure 1A) increased significantly after statin treatment (from 0.20 ± 0.02 mL in untreated, to 0.51 ± 0.06 mL with AV, to 0.47 ± 0.05 mL with SV, and to 0.43 ± 0.05 mL with PV; *n* = 10, *P* < 0.05). In diabetic rats ejection fraction was lower than in CT (Figure 1B; 44.93% ± 3.03% *vs* 70.67% ± 2.11%; *n* = 10, *P* < 0.05), but also improved after statin treatment (to 59.92% ± 2.98 % with AV, to 60.13% ± 3.55% with SV, and to 56.85% ± 4.45% with PV; *n* = 10, *P* < 0.05). Similarly, cardiac output index (mL/min per 100 g BW) improved after statins treatment in diabetic rats (from 24.74 ± 3.52 in untreated to 57.65 ± 6.59 with AV, to 60.13 ± 4.10 with SV and to 53.25 ± 6.19 with PV; *n* = 10, *P* < 0.05) (Figure 1C).

SBP (Figure 2) was higher in diabetic rats than in CT (116.52 ± 3.81 mmHg in STZ *vs* 82.72 ± 2.36 mmHg in CT; *n* = 10, *P* < 0.05. Administration of statins significantly reduced this variable in diabetic rats (to 100.91 ± 5.15 mmHg with AV, 93.17 ± 3.31 mmHg with SV, and 106.44 ± 4.21 mmHg with PV; *n* = 10, *P* < 0.05).

The maximal relaxation (*E*_{max}) induced by ACh (Figure 3) was significantly reduced in the aortic rings from diabetic rats compared to those from aged-matched CT (53.70% ± 4.07% *vs* 74.61% ± 3.27%; *n* = 10, *P* < 0.05). This finding confirms that, at four weeks

Table 4 Effect of chronic statin treatment on EC₅₀ and E_{MAX} values following ach-induced relaxation in diabetic and control rats

Condition	E _{max} relaxation, %	EC ₅₀ , μ mol/L
CT none	74.61 \pm 3.27	0.56 \pm 0.11
CT + AV	70.75 \pm 3.99	0.68 \pm 0.22
CT + SV	70.76 \pm 4.16	1.15 \pm 0.47
CT + PV	71.16 \pm 4.30	0.72 \pm 0.20
Diabetic none	53.70 \pm 4.07 ^a	0.41 \pm 0.10
Diabetic + AV	82.13 \pm 7.01 ^c	0.84 \pm 0.32
Diabetic + SV	84.63 \pm 6.51 ^c	0.40 \pm 0.21
Diabetic + PV	83.88 \pm 6.83 ^c	0.66 \pm 0.35

Values shown are the means \pm SEM of an average of 10 animals per group. ^a*P* < 0.05 when *vs* age-matched treated and untreated CT; ^c*P* < 0.05 when *vs* age-matched untreated diabetic rats. No statistically significant differences were found between treated diabetic rats and treated CT. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

following diabetes induction, endothelial dysfunction is present in the aorta of diabetic rats. The tested statins significantly improved E_{max} values in diabetic rats (82.13% \pm 7.01% with AV, 84.63% \pm 6.51% with SV, and 83.88% \pm 6.83% with PV; *n* = 10, *P* < 0.05), but did not modified this value in CT. Moreover, a 45-min incubation period with 1 mmol/L L-NAME completely abolished the ACh-induced relaxation, indicating that the effect of these statins on vascular relaxation is NO-mediated. EC₅₀ values, by contrast, were not modified by any statin in either diabetic rats or CT (Table 4).

MDA and 4-HAE (μ mol/g protein), which are oxidative stress markers were higher in aortic homogenates (Figure 4A) from diabetic rats than in those from CT (6.49 \pm 1.24 *vs* 3.69 \pm 0.58; *n* = 8, *P* < 0.05). In diabetic rats, but not in CT, all statins significantly reduced MDA and 4-HAE levels (2.69 \pm 0.42 with AV, 3.59 \pm 0.47 with SV, and 4.03 \pm 0.40 with PV; *n* = 8, *P* < 0.05). In cardiac homogenates (Figure 4B), by contrast, MDA and 4-HAE levels were similar in untreated diabetic (1.42 \pm 0.12) and CT (1.10 \pm 0.12; *n* = 8, *P* > 0.05), and statin treatment did not modify these parameters.

Similar segments of the thoracic aorta from STZ-diabetic rats and CT were investigated to assess the effects of statins on vascular remodeling. In untreated diabetic rats, perivascular fibrosis (Figure 5A) was higher than in CT (10.59 \pm 0.40 μ m/100 g BW *vs* 4.21 \pm 0.22 μ m/100 g BW; *n* = 5, *P* < 0.05). All statins reduced perivascular fibrosis in diabetic rats (8.99 \pm 0.33 μ m/100 g BW with AV, 8.75 \pm 0.43 μ m/100 g BW with SV, and 9.04 \pm 0.39 μ m/100 g BW with PV; *n* = 5, *P* < 0.05). Perivascular fibrosis in CT, by contrast, was not modified by any of the statins. In addition, media thickness, which was thicker in diabetic rats than in age-matched CT (49.70 \pm 1.10 μ m/100 g BW *vs* 46.03 \pm 0.67 μ m/100 g BW; *n* = 5, *P* < 0.05), was significantly reduced by all the statins in diabetic rats (44.93 \pm 0.76 μ m/100 g BW with AV, 47.15 \pm 0.48 μ m/100 g BW with SV, and 46.78 \pm 0.67 μ m/100 g BW with PV), but

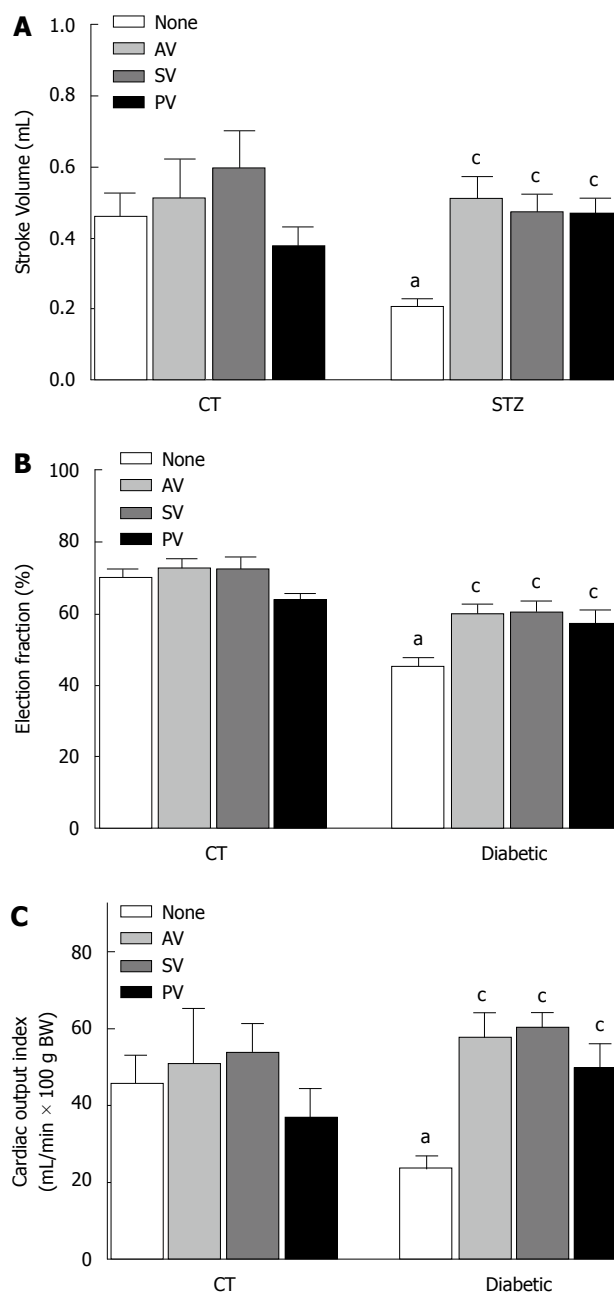


Figure 1 Effects of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on diabetic rats and control. A: Stroke volume (mL); B: Ejection fraction (%); C: Cardiac output index (mL/min \times 100 g BW). The results represent the mean \pm SEM of 8 animals per group. All the statins significantly improved these CV parameters in diabetic rats. ^a*P* < 0.05 for diabetic rats *vs* CT; ^c*P* < 0.05 for untreated diabetic rats *vs* treated diabetic rats. STZ: Streptozotocin; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

not in CT.

The effect of chronic statin treatment on iNOS and eNOS protein levels (% relative to CT) was evaluated in aortic (Figure 6) and cardiac (Figure 7) tissue from diabetic rats and CT. Comparing the two groups, iNOS levels were similar in aortic tissue (115.40% \pm 48.08% in diabetic *vs* 100% in CT; *n* = 5, *P* > 0.05) and in cardiac tissue (155.30% \pm 54.47% in diabetic *vs* 100% in CT; *n* = 5, *P* > 0.05). Whereas eNOS levels in cardiac tissue also did not differ (92.16% \pm 16.07% in diabetic

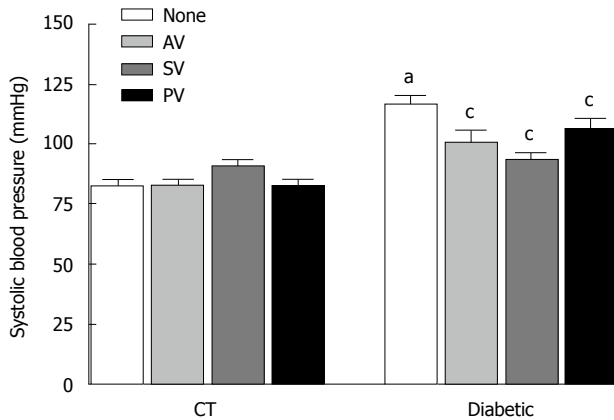


Figure 2 Effects of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on systolic blood pressure (mmHg) in diabetic rats and control. The values shown are the means \pm SEM of 10 animals per group. All statins significantly decreased blood pressure in diabetic rats. ^a $P < 0.05$ for diabetic rats vs CT; ^c $P < 0.05$ for untreated diabetic rats vs treated diabetic rats. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

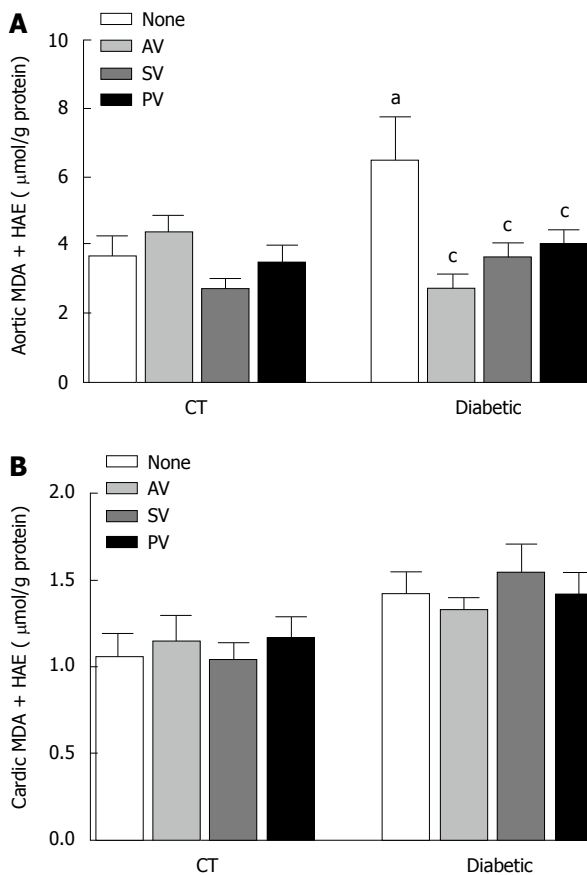


Figure 4 Effect of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on malondialdehyde + 4-hydroxyalkenal levels in aortic homogenates (A) and in cardiac homogenates (B) from diabetic rats and control. For diabetic rats, all statins equally reduced lipid peroxidation levels in aortic homogenates, but had no effect on these levels in cardiac homogenates. For CT, no effect of statins was observed in either aortic or cardiac homogenates. The values shown are the means \pm SEM of 8 animals per group. ^a $P < 0.05$ for diabetic rats vs CT; ^c $P < 0.05$ for untreated diabetic rats vs treated diabetic rats. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

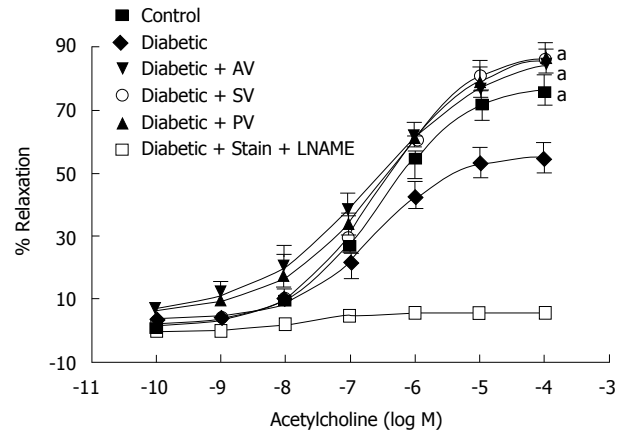


Figure 3 Cumulative concentration response curves for acetylcholine-induced relaxation of aortic rings from diabetic rats after four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day). Aortic rings were precontracted with 0.1 $\mu\text{mol/L}$ norepinephrine (NE) before the addition of cumulative concentrations of ACh. Note that the addition of 1 mmol/L L-NAME to the incubation bath inhibited ACh-induced relaxation. The values shown are the means \pm SEM of 10 animals per group. ^a $P < 0.05$ for E_{MAX} between untreated diabetic rats and treated diabetic rats. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; ACh: Acetylcholine.

vs 100% in CT; $n = 5$, $P > 0.05$), the levels were reduced in aortic tissue ($54.37\% \pm 7.29\%$ in diabetic vs 100% in CT; $n = 5$, $P < 0.05$). Nevertheless, statin treatment had no effect on either aortic eNOS or iNOS protein levels.

For all tested variables, no significant differences were found between the effects of the three statins. AV, SV, and PV equally improved cardiac function, vascular function, and reduced perivascular fibrosis and oxidative stress.

DISCUSSION

In this study, we compared the effects of AV, SV, and PV on CV performance of Type 1 diabetic rats. For the first time, we report that these three statins similarly improve the CV function of this animal model at a low dose of 10 mg/kg per day. Each statin improves ACh-induced relaxation and CV function, and reduces aortic oxidative stress and remodeling, without lowering cholesterol levels.

In both, patients and animal models of diabetes the beneficial effects of statins improved vascular dysfunction. In diabetic rats, a 50 mg/kg per day dose of AV improves ACh-dependent relaxation^[22]. In spontaneously hypertensive rats, a lower dose of 20 mg/kg also improves vascular function^[13]. Improvements of vascular function are also observed in Type 1 diabetic patients, where both AV (40 mg/d) and PV (40 mg/d per 1 mo) normalize flow-mediated dilatation^[23,24]. Moreover, SV (40 mg/d per 8 wk) improves endothelial-dependent relaxation in hypercholesterolemic patients^[25]. In the current study, we demonstrated that all three statins tested (AV, SV,

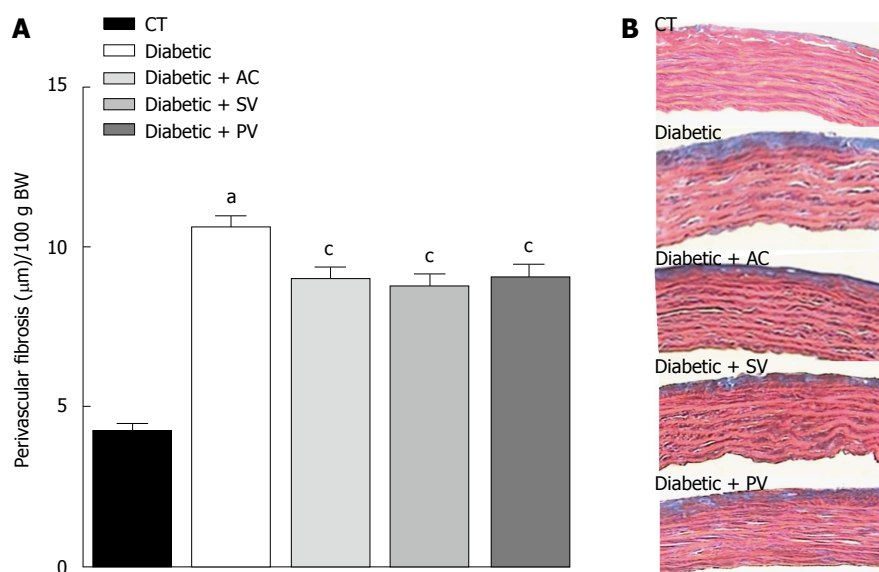


Figure 5 Representative histological sections of aortic segments from untreated and statin-treated diabetic rats, and untreated control. A: Quantified thickness of perivascular fibrosis in comparable aortic segments from treated diabetic rats and untreated diabetic rats. Perivascular fibrosis was higher in untreated diabetic rats than in CT. All statins decreased perivascular fibrosis in diabetic rats. The values shown are the means \pm SEM of 5 animals per group, with the mean value for each animal based on five measurements of its aortic segment. ^a $P < 0.05$ for untreated diabetic rats vs untreated CT; ^c $P < 0.05$ for untreated diabetic rats vs treated diabetic rats; B: Representative histological sections ($\times 40$, Azan-Mallory stain) of aortic segments from untreated diabetic rats and treated diabetic rats, demonstrating the typical reduction in perivascular fibrosis after treatment with each individual statin. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

and PV) improve endothelium-dependent relaxation equally in the aortic rings of Type 1 diabetic rats, but at a low dose of only 10 mg/kg per day.

Nevertheless, controversy still exists regarding the beneficial effects of statins on vascular function. For example, among Type 2 diabetic patients with normal cholesterol levels, endothelial function is not restored after the administration of AV (40 or 80 mg/d per 30 wk)^[26], or SV (40 mg/d per 6 wk)^[27]. Similarly PV (40 mg/d per 8 wk) was ineffective in improving endothelial-induced relaxation in patients with coronary heart disease^[28]. The lack of effect of statins in these cases may be due, at least in part, to differences among the experimental models, patient co-morbidities, statin doses, and treatment duration.

The EC₅₀ for the ACh-induced relaxation curves is not modified by any of the three statins tested, indicating that the mechanisms by which these drugs improve endothelial function do not include changes in ACh affinity for the muscarinic receptor. The improvement, however, is fully abolished by L-NAME, suggesting that AV, SV, and PV improve vascular function by increasing NO availability. Whereas all three statins reduce lipid peroxidation markers in the aorta, none modify cardiac or vascular eNOS or iNOS protein levels. Thus, the observed CV improvements at this low dose are most likely secondary to the antioxidant properties of the statins, rather than due to their direct stimulation of NO production. In addition, although the etiology of hypertension is largely unknown, oxidative stress, endothelial dysfunction, and structural alterations of the vasculature have been associated with hypertensive pathophysiology. Thus, the reduction of oxidative stress

and vascular remodeling, together with the improved endothelial dysfunction observed following statin treatment, may underlie the reduced SBP found in diabetic rats.

The results of some studies differ from ours, however. Wenzel *et al.*^[29] found that AV (20 mg/kg per day per 7 wk) decreases eNOS uncoupling in Type 1 diabetic rats. In addition, Ito and colleagues^[30] reported that in the kidney of spontaneously hypertensive rats, AV (20 mg/kg per day per 8 wk) increases eNOS and nNOS expression. Moreover, in endothelial cell cultures from human saphenous vein SV (1 μ mol/L) increases eNOS mRNA and function^[31]. It is possible that statins modify NOS activity and/or expression in a dose-dependent manner. If such is the case, the lack of effect on eNOS and iNOS activity observed in the current study may be due to dosage differences. Alternatively, or in addition, experimental models (*e.g.*, *in vivo* vs *in vitro*) and treatment duration are likely to be major factors underlying this discrepancy.

CV status is deteriorated in diabetic rats by four weeks after induction of diabetes^[19,32]. That AV, SV, and PV equally increasing ejection fraction, stroke volume, and cardiac output suggest that the cardioprotective effect of statins is class-related rather than drug-specific. In addition, this pleiotropic effect appears to be independent of the ability of these drugs to lower cholesterol levels. Improvement of systolic function may result from reductions in peripheral resistance secondary to increased endothelial function, decreased blood pressure, and the vascular remodeling regression observed with all three statins. In line with our results, SV (10 mg/kg per day per 8 wk)

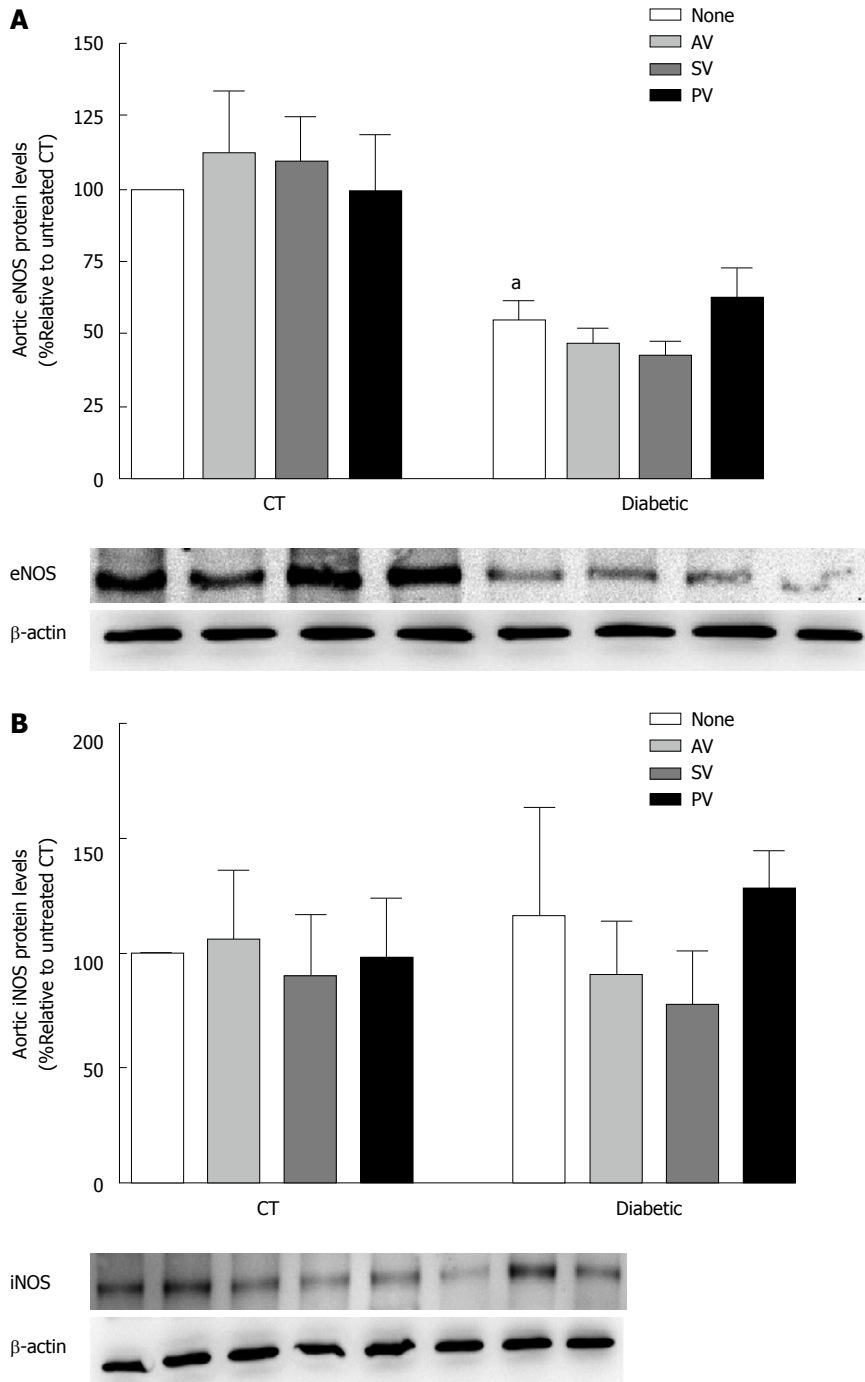


Figure 6 Effect of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on endothelial nitric oxide synthase (A) and inducible nitric oxide synthase (B) protein levels in aortic homogenates from treated and untreated diabetic rats, and untreated control. Data represent values normalized against β -actin and expressed as percent change relative to untreated CT. The values shown are the means \pm SEM of five animals per group; ^a $P < 0.05$ for untreated diabetic rats vs untreated CT. Bottom: Representative Western blot for eNOS and iNOS of homogenized aortic tissue; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control; eNOS: Endothelial nitric oxide synthase; iNOS: Inducible nitric oxide synthase.

increases ejection fraction and prevents left ventricular hypertrophy and fibrosis in rabbits with non-ischemic heart failure^[33]. Improved vascular function, including augmented ACh-induced relaxation and reduced perivascular fibrosis, may increase cardiac function by reducing total peripheral resistance and reducing cardiac work. Alternatively, the beneficial effects of these statins on cardiac performance may include the preservation of myocardial contractility, which is

deteriorated in diabetes. Indeed, in hearts from diabetic hypercholesterolemic rats, SV (10 mg/kg per day per 5 d) improves cardiac contractility without reducing cholesterol levels^[34]. Statins, however, do not appear to be effective in improving particular aspects of cardiac dysfunction associated with diabetes. The appearance of diastolic dysfunction in Type 2 diabetic rats was not prevented by 100 mg/kg AV^[35]. Furthermore, although AV improves cardiac function, it does not prevent the

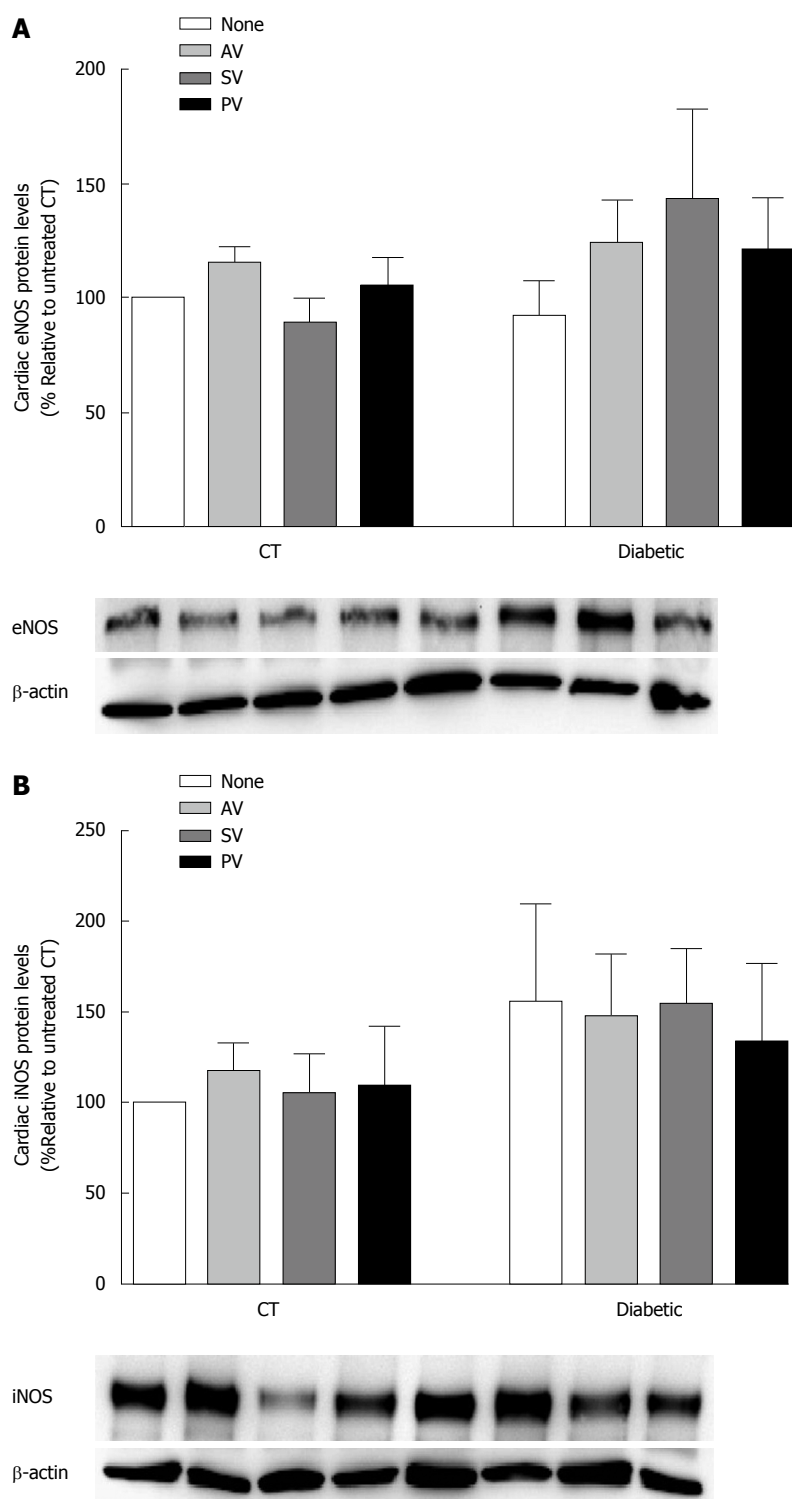


Figure 7 Effect of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on endothelial nitric oxide synthase (A) and inducible nitric oxide synthase (B) protein levels in cardiac homogenates from treated and untreated diabetic rats, and untreated control. Data represent values normalized against β -actin and expressed as percent change relative to untreated CT. The values shown are the means \pm SEM of five animals per group. No statistically significant differences were found. Bottom: Representative Western blot for eNOS and iNOS of homogenized cardiac tissue; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control; eNOS: Endothelial nitric oxide synthase; iNOS: Inducible nitric oxide synthase.

onset of cardiomyopathy in Type 1 diabetic rats^[20].

Although the STZ-induced diabetic rat has proven to be an effective animal model for the study of Type 1 diabetes^[36], it has several limitations that must be taken into consideration. Reductions in effective circulating

volume due to glycosuria introduce an additional variable because cardiac and vascular RAS become activated. Autonomic dysfunction, which is present in this model, also may cause a reduction in cardiac vagal tone, without changing sympathetic tone^[37].

Moreover, due to its chemical structure, STZ down-regulates glucose and lipid metabolism genes before hyperglycemia appears, suggesting that this compound can affect gene expression in a hyperglycemia-independent manner^[38]. Despite these limitations, the STZ-diabetic rat is widely used in experimental studies because it replicates both Type 1 diabetes and poorly controlled Type 2 diabetic conditions, making it a useful model in the study of diabetes-related pathophysiology.

The current study demonstrates that AV, SV, and PV are equally effective in improving CV performance in Type 1 diabetic rats. The observed hemodynamic benefits are cholesterol-independent. These benefits appear to be secondary to improved vascular function which, in turn, results from reduced oxidative stress. Although the etiology of Type 1 and Type 2 diabetes is different, in both conditions oxidative stress is high. Thus, it is plausible to postulate that Type 2 diabetics also may benefit from statin treatment. If our findings for diabetic rats are applicable to humans, the benefits of statins to diabetics who are predisposed to develop cardiac complications may extend beyond cholesterol reduction. In addition, even at low doses, statins may be useful for improving the CV profile of diabetics.

COMMENTS

Background

Although there is evidence that statins are useful in the treatment of diabetes, whether cardiovascular (CV) improvement is class-related or drug-specific is unknown. To address the issue, this study tests how low doses of the class-related atorvastatin, simvastatin, and pravastatin improve CV performance in Type 1 diabetic rats.

Research frontiers

Knowledge of the mechanisms underlying statin improvement of CV function, whether these effects are drug-specific or class dependent, and which statin is most effective should result in significant advancements in the current treatment of diabetes.

Innovations and breakthroughs

The beneficial cardioprotective effect of statins is revealed to be class-related, rather than drug-specific. Moreover, this beneficial effect is secondary to reductions in oxidative stress and vascular remodeling, and appears to be independent of the ability of these drugs to lower cholesterol levels.

Applications

If these findings for Type 1 diabetic rats prove to be applicable to humans, the benefits of statins for diabetic patients who are prone to develop CV complications may extend beyond cholesterol reduction.

Terminology

Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

Peer-review

The authors concluded the benefits appear to be secondary to the improved endothelial function, and to the reduced vascular tone and remodeling that result from decreased oxidative stress. The findings are interesting.

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

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Rajeev K Singla, Division of Biotechnology, Netaji Subhas Institute of Technology, Sector-3, Dwarka, New Delhi 110078, India

Prakash Katakam, Faculty of Pharmacy, University of Zawia, Az-Zawiya 13, Libya

Shanta K Adiki, Nirmala College of Pharmacy, Guntur 522503, Andhra Pradesh, India

Author contributions: De B developed the central theme of the manuscript under the guidance of Chakravorty N, Katakam P and Mitra A; Bhandari K, Mukherjee R, Gundamaraju R and Singla RK contributed in literature review, and provided their valuable suggestions; De B developed the manuscript under the guidances of Chakravorty N, Shanta KA and Mitra A; Ghosh B prepared the pictorial presentations of receptor pathways and gave his suggestions.

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Correspondence to: Analava Mitra, MBBS, PhD, School of Medical Science and Technology, B-145, IIT Campus, IIT

Kharagpur 721302, India. analavamitra@gmail.com
Telephone: +91-94-75258298
Fax: +91-32-22279970

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Abstract

Despite tremendous strides in modern medicine stringent control over insulin resistance or restoration of normoglycemia has not yet been achieved. With the growth of molecular biology, omics technologies, docking studies, and *in silico* pharmacology, modulators of enzymes and receptors affecting the molecular pathogenesis of the disease are being considered as the latest targets for anti-diabetic therapy. Therapeutic molecular targets are now being developed basing on the up or down regulation of different signaling pathways affecting the disease. Phytosynergistic anti-diabetic therapy is in vogue both with classical and non-classical medicinal systems. However its chemo-profiling, structural and pharmacokinetic validation awaits providing recognition to such formulations for international acceptance. Translational health research with its focus on benchside product development and its sequential transition to patient bedside puts the pharma RDs to a challenge to develop bio-waiver protocols. Pharmacokinetic simulation models and establishment of *in vitro-in vivo* correlation can help to replace *in vivo* bioavailability studies and provide means of quality control for scale up and post approval modification. This

review attempts to bring different shades highlighting phyto-synergy, molecular targeting of antidiabetic agents *via* different signaling pathways and bio-waiver studies under a single umbrella.

Key words: *In silico* pharmacology; Phytosynergistic; Anti-diabetic; Simulation models; Translational health research; Bio-waiver; Signaling pathways

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Core tip: The current research scenario on anti-diabetic drug development pipeline focuses on pharmacological targets influencing the molecular pathogenesis of the disease. It encompasses receptors and enzymes that will increase insulin sensitivity, intracellular insulin signaling, enhance peripheral glucose utilizations, suppress hepatic glucose production and reduce circulating triglycerides levels. Combination therapy has gained significance either with herbal or synthetic drugs, though "phytosynergy" awaits proper validation to give rise to new generations of "phytopharmaceuticals". Pharmacokinetic simulation models and established *in vitro-in vivo* correlation that may be extrapolated to humans can serve the purpose of bio-waiver in product transition from lab bench to patient bedside.

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INTRODUCTION

The constant escalations in the number of diabetics worldwide has given an alarming signal and fueled intensified research for the development of new therapeutic entities and latest effective therapeutic regimen. The statistics of the global diabetic population is expected to show a steady growth to 366 million by 2030 of which 90% will be type 2 diabetics. The international diabetes federation has estimated the number of diabetics in India to be 40.9 million, which is expected to grow to 60.9 million by 2025^[1-3]. Diabetes is a common metabolic disorder with abnormal elevations in the blood-gluco-lipid profile, leading to major complications like diabetic neuropathy, nephropathy leading to end stage renal disease, retinopathy leading to blindness and diabetic foot ulcers necessitating limb amputations^[1,2]. Type 2 diabetes is characterized by the hallmark of insulin resistance and β -cell dysfunction and ultimate destruction of pancreatic insulin secreting cells. Combating insulin resistance with the existing pharmacological approaches are unsatisfactory primarily

because although they may compensate for the defects in insulin secretion and action, but are ineffective in counteracting β -cell dysfunction and handling the secondary complications of type 2 diabetes^[1-3]. While developing a novel anti-diabetic chemical entity, latest drug design approaches focuses on activation-inhibition of enzymes in insulin-sensitive cells, minimization of associated side effects like obesity, substitution or antagonizing of physiological hormones and their pathways. With the advancements in high throughput screening, proteomics, genomics, molecular docking, and combinatorial chemistry, new therapeutic entities are being developed that influence enzyme activities, signaling receptors and pathophysiological pathways^[1,2]. Modern day quantitative structural activity relationship and docking studies are enabling development of bio-active molecules that can achieve structural modifications and thereby alter their pharmacological actions and pharmacokinetic profile so as to maximize bioavailability and minimize the side effects^[4-10].

Latest anti-diabetic drug development pipeline focuses on pharmacological targets which include receptors and enzymes that will increase insulin sensitivity, intracellular insulin signaling, enhance peripheral utilizations of glucose, suppress hepatic glucose production and reduce the levels of circulating tri glycerides^[4-10].

Medicine in recent times, whether western classical or phyto-therapy, advocates for combination therapy, instead of single approach. Synergy research in phyto-therapy, with the aid of "omics technologies" needs a rationale for establishing its pharmacological and therapeutic superiority to treat diseases which have hitherto been treated using synthetic drugs alone^[11-15].

Along with the paradigm of translational health research with the perspectives of bench to bedside approach; all pharmaceutical RDs target to develop robust, cost effective, enhanced throughput *in vitro* assays which may be extrapolated to humans and serve the purpose of bio-waiver. The development of increased number of new chemical entities obviates the need of enhanced pharmacokinetic studies. Though human pharmacokinetic *in vivo* studies are often considered as the "gold standard" for assessment of bioequivalence but it is expensive, time consuming and difficult to handle enormous amount of pharmacokinetic data. Development of pharmacokinetic simulation models which are computational or mathematical tools help to interpret drug kinetics in living environment under specified conditions and can waive off bioequivalence requirements called bio-waiver studies. Establishment of *in vitro-in vivo* correlation (IVIVC) provides a justified explanation for bio-waiver during scale up or post approval changes^[16-25].

Thus the editorial encompasses the broad areas highlighting phyto-synergy, targeting of different signaling pathways of type 2 diabetes and how computational pharmacokinetics and development of IVIVC serves the purpose of bio-waiver.

MOLECULAR PATHOGENESIS OF TYPE 2 DIABETES

Treatment regimen of type 2 diabetes advocates two different approaches, one recommending the sequential use of anti-diabetics and another is a pathophysiologic approach which aims to control the disease conditions basing on pathogenesis with a comparative preference on combination therapy.

American Diabetes Association guidelines incorporated an individualized ABCDE anti-diabetic therapy approach where each alphabet refers to A-age, B-body weight, C-complications (micro and macro vascular), D-disease duration and E-life expectancy and expense. Progressive β -cell destruction coupled with the development of insulin resistance in liver, muscles and adipocytes, subsequent elevation in glycated hemoglobin level being the common pathogenic hallmark in all type 2 diabetes mellitus, though variations are reported amongst different ethnic groups^[4-6].

Apart from insulin resistance, a host of cardiovascular co-morbidities like dyslipidemia, hypertension, and central visceral adiposity occur in type 2 diabetes. Evidence based contemporary research paradigms have shown that intra abdominal or visceral fat depots synergize defective insulin action and secretion. Moreover leptins, adiponectins, tumor necrosis factor- α , resistin which are secreted from the adipose tissues interfere with glucose metabolism and insulin sensitivity giving rise to the concept of lipotoxicity in type 2 diabetes. These adipokines greatly modify insulin signaling pathways and promote development of insulin resistance. A triadic relation is found to exist amongst β -cell destruction, insulin resistance and adiposity^[4-6].

Sedentary lifestyle, westernized dietary pattern, stress, anxiety, depression, smoking and alcohol consumption are other contributing risk factors of type 2 diabetes. Obesity is also found to be associated with endothelial dysfunction, impairs muscle microcirculation, retards entry of insulin and blood glucose into skeletal muscle and decreases their availability to muscle cells. Lack of physical activity is an important risk factor in type 2 diabetes. Daily physical activities decreases visceral and body fat, increase glycogen synthase (GS) content of the muscle, promotes non-oxidative disposal of glucose as glycogen and activates glucose transporter subtype 4 (GLUT4) to enhance peripheral glucose utilization. Physical activity up regulates expression and activity of proteins involved in insulin signal transduction, improves oxidative capacity of the skeletal muscles, decreases free fatty acid concentrations and enhances the increased expression of downstream signaling components of insulin. Regular exercises also trigger the release of anti-inflammatory cytokines, a protective role against insulin resistance^[1,4-6].

An insight into the genetics of type 2 diabetes showed that genes encoding proteins are involved in insulin signaling, glycogen synthesis and glucose transportation, fatty acid uptake and synthesis, adipocyte differentiation

and thus suggests associations with diabetes. A clear understanding of human genome sequence is necessary which will help in rapid identification of the genes associated with diabetes. Mutations of five genes viz. glucose metabolizing enzyme glucokinase, transcription factors hepatocyte nuclear factor (HNF) 1 α and β , HNF4 α and insulin promoter factor 1 (IPF1) affect moderate to significant reductions in insulin secretions. Latest research reporting does have mentioned that genetic variation of newly encoded gene Calpain, called as CAPN10 gene can cause diabetes^[5-9].

THERAPEUTIC MOLECULAR TARGETS BASED ON RECEPTOR SIGNALING PATHWAYS IN TYPE 2 DIABETES

Amongst the Oral Hypoglycemic Agents (OHAs) mostly recommended for type 2 anti-diabetic therapy, sulfonylureas (e.g., tolbutamide, glibenclamide, acetohexamide) and biguanides (e.g., phenformin, metformin) are in wide use followed by thiazolidinediones (also known as glitazones, e.g., Rosiglitazone, Pioglitazone) and alpha glucosidase inhibitors (acarbose, miglitol, voglibose). Sulphonyl ureas work primarily by stimulating pancreatic insulin secretion and reduce the hepatic glucose output and enhance peripheral glucose utilizations. Biguanides are anti-hyperglycemic agents rather than hypoglycemics, suppress excessive hepatic glucose production, increases peripheral glucose utilizations to a lesser extent, reduce intestinal glucose absorption by reducing food intake. Alpha glucosidase inhibitors delay the breakdown of disaccharides and polysaccharides and hence glucose absorption is delayed. Thiazolidinediones enhance insulin sensitivity in peripheral tissues.

However, the available pharmacological approaches for anti-diabetic therapy are not successful enough to put a stringent control on insulin resistance. Instead of mono therapy now combination therapy and multi-drug formulations are in vogue. With the development of proteomics, genomics and a thorough understanding of the molecular pathways, the development of new molecular targets with anti-diabetic potentials focuses in modulating pharmacokinetics, cellular location, overall distribution etc. Modulators of enzymes and receptors are now becoming the molecular targets for any disease therapy^[8-10].

The three targeted tissues of insulin action include skeletal muscle, adipose tissue and liver. Insulin binds with the target cell surface receptor and activates the tyrosine kinase which is a constituent of the receptor molecule. Tyrosine residues of the insulin receptors undergo autophosphorylation and the serine/threonine residues become phosphorylated^[7-10]. In type 2 diabetes elevated levels of insulin stimulates serine kinases via IGF-1 receptor (Insulin like growth factor 1) leading to insulin resistance^[7-10]. Protein kinase C (PKC) is known to play a significant role in developing

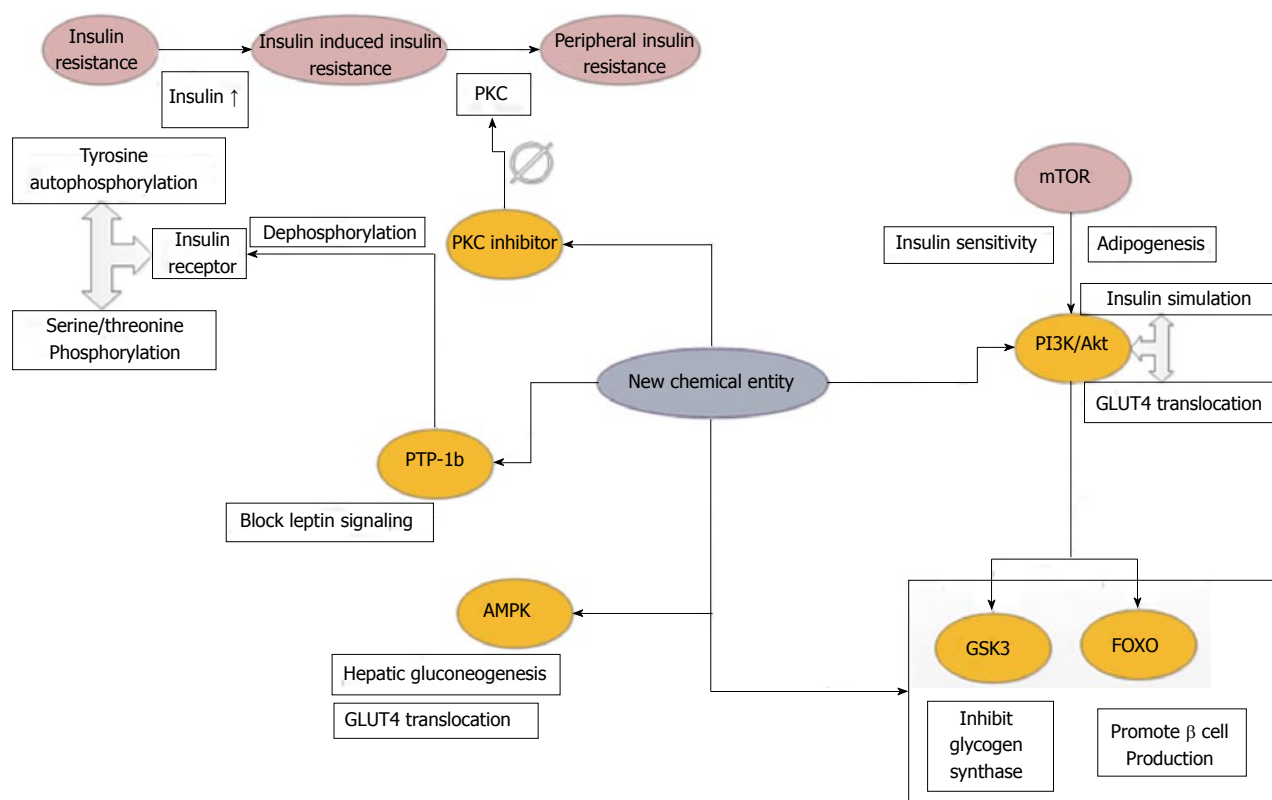


Figure 1 Schematic representation of different receptor signaling pathways in type 2 diabetes mellitus which can be targeted by new chemical entity. PKC: Protein kinase C; Akt: Also known as protein kinase B (PKB); GSK3: Glycogen synthase kinase 3; mTOR: Mammalian target of rapamycin; FOXO: Forkhead box subgroup O; PTP-1b: Protein tyrosine phosphatase-1b; GLUT4: Glucose transporter Subtype 4; PI3K: Phosphoinositide 3 kinase; AMPK: AMP activated protein kinase.

peripheral insulin resistance. Thus inhibition of PKC or its reduced expression may enhance insulin sensitivity and insulin receptor tyrosine kinase activity which can be an effective therapeutic strategy against type 2 diabetes. Protein tyrosine phosphatase-1b (PTP-1b) causes dephosphorylation of insulin receptor and is a negative regulator of the insulin signaling. It enhances insulin activity and is resistant to the development of obesity. PTP-1b down regulates or blocks leptin signaling by dephosphorylating Janus kinase (JAK). Thus PTP-1b serves as an essential therapeutic target. Phosphoinositide 3 kinase (PI3K) plays a significant role in the glucose uptake *via* insulin stimulation and GLUT4 translocation. PI3K is down regulated by two classes of serine/threonine kinases, Akt, also known as protein kinase B (PKB) and the isoforms of PKC^[7-10]. Akt and isoforms of PKC are known to facilitate GLUT4 translocation. P70s6k directly phosphorylates IRS (insulin receptor substrate) which inhibits its activity and hinders Akt actively. Mammalian target of rapamycin (mTOR) has a significant role in obesity and IR and activates both Akt and p70s6k. The essential targets for Akt include the transcription factors, glycogen synthase kinase 3 (GSK3) and the forkhead box subgroup O (FOXO). GSK3 can phosphorylate and inhibit GS. Now phosphorylation of Akt inactivates GSK3 and leads to an increase in glycogen synthesis^[7-10]. Akt phosphorylation also targets FOXO mediated transcription of target genes that promote the production of β -cells. To coun-

teract IR and restore insulin sensitivity therapeutic agents should target to increase PI3K/Akt activity. Lipid phosphatase PTEN (phosphatase and tensin homolog) dephosphorylates phosphatidylinositol (3,4,5) trisphosphate (PIP3) making it less available to recruit Akt. Also downstream regulation of mTOR can regulate adipogenesis and insulin sensitivity^[7-10].

AMPK (AMP activated protein kinase) regulates hepatic gluconeogenesis and increase muscle glucose uptake by translocation of GLUT4 which also serves the purpose of an essential therapeutic target^[10]. A comprehensive scheme of the different receptor signaling pathways have been presented below in Figure 1.

Some of the novel molecular targets for anti-diabetic therapy have been mentioned in Table 1.

PHYTOSYNERGY IN TYPE 2 DIABETES

Synergy refers to the increased effectiveness that results when two or more elements work together, though here we will refer to phytochemical constituents. Synergism is the total outcome of a cumulative effect which is greater than the sum of individual effects. From the dimensions of pharmacology, molecular biology or clinical research, synergism can be either in the form of multi target effect where different phytoconstituents of a single extract or a composite extract will affect more than one targets agonistically and exhibit

Table 1 Possible therapeutic molecular targets for type 2 anti-diabetic therapy

Type	Target for action	Nature of action	Effect produced
Protein kinases	Protein kinase C	Inhibitory	Block receptor desensitization
	AMP activated kinase	Activator	Enhance glucose transport
	GSK-3	Inhibitory	Activate glycogen synthase
	MAP kinase	Inhibitory	Block receptor desensitization
Protein phosphatases	PTP-1b	Inhibitory	Block receptor dephosphorylation
	PP1	Activator	Activate glycogen synthase
	LAR	Inhibitory	Block receptor dephosphorylation
Lipid phosphatases	PTEN	Inhibitor	Increase PIP3-stimulated glucose transport
Cell surface receptors	Insulin receptor	Agonist	Insulin mimetic
	Glucagon receptor	Antagonist	Low fasting glucose
	GLP receptor	Agonist	Increase insulin secretion
Ion channels	β -3 adrenergic receptor	Agonist	Increase lipolysis
	Sulphonyl urea receptor	Inhibit K channel	Increase insulin secretion
Transcription factors	PPAR- γ	Selective modulator	Insulin sensitizer
	HNF4	Selective modulator	Increase insulin secretion

AMP: Adenosine monophosphate activated kinase; GSK-3: Glycogen synthase kinase 3; MAP: Mitogen activated protein; PTP-1b: Protein tyrosine phosphatase-1b; PP1: Protein phosphatase 1; LAR: Leukocyte antigen related; PTEN: Phosphatase and tensin homolog; PPAR- γ : Peroxisome proliferator-activated receptor; GLP: Glucagon-like peptide.

synergism^[11,12]. Synergy can give better outcomes in terms of pharmacokinetic profile or physicochemical effects based on enhanced solubility profile, improved absorption and ultimately better bioavailability. Use of synergistic combinations also helps to restrict the development of resistance due to single prolonged drug use. While synthesizing or processing a single entity, unwanted adverse effects may develop due to either the extraction procedure or synthetic scheme being followed, or development of any by products; such adverse effects can be minimized or eliminated by use of combo formulations. Moreover stability issues of one to several bio-actives on long storage are more protected in combined form than in isolated form^[11,12].

Combination therapy has made its way in the treatment of type 2 diabetes whether it is western classical medicine or herbal formulations. Resveratrol, a phytoalexin found in grapes which acts on various molecular targets in adipocytes and osteoblasts decreases the number of adipocytes and acts synergistically with quercetin and genistein to reduce adipogenesis^[12]. Evidence based clinical research results have shown that miglitol in combination with metformin provides a better glycemic control than metformin

monotherapy which is an example of synergism in anti-diabetic therapy with western medicine. Oleanolic acid, a pentacyclic triterpene, a natural component of many medicinal herbs in combination with metformin, first line antidiabetic drug showed synergistic anti-diabetic potentials in animal studies^[13]. Experimental results showed that the combination reduced hepatic gluconeogenesis by decreasing mRNA expressions of PGC-1 α , G-6-Pase and PEPCK (Phosphoenol pyruvate carboxykinase 1). The combination is also found to stimulate the PI3K pathway that phosphorylates Akt and down regulates mTOR to improve insulin resistance. Sesame oil, an edible oil rich in mono and polyunsaturated fatty acids is found to show synergistic anti-diabetic potentials with sulphonyl ureas *viz.* glibenclamide^[14]. In case of allopathy, results of clinical trials have shown that combination therapy with miglitol and metformin was found to be more effective than the use of single drug alone^[15].

Establishment of standard quality control profile in global context to confirm the validity and reproducibility of phytochemical constituents in the form of processed extract rather than single isolated compound; proper analytical and spectroscopic method development for structural characterizations in combined forms; rigorous validation of safety profile and pharmacokinetic parameters is essential to find a scientific basis of phytosynergy which may give rise to a new generation of medicinal products - phyto-pharmaceuticals^[11].

BIOWAIVER-COMPUTATIONAL PHARMACOKINETICS AND IVIVC

Drug development procedure is very tedious and expensive and in many cases due to lack of adequate pharmacokinetic data of the candidate drug, completion of further research becomes questionable. With the vast expansions in the research arenas undertaking the development of new chemical entities, bioequivalence studies are of vital concern in drug development especially when there are absolute new entities or having narrow therapeutic index. Though *in vivo* animal experimentation for establishing the pharmacokinetic profile is still the surrogate, yet it's very tedious, expensive, and time consuming to handle enormous amount of data. Along with the development of *in silico* pharmacology, computational modeling now finds applications in pharmacokinetics and dynamics, as well as toxicokinetics and dynamics. Many multinational pharma R&Ds are now focusing on bio-waiver where in many cases *in vitro* results were considered more acceptable in different dosage formulations especially immediate release solid dosage forms^[16-18]. In that condition to proceed with a bio-waiver study there's a need to establish dissolution profile and is to be characterized with both model dependent and independent approaches. *In vivo* performance of a dosage formulation or new chemical entity can be

Table 2 Types of *in vitro-in vivo* correlation and the parameters used

Level	<i>In vitro</i> parameters	<i>In vivo</i> parameters	Utility
Level A: direct relationship with <i>in vivo</i> data based on <i>in vitro</i> measurement alone	Dissolution curves	Absorption curves	Highest level of correlation depicting point to point relation between <i>in vitro</i> dissolution rate and <i>in vivo</i> input rate of drug from dosage form. Marks <i>in vitro</i> dissolution as the surrogate of <i>in vivo</i> performance
Level B: relation based on statistical moments analysis	MDT	MAT; MRT	Mean <i>in vitro</i> dissolution time of the product compared to mean <i>in vivo</i> residence time or mean <i>in vivo</i> dissolution time
Level C: relates one dissolution time point ($t_{50\%}$, $t_{90\%}$, etc.) to one mean pharmacokinetic parameter (AUC, C _{max} , t _{max})	Disintegration time, time to have 10%, 50%, 90% dissolved, dissolution rate, dissolution efficiency	C _{max} , T _{max} , K _a , time to have 10%, 50%, 90% absorbed, AUC (total or cumulative)	Single point weak correlation showing a partial relation between absorption and dissolution. Used in early stages of formulation development before pilot production

MDT: *In vivo* measurement of the dissolution rate in the digestive tract; MRT: The mean time that the drug resides in the body, MRT may also be the mean transit time; MAT: The mean time required for drug to reach systemic circulation from the time of drug administration. It is actually the mean time involved in the *in vivo* release and absorption processes as they occur in the input compartment and is estimated as $MAT = MRT - MRT_{Total}/i.v.$; AUC: In pharmacokinetics, AUC is the area under the curve (mathematically known as definite integral) in plot of concentration of drug in blood plasma against time, it reflects the actual body exposure to drug after administration of a dose of the drug and expressed in $mg \times h/L$; K_a: It is the absorption rate constant which is a proportionality constant that relates the rate of drug absorbed in the body; C_{max}: It refers to peak serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and prior to the administration of a second dose; T_{max}: It is the time after administration of a drug when the maximum plasma concentration, C_{max} is reached and during which rate of absorption is equal to the rate of elimination. MDT: Mean dissolution time; MAT: Mean absorption time; MRT: Mean residence time.

simulated from the *in vitro* dissolution data after establishing a definitive IVIVC^[19-23].

The biopharmaceutics classification system (BCS) proceeds with a predictive approach for developing correlation between physicochemical criteria of drug formulations and its *in vivo* bioavailability. BCS is not the direct IVIVC; IVIVC develops a mathematical relation between *in vitro* and *in vivo* data by either linear or non-linear correlation^[19-25]. As per FDA guidelines IVIV correlation ranges from A-D with multiple level C correlation, the details of which have been presented in Table 2.

Apart from these three types of correlation, level D correlation is a rank order and qualitative method which may be applicable in some steps of formulation development but not recommended for regulatory purposes. A multiple point level C correlation is really a justified bio-waiver where correlation is established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. This correlation is based on three dissolution points (early, middle and end stages) and on achievement of this correlation level, the level A correlation is also likely to develop^[19-21].

Even after the attainment of high level of correlation, till date no *in vitro* method can exactly simulate physiological conditions *in vivo* especially when it comes to replicate the exact gastro-intestinal (GI) conditions *in vitro viz.* appropriate amount, pH and exact physiological amounts of enzymes needed for digestion, physiological transits during digestion process, exact replication of peristalsis, food - drug interactions and its impact on dosage formulations. An artificial digestive system known as TIM1 have been developed by TNO Nutrition and Food Research mimicking the human stomach and three segments of small intestine where pH is monitored and computer controlled, constant

generation of water pressure ensures mixing of enzymes by alternate compression and relaxation of flexible walls and removal of water and small molecules from lumen compartment by pumping dialysis fluid mimics the GI motility. Though such artificial models find applications in nutrition research but to be an effective quality control tool in drug development studies warrants further research^[21].

CONCLUSION

Translational health research is the latest buzzword in the field of biomedicine which aims to bridge basic research with medical innovation with the perspectives of sequential development of products from lab bench to patient bedside. The landscape of drug discovery which is just the initiation of creating new chemical entities has undergone a drastic change after the emergence of computational biology, combinatorial chemistry and *in silico* docking studies. Now drug molecules are tailored as per requirements for maximizing bioavailability and stringent control over pharmacokinetics. Combination therapies with synergistic potentials are finding more prominence than monotherapy and even documentations are available in some anti-diabetic medications where combination of natural and synthetic medicine showed better results. However to capture the international pharma market and speed up the pilot scale production, there is an urgent need to boost bio-waivers which necessitates to develop robust and reproducible *in vitro* models simulating *in vivo* conditions.

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Incretins and selective renal sodium-glucose co-transporter 2 inhibitors in hypertension and coronary heart disease

Ramiro A Sanchez, Hugo Sanabria, Cecilia de los Santos, Agustin J Ramirez

Ramiro A Sanchez, Agustin J Ramirez, Arterial Hypertension and Metabolic Unit, University Hospital, Fundación Favaloro, Buenos Aires 1093, Argentina

Hugo Sanabria, Diabetes Unit, Instituto Cardiovascular de Buenos Aires, Buenos Aires 1428, Argentina

Cecilia de los Santos, Medical Affairs Department, Boehringer Ingelheim, Munro 1605, Argentina

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Correspondence to: Ramiro A Sanchez, MD, PhD, Professor, Head, Arterial Hypertension and Metabolic Unit, University Hospital, Fundación Favaloro, Av. Belgrano 1782, 4.º, Buenos Aires 1093, Argentina. rsanchez@ffavaloro.org
 Telephone: +54-11-4371337

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Abstract

Hyperglycemia is associated with an increased risk of cardiovascular disease, and the consequences of

intensive therapy may depend on the mechanism of the anti-diabetic agent(s) used to achieve a tight control. In animal models, stable analogues of glucagon-like peptide-1 (GLP-1) were able to reduce body weight and blood pressure and also had favorable effects on ischemia following coronary reperfusion. In a similar way, dipeptidyl peptidase IV (DPP-IV) showed to have favorable effects in animal models of ischemia/reperfusion. This could be due to the fact that DPP-IV inhibitors were able to prevent the breakdown of GLP-1 and glucose-dependent insulintropic polypeptide, but they also decreased the degradation of several vasoactive peptides. Preclinical data for GLP-1, its derivatives and inhibitors of the DPP-IV enzyme degradation suggests that these agents may be able to, besides controlling glycaemia, induce cardio-protective and vasodilator effects. Notwithstanding the many favorable cardiovascular effects of GLP-1/incretins reported in different studies, many questions remain unanswered due the limited number of studies in human beings that aim to examine the effects of GLP-1 on cardiovascular endpoints. For this reason, long-term trials searching for positive cardiovascular effects are now in process, such as the CAROLINA and CARMELINA trials, which are supported by small pilot studies performed in humans (and many more animal studies) with incretin-based therapies. On the other hand, selective renal sodium-glucose co-transporter 2 inhibitors were also evaluated in the prevention of cardiovascular outcomes in type 2 diabetes. However, it is quite early to draw conclusions, since data on cardiovascular outcomes and cardiovascular death are limited and long-term studies are still ongoing. In this review, we will analyze the mechanisms underlying the cardiovascular effects of incretins and, at the same time, we will present a critical position about the real value of these compounds in the cardiovascular system and its protection.

Key words: Incretins; Hypertension; Cardiovascular effects; Dipeptidyl peptidase 4 inhibitors; Sodium-glucose co-transporter 2

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Core tip: The dipeptidyl peptidase IV inhibitors prevent the breakdown of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide, but also decrease the degradation of several vasoactive peptides. Dipeptidyl peptidase IV inhibitors have shown to have favorable effects in animal models of ischemia/reperfusion and in hypertension. Clinical studies are most under way and final results could give reliable information on cardiovascular protection. Selective inhibitors of renal sodium glucose transport 2 have been also evaluated in the prevention of cardiovascular outcomes in type 2 diabetes. However, data on cardiovascular outcomes and cardiovascular death are limited and long term studies are on-going, therefore it is premature to draw conclusions.

Sanchez RA, Sanabria H, de los Santos C, Ramirez AJ. Incretins and selective renal sodium-glucose co-transporter 2 inhibitors in hypertension and coronary heart disease. *World J Diabetes* 2015; 6(11): 1186-1197 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i11/1186.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i11.1186>

INCRETINES IN THE CARDIOVASCULAR SYSTEM

As a cardiovascular risk factor, hypertension together with dysglycemia, hyperlipidemia and overweight, is one of the components of the so-called metabolic syndrome. From these four, hypertension takes the first position in mortality, particularly in middle- and low-income countries. Regarding disabilities, hypertension ranks in the third position after malnutrition and risky sex behavior^[1-11]. As it is well known, diabetes mellitus is closely linked to cardiovascular diseases, and hypoglycemic agents may have either positive or negative effects on cardiovascular outcomes. Consequently, there is a growing interest in the evaluation of new compounds as therapeutic tools or with relation to side effects and interactions.

Incretins, either glucagon-like peptide-1 (GLP-1) analogues or dipeptidyl peptidase IV (DPP-IV) inhibitors, are just a new group of hypoglycemic drugs and their cardiovascular effects are being evaluated in different trials.

At the gastrointestinal level, incretins are able to increase insulin release after food intake in a glucose-dependent manner^[12]. From these hormones, the most widely known ones are GLP-1 and the gastric inhibitory polypeptide.

The role of endogenous GLP-1 in the metabolic and cardiovascular systems has been intensively studied^[13] with specific receptor antagonists (GLP-1R antagonists), with special attention to the cardiac effects of GLP-1 in different animal models. In conscious dogs with induced

cardiomyopathy^[14], GLP-1 infusion improved left ventricular contractility in 90%, stroke volume in 100% and cardiac output in 50%. Furthermore, an enhanced oxidative phosphorylation effect as a consequence of an increase in myocardial glucose uptake and oxygen consumption was also reported. Some authors suggested that the beneficial cardiovascular effects of GLP-1R stimulation are primarily due to the modulation of myocardial metabolism rather than direct mechanisms^[14].

Other studies suggest that GLP-1 may induce vasodilation, possibly through the activation of specific endothelial and cardiovascular myocyte receptors^[15].

In recent studies that used a mouse isolated heart preparation, both GLP-1 and its analog exenatide improved cardiac function following ischemia/reperfusion^[16]. Moreover, data reported that GLP-1 cardioprotective effects result from additional mechanisms over the GLP receptor activation, affecting the GLP-1 degradation pathway^[16-18]. Thus, the improvement of ischemic injury by coronary vasodilation induced by the metabolite GLP-1 seems to be mediated by a nitric oxide GLP-1 receptor-independent mechanism.

Studies in human beings seemed to have similar effects than those found in animal models. As an example of this, a significant improvement of left ventricular ejection fraction and wall motion scores were reported in a pilot study^[19] in which 10 patients with acute myocardial infarction and coronary arterial graft surgery were perfused for three days with recombinant human GLP-1. These effects were independent from the infarction location or the diabetes history and, in some patients; they were detectable even months after cessation of the infusion. Similarly, the GLP-1 infusion improved left ventricular ejection fraction and exercise capacity in both diabetic and non-diabetic patients with congestive heart failure^[15]. Finally, in diabetic patients with coronary heart disease that were pretreated with GLP-1 before cardiac surgery, an improvement of glycemic control and hemodynamic recovery indexes were reported^[20].

In type 2 diabetes, endothelial dysfunction is an early alteration of the consecutive vascular disease that is responsible for an increase in cardiovascular (CV) morbidity and mortality. Furthermore, endothelial dysfunction, as a cluster of the metabolic syndrome, together with postprandial hyperglycemia and postprandial hypertriglyceridemia are commonly associated with oxidative stress, decreased fibrinolysis, sympathetic activation, and increased atherosclerotic coronary plaque burden^[21]. It is interesting that incretins play a role in reducing endothelial dysfunction in experimental studies. In accordance with this information, Basu *et al*^[22] reported that administration of GLP-1 enhanced forearm vasodilator response to intra-arterial acetylcholine but not to nitroprusside, which was consistent with a nitric oxide synthase-dependent effect. However, whether the role of GLP-1 or the products of its degradation mediated these effects was not

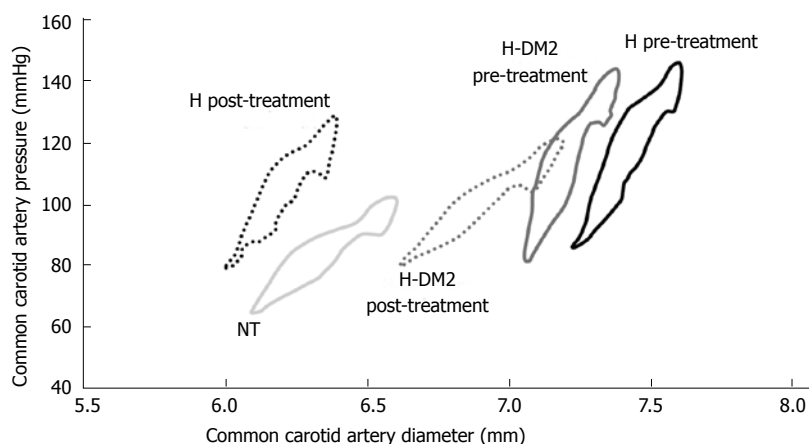


Figure 1 Pressure and diameter plot. The instantaneous pressure-diameter loops are shown, which were obtained from: H pre-treatment: Hypertensive patients without diabetes mellitus (DM) before administration of ramipril; H post-treatment: Hypertensive patients without DM after administration of ramipril; H-DM2 pre-treatment: Hypertensive patients with type 2 DM before ramipril administration; H-DM2 post-treatment: Hypertensive patients with type 2 Diabetes after ramipril administration; NT: Normotensive subjects. Adapt from Christen *et al*^[23].

evaluated. In a review published by our group^[23] that included patients with type 2 diabetes, we examined the endothelial function and the effects of treatment (Figure 1). The endothelial function was improved with ramipril, an angiotensin-converting enzyme inhibitor (ACEI), suggesting that GLP-1 may have endothelial effects that are similar to the ones of ACEI. In another study of Japanese diabetic patients with coronary artery disease, changes in endothelial function^[24] were studied when patients were treated for 6 mo either with sitagliptin or conventional therapy. Patients receiving sitagliptin experienced a greater reduction in the C-reactive protein and systolic blood pressure (~7 mmHg), whereas hemoglobin A1c did not present any changes after treatment when compared to the control group. The authors concluded that sitagliptin, beyond its hypoglycemic action and blood pressure reduction, significantly improved the endothelial function and inflammatory state.

In conclusion, incretins as a family of anti-diabetic drugs may have additional protective effects on the cardiovascular system not only by improvement of glycemic control. In this regard, the mechanisms involved could be: the optimization of the endothelial function and the reduction of the inflammatory process with a subsequent improvement of the arterial and cardiac dynamics.

INCRETINS ON BLOOD PRESSURE

In addition to the well-demonstrated metabolic actions, incretins can reduce blood pressure as shown in different animal models of arterial hypertension. In Dahl salt-sensitive (DSS) rats, infusion of recombinant GLP-1 induces a reduction in blood pressure with concomitant attenuation of the development of hypertension^[25]. This effect was related to higher levels of urine flow and sodium excretion, known as the natriuretic effect. In addition, a decrease in LV hypertrophy was observed.

Similarly, in another study with DSS^[26], a blunting effect of development of hypertension and cardiac left ventricular hypertrophy was described when the animals were pretreated with an exenatide-related GLP-1 receptor agonist. This was further confirmed during the pre-hypertensive period in spontaneously hypertensive rats^[27] in which the administration of sitagliptin increased the levels of biologically active intact GLP-1 and significantly reduced the increase of blood pressure. These effects do not seem to be the only mechanisms involved in blood pressure reduction since, by using a mouse transgenic model, cardiac GLP-1R activation was able to induce the plasma levels of atrial natriuretic peptide (ANP) together with a decrease in blood pressure. Conversely, in GLP-1R-deficient mice, the GLP-1R agonist liraglutide failed to induce ANP secretion, vasodilation and blood pressure reduction. This supports the idea that different mechanisms of action like a gut-heart GLP-1R and an ANP-dependent axis are involved in blood pressure regulation with these compounds.

Studies of the stable GLP analogues on blood pressure were also performed in human beings^[28]. Data obtained in six studies involving type 2 diabetic patients^[29] showed that 6 mo of treatment with exenatide significantly reduces systolic blood pressure. Similarly, liraglutide in combination with other anti-diabetic drugs like metformin^[30] also demonstrated the ability to reduce systolic blood pressure in diabetic hypertensive patients. In the LEAD-3 Mono trial^[31], treatment with liraglutide vs glimepiride significantly decreased blood pressure. In a different study, Okerson *et al*^[29] reported that six-month treatment with exenatide reduced systolic blood pressure when patients are pretreated with either insulin or placebo. The authors of these studies postulated that the exenatide antihypertensive effect seems to be partly independent from its metabolic activity. However, the weight loss effect cannot be ruled out^[29] (Figure 2), raising one

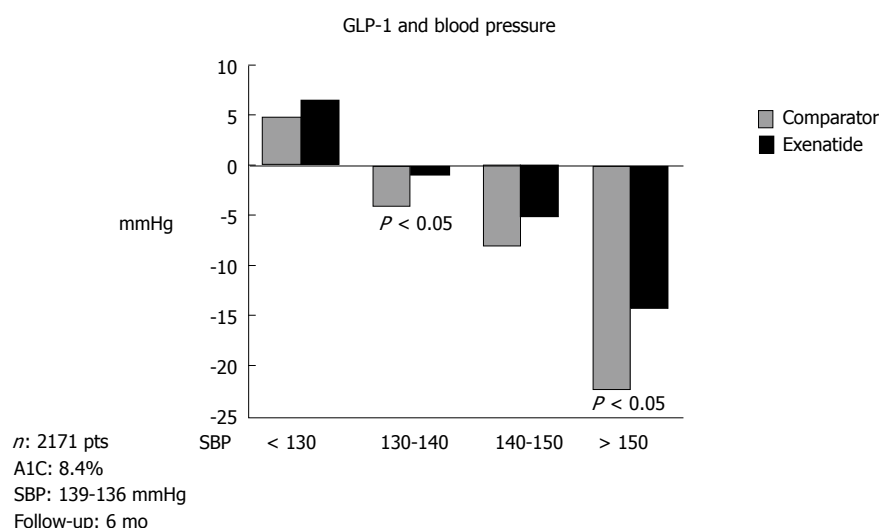


Figure 2 Glucagon-like peptide-1 and blood pressure. Summary of changes in systolic blood pressure (SBP) after the 6-mo study end point in subjects with type 2 diabetes treated with exenatide vs placebo. Data are presented as differences between baseline-to-end point in the least squares (mean \pm SE). Adapt from Okerson *et al*^[29]. GLP-1: Glucagon-like peptide-1.

important point of discussion: How weight loss may contribute to lowering blood pressure and whether this reduction is linked to the antihypertensive effect. In fact, in the Okerson study^[29] the decrease observed in systolic blood pressure was significantly related to weight loss. Likewise, in the LEAD-3 trial^[32], liraglutide treatment significantly reduced weight, whereas glimepiride did not. However, in another study^[33], a decrease in blood pressure was observed prior to a decrease in body weight. Thus, the real association between weight reduction and blood pressure reduction is not yet clear.

Different studies re-analyzed the effects of the pressure-natriuretic mechanism in lowering of blood pressure by both GLP-1 analogues^[34] and DPP-IV inhibitors^[35]. In addition, Crajoinas *et al*^[35] recently suggested that the activation of the cAMP/PKA signaling pathway by incretins interferes with the normal Na⁺ transport in the proximal tubule that decreases sodium and water reabsorption, thus giving further support to the role of the natriuretic effect to the lowering of blood pressure through incretins.

ANTI-HYPERTENSIVE EFFECT OF DPP-IV INHIBITORS IN METABOLIC SYNDROME IN DIABETIC PATIENTS

Although a blood pressure decrease was reported in clinical studies with DPP-IV inhibitors in diabetes, these studies were not designed to evaluate the blood pressure effects and the conclusions were weak and failed to give support to the effect^[36]. In this regard, patients with metabolic syndrome either under placebo or incomplete ACE inhibition were evaluated in one study carried out by Marney *et al*^[37], who examined the interactive effect on blood pressure of the acute inhibition of both ACE and DPP-IV. The administration

of sitagliptin was effective in lowering blood pressure. Yet, during maximal ACE inhibition sitagliptin had the opposite effect: It increased blood pressure with a concomitant increase in heart rate and circulating norepinephrine concentrations. These findings were similar to data previously reported in rats^[38], where a dose-dependent decrease in blood pressure was observed with DPP-IV inhibition but later, when animals were pretreated with the ACE inhibitor captopril, the DPP-IV inhibition caused an increase in blood pressure. This effect was prevented with the blockade of the Neuropeptide Y (NPY1) receptors, thus suggesting that the combined inhibition of ACE and DPP-IV could raise blood pressure through their synergistic effects on substance P degradation. Moreover, Shah *et al*^[39] showed that the inhibition of DPP-IV, similarly to GLP-1, is able to induce vasodilation (nitric oxide effect) with a consequent decrease in peripheral vascular resistance. Despite these controversial results, many investigators still favor the use of GLP-1 analogues and DPP-IV inhibitors for a better control of blood pressure in patients with diabetes and arterial hypertension^[40,41]. In different studies performed in non-diabetic patients, sitagliptin^[42] was associated with a 2-3 mmHg reduction in mean systolic blood pressure, assessed by 24-h ambulatory blood pressure monitoring and, in diabetic patients with inadequate glycemic control^[43] that were receiving metformin, the addition of vildagliptin induced a dose-dependent decrease in both systolic and diastolic blood pressure.

Despite the data presented above, the ability of incretins to reduce blood pressure is still limited. Further studies must be performed in order to elucidate the real efficacy of GLP-1 analogues and DPP-IV inhibition on hypertension. Consequently, randomized trials in patients with either hypertension or diabetes and also with both hypertension and diabetes must be performed

in order to elucidate this important question.

ANTI-INFLAMMATORY EFFECTS OF INCRETINS IN THE CARDIOVASCULAR SYSTEM

Clinical studies of DPP-IV inhibitors on cardiovascular outcomes

Although the CV protective effects of DPP-IV inhibitors seem to be a result of an improvement of type 2 diabetes, the accumulating evidence that was mentioned earlier also suggests a possible direct myocardial effect of GLP-1 on the improvement of the endothelial function, lowering blood pressure and preventing myocardial injury^[44,45].

Another important mechanism of cardiovascular protection is associated with the immune modulatory role of DPP-IV on cardiovascular inflammation. Even though this concept has been minimally investigated, this seems to be an area of emerging importance to evaluate the role of DPP-IV inhibitors in the modulation of innate and adaptive immunity^[46-50]. In this regard, the decreased accumulation of specific inflammatory macrophages present in adipose tissue or atherosclerotic lesions related to the DPP-IV inhibitor treatment was studied^[51,52]. The data provided raises the possibility of a DPP-IV facilitatory interaction with inflammatory related macrophages, resulting in an impairment of inflammation. On the other hand, since DPP-IV activity in serum and tissues is markedly increased in obesity in both animal models and human beings^[53-55], the inhibition of DPP-IV might offer a novel strategy for suppression of low-grade inflammation present in diabetes and associated tissue insulin resistance with favorable effects that improve heart and coronary artery function. Thus, it is possible that the common effects of DPP-IV inhibition/GLP-1 signaling, in opposition to angiotensin II/aldosterone effects, contribute to the beneficial modulation of immune responses in the cardio-renal system^[56-58].

On the other hand, the vasodilator effect of both GLP-1 and DPP-IV inhibitors correlate with an increase in cGMP release, which is attenuated by the pre-incubation with nitric oxide synthase inhibitors, suggesting that at least part of their vasodilator mechanism is nitric oxide/cGMP-dependent. In addition, it seems that the anti-inflammatory effect precedes the blood pressure effect and mediates early improvements in endothelial function and atherosclerosis. Important *in vitro* studies with linagliptin performed in a mouse model of diabetic nephropathy^[59] showed anti-inflammatory^[49,60] and antioxidant^[61] properties, improved re-epithelialization and healing of diabetes-related wounds^[60] and, in a chronic renal failure rat model^[62], renoprotective effects that were not linked to the worsening of glomerular and tubular pathological markers. In addition, in a uremic cardiomyopathy rat model, linagliptin significantly reduced the RNA messenger (mRNA) levels of several

cardiac fibrosis markers and of a marker of left ventricular dysfunction. These results would demonstrate an important anti-fibrotic property of linagliptin^[62].

In clinical studies, incretins seemed to reduce cardiovascular outcomes when compared to other hypoglycemic drugs as shown in a meta-analysis^[63] in which the treatment with DPP-IV was associated with reduced CV events. The overall use of DPP-IV inhibitors compared to placebo or other oral hypoglycemic agents, apart from decreasing adverse CV effects, it was also able to reduce the risk of non-fatal myocardial infarction (MI) and acute coronary syndrome (ACS). Moreover, with DPP-IV inhibitor therapy the risk of adverse CV events was not significantly different compared to placebo, but was significantly lower compared to metformin and other oral hypoglycemic agents, including sulfonylureas and thiazolidinediones. In another small study^[64] comparing sitagliptin vs placebo in patients with coronary artery disease and preserved left ventricular function awaiting revascularization, increased ejection fraction from 64.0% \pm 8.0% to 73.0% \pm 7.0% and increased plasma GLP-1 levels at peak stress (from 10.0 \pm 9.0 pg/mL to 17.0 \pm 11.0 pg/mL; $P \leq 0.003$) and at rest (from 9.0 \pm 6.0 pg/mL to 12.0 \pm 6.0 pg/mL) were reported.

In a large meta-analysis^[65] of 25 phase III studies, vildagliptin was administered either as monotherapy or in combination therapy for a period of 12 wk to 2 years and the drug safety was compared to a pool of placebo and active comparators. Relative to all comparators, the RRs for the composite endpoint were < 1 for both vildagliptin 50 mg *qd* and vildagliptin 50 mg *bid*, and the results were consistent across subgroups defined by age, gender and CV risk status, including the higher CV risk subgroups of elderly patients, males, or patients with a high CV risk status. The exposure-adjusted incidences of each component of the composite endpoint for vildagliptin 50 mg *bid* were also lower than or similar to those of all comparators. Based in these results, it was concluded that vildagliptin is a safe drug in the broad population of type 2 diabetes mellitus (T2DM), including patients at a higher risk of CCV events.

The incidence of major side effects (MACEs) was also evaluated in different studies with DPP-IV inhibitors. A meta-analysis^[66] conducted to assess the effect of DPP-IV inhibitors on the incidence of MACE, cancer and pancreatitis compared to placebo or other treatment, determined that they were associated with a similar risk of cancer and pancreatitis and with a reduced risk of MACE. Frederich *et al.*^[67] analyzed eight randomized double-blind, phase II and III trials of patients with T2DM treated with saxagliptin, placebo, metformin, or glyburide. Cox proportional regression hazard model showed a 41% RR reduction of CV events with saxagliptin vs the comparators. The composite endpoint of CV death, MI or stroke was confirmed in 40 patients from whom 0.7% received saxagliptin and 1.4% received other comparator. The Cox RR

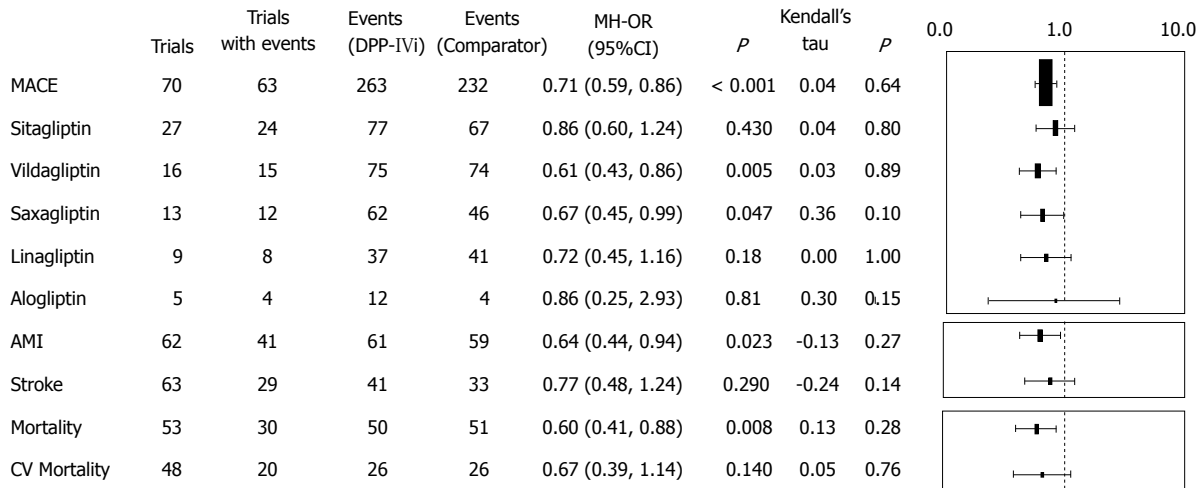


Figure 3 Mantel-Haenzel odds ratio for major cardiovascular events, acute myocardial infarction, stroke, mortality and cardiovascular mortality with 95%CI. Adapt from Monarini *et al*^[60]. DPP-IVi: Dipeptidyl peptidase-IV inhibitors; MH-OR: Mantel-Haenzel odds ratio; CV: Cardiovascular; MACE: Major adverse CV events; AMI: Acute myocardial infarction.

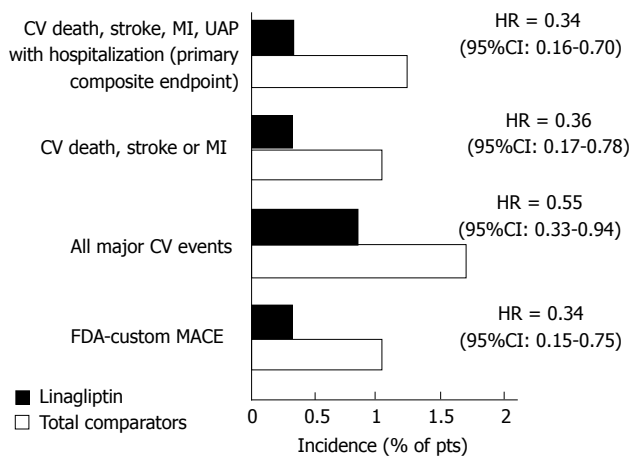


Figure 4 Cardiovascular tolerability profile of linagliptin in adults with type 2 diabetes mellitus. Results of a pre-specified meta-analysis of eight randomized, double-blind trials in which patients treated with linagliptin 5 or 10 mg/d ($n = 3159$ and 160), glimepiride 1-4 mg/d ($n = 781$), voglibose 0.6 mg/d ($n = 162$) or placebo ($n = 977$) as monotherapy or in combination with other oral anti-hyperglycemia drugs for 18-52 wk^[64]. It shows the incidence of primary and secondary composite endpoints in the linagliptin and total comparators group (primary analysis), together with corresponding hazard ratios and 95%CI. Adapted from Deeks^[74]. CV: Cardiovascular; FDA: Food and Drug Administration; MACE: Major adverse CV events; MI: Myocardial infarction; pts: Patients; UAP: Unstable angina pectoris.

estimate was 0.43 translating to a 57% risk reduction in patients assigned to saxagliptin. Thus, no CV harm and a potential for an actual reduction in CV events with saxagliptin was suggested^[67].

Pooled information of MACEs^[68-70] from different DPP-IV inhibitors is shown in Figure 3.

More recently, in a pre-specified meta-analysis assessing cardiovascular safety^[71], cardiovascular risk did not increase with linagliptin 5 or 10 mg once daily (as monotherapy). Additional data suggested that linagliptin was not associated with a significantly greater risk of the primary composite endpoints, regardless of age, gender, and race, use of rescue therapy, hypoglycemia

or cardiovascular risk. In an extension of one clinical trial^[72], after receiving linagliptin monotherapy, the rate of patients reporting cardiovascular/cerebrovascular events was 4.1% and the rate of those with ischemic events amounted up to only 1.9%.

Finally, in a study^[73] of 52 wk of follow-up in which 2.9% of the patients had severe renal impairment (a population with high cardiovascular risk), linagliptin was added to their hypoglycemic therapy, and the rate of death from cardiovascular causes was significantly lower and did not differ from the one observed with placebo.

Figure 4 shows safety indicators in other studies with linagliptin compared to other hypoglycemic drugs^[74].

Trials specifically designed to evaluate the cardiovascular impact of DPP-IV inhibitors

In the SAVOR-TIMI 53 study^[75], 16492 patients with type 2 diabetes and established atherosclerotic disease or high cardiovascular risk were randomized to receive saxagliptin or placebo. The primary endpoint was a composite of cardiovascular death, myocardial infarction or ischemic stroke, with a follow up of 2.1 years. No difference was observed for the primary endpoint when comparing saxagliptin to placebo (7.3% vs 7.2%, HR = 1.00, 95%CI: 0.89-1.12, $P = 0.99$ for superiority, $P < 0.001$ for non-inferiority). Surprisingly, a higher amount of hospitalizations due to heart failure were reported under saxagliptin compared to placebo (3.5% vs 2.8%; HR = 1.27, 95%CI: 1.07-1.51, $P = 0.007$). However, mortality secondary to heart failure did not increase (Figure 5).

The EXAMINE study^[76] evaluated cardiovascular endpoints using alogliptin in patients with diabetes at very high cardiovascular risk. It randomized 5380 patients with diabetes and history of acute coronary syndrome. At a mean follow-up of 18 mo and compared to placebo, there was no difference in a composite of death from cardiovascular causes, non-fatal myocardial infarction, non-fatal stroke (11.3% vs 11.8%, HR =

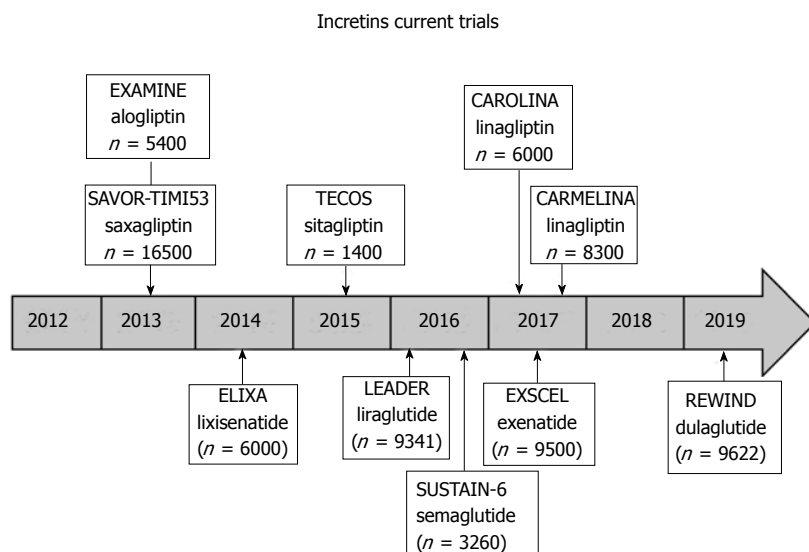


Figure 5 Flowchart of the clinical investigational trials which are completed or ongoing.

0.96; $P < 0.001$ for non-inferiority), in the different components of the primary endpoint nor in the incidence of heart failure.

TECOS: In this randomized, double-blind study recently published, 14671 patients were assigned to add either sitagliptin or placebo to their existing therapy. The primary cardiovascular outcome was a composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for unstable angina. Sitagliptin was noninferior to placebo for the primary composite cardiovascular outcome (HR = 0.98; 95%CI: 0.88-1.09; $P < 0.001$). Rates of hospitalization for heart failure did not differ between the two groups (HR = 1.00; 95%CI: 0.83-1.20; $P = 0.98$)^[77].

The interim analysis of results of SITAGRAMI: Safety and Efficacy of Sitagliptin plus Granulocyte Colony-Stimulating Factor in Patients Suffering from Acute Myocardial Infarction^[78]. It is a phase III multicenter trial testing the myocardial regenerating effects of Sitagliptin combined with G-CSF after an acute MI. The results are encouraging, but they still need to be confirmed once the long-term study has been analyzed.

Others ongoing multicenter clinical trials

CAROLINA: Cardiovascular Outcome Study of Linagliptin vs Glimepiride in Patients with T2DM^[79]. It is a long-term multicenter study planning to enroll 6000 patients with an expected completion date in September 2018.

CARMELINA: Cardiovascular safety and Renal Microvascular outcome with linagliptin patients with T2DM at high vascular risk. It is a long-term study investigating the efficacy and safety of linagliptin vs placebo on cardiovascular and renal micro-vascular outcomes in patients with type 2 diabetes and risk of cardiovascular events. The study will randomize patients

with type 2 diabetes and previous CV complications and albuminuria [urinary albumin creatinine ratio (UACR) ≥ 30 mg/g] with or without evidence of micro-vascular related end-organ damage and an estimated glomerular filtration rate (eGFR) between 15 and 45 mL/min and an UACR > 200 mg/g or eGFR ≥ 45 -75. The study will include more than 8000 adults with type 2 diabetes. The primary endpoint will be the time to the first occurrence of either CV death (including fatal stroke and fatal MI); non-fatal MI; non-fatal stroke; or hospitalization for unstable angina pectoris. The renal outcome is measured as a composite of renal death, sustained end-stage renal disease and sustained decrease of $\geq 50\%$ eGFR. The study will be completed in 2018. This kind of study could provide us with answers regarding the CV and renal outcomes for this type of drugs.

CARDIAC AND BLOOD PRESSURE EFFECTS OF RENAL GLUCOSE TRANSPORT INHIBITORS

The glucose reabsorption regulation is mainly performed in the kidneys where more than 99% of the plasma glucose that filters through the kidneys is reabsorbed. There are two transporters of glucose across cell membranes, the GLUTs, facilitative glucose transporters and an active sodium-dependent transport process mediated by the sodium/glucose co-transporters (SGLTs). These are a large family of intestinal epithelium and of the proximal renal tubules membrane proteins involved in the transportation of glucose, amino acids, vitamins, osmolytes, and some ions^[80].

The high-capacity, low-affinity transporter sodium-glucose co-transporter 2 (SGLT2) is expressed primarily in the kidney, while SGLT1 plays an important function in the absorption of glucose in the intestine. The issue of gene expression and the possibility of SGLT

adaptation to chronic hyperglycemia is an area for further investigation. A small amount of adaptation and a near two-fold increase in the SGLT2 mRNA expression in diabetes animal models was shown. The induction of diabetes in rats increased mRNA expression of both SGLT2 and hepatocyte nuclear factor-1 α in the renal cortex. Glycemic control was improved after 6 d of treatment with insulin or phlorizin accompanied by a reduced expression of SGLT2 and hepatocyte nuclear factor-1 α to near-normal levels^[81].

SGLT2 inhibitors are a new class of anti-diabetic drugs that reduce renal glucose reabsorption selectively in the proximal convoluted tubule leading to an increased urinary glucose excretion without potential gastrointestinal side effects. The SGLT2 inhibitors that are currently under investigation are dapagliflozin, a C-Aryl glucoside, empagliflozin and sergliflozin, an O-glucoside and canagliflozin^[82,83] and represent an interesting and important tool to be added for the treatment of hyperglycemia. Additionally, SGLT2 inhibitors were associated with a reduction in systolic blood pressure compared to placebo (mean difference: -3.77 mmHg) and active comparators (mean difference: -4.45 mmHg). Diastolic blood pressure was also reduced with SGLT2 inhibitors compared to placebo (mean difference: -1.75 mmHg) and other anti-diabetic agents (mean difference: -2.01 mmHg). Risk of bias was high for both systolic and diastolic blood pressure analyses^[84,85].

To be taken into account is the fact that SGLT2 inhibitors, like metformin, are associated with weight loss and also act as osmotic diuretics, resulting in a lowering of BP. While not approved for BP lowering, they may potentially aid BP goal achievement in people with a target reduction within 7-10 mmHg^[86,87]. However, more studies are needed in order to determine a positive antihypertensive action of these compounds.

Regarding potential cardiovascular effects of SGLT2, different meta-analysis were performed: for dapagliflozin, the meta-analysis was based on 14 trials including 6300 patients. An OR of 0.73 (95%CI: 0.46-1.16) compared with the control group was reported, supporting the idea of an absence of cardiovascular risk. In a pooled analysis of two dapagliflozin trials^[87] involving patients with established cardiovascular disease, the hazard ratio (HR) for the composite cardiovascular endpoint (cardiovascular death, myocardial infarction, stroke, and hospitalization for unstable angina) was 1.07 (95%CI: 0.64-1.72) compared to placebo. In another study that included data from 10 trials (10474 patients, OR = 0.95), of canagliflozin compared with placebo, no association of an increased risk for the composite cardiovascular outcome compared to placebo or an active comparator was found. Similarly, in the United States Food and Drug Administration report^[87], the HR for non-fatal stroke was higher in patients receiving canagliflozin (6876 patient-years) than in the control groups (3470 patient-years; HR = 1.46; 95%CI: 0.83-2.58). On the other hand, an imbalance in the

incidence of cardiovascular events was observed during the first 30 d^[88] for canagliflozin (13 of 2886 patients) or placebo (1 of 1441 patients), which resulted in an HR = 6.50 (95%CI: 0.85-49.66). It was explained that this high risk of events resulted from volume depletion after the initiation of canagliflozin treatment, which failed to be observed after 30 d of treatment. In another recent study, systolic and diastolic blood pressure analyses were performed in response to empagliflozin during the euglycemic clamp in hypertensive patients. A reduction in systolic blood pressure was reported, as well as a decreased augmentation index at the radial, carotid and aortic arteries. Similar effects on arterial stiffness were observed, without changes in blood pressure. Carotid-radial pulse wave velocity decreased significantly under both glycemic conditions ($P \leq 0.0001$), whereas declines in carotid-femoral pulse wave velocity were only significant during clamped hyperglycemia. Finally, HRV, plasma noradrenalin and adrenaline remained unchanged under both euglycemic and hyperglycemic clamp conditions^[89].

CONCLUSION

These new anti-diabetic compounds have shown additive CV protective effects in T2DM. Additional benefits include lowering of blood pressure, improvement of lipid profile and endothelial dysfunction, decrease in the macrophage-mediated inflammatory response, and reduction of myocardial injury. All these effects were mainly evaluated in animal models, since human clinical studies that include a high number of participants are still missing.

On the other hand, there are ongoing studies that aim to evaluate the CV effect and the safety of DPP-IV inhibitors. From the last studies that were published in which DPP-IV inhibitors were used, SAVOR TIMI, TECOS and EXAMINE, it seems that a neutral cardiovascular effect rather than a benefit is expected for these compounds. There are other studies with DPP-IV, which are still being developed, such as CAROLINA and CARMELINA, so additional effects could still be assessed.

As it was previously mentioned, further investigations in large cohorts of diabetic patients are needed in order to assess the exact mechanisms of CV protective effects held by renal glucose transport inhibitors. The reason supporting this need is based on the fact that these compounds have shown interesting natriuretic effect resulting in blood pressure decrease and loss of weight. Further trials may endorse these clinical features.

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Initial validation of the Yin-Yang Assessment Questionnaire for persons with diabetes mellitus

Yee Chi Peggy Wong, Mei Che Samantha Pang

Yee Chi Peggy Wong, Dietetic Department, Tuen Mun Hospital, Tuen Mun, New Territories, Hong Kong, China

Mei Che Samantha Pang, School of Nursing, Hong Kong Polytechnic University, Hong Kong, China

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Correspondence to: Yee Chi Peggy Wong, Dr, Dietetic Department, Tuen Mun Hospital, No. 1, Tsing Chung Koon Road, Tuen Mun, New Territories, Hong Kong, China. wongp@ha.org.hk
Telephone: +852-24685108
Fax: +852-24631314

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Abstract

AIM: To initially test for the content validity, comprehensibility, test-retest reliability and internal consistency reliability of the Yin-Yang Assessment Questionnaire (YY-AQ).

METHODS: The process of initial validity and reliability test covered: (1) content validation from the findings of 18 multiple-case studies, validated Yin- and Yang-deficiency assessment questionnaires, relevant literatures and registered Chinese medicine practitioners; (2) comprehension with the levels of comprehensibility for each item categorized on a 3-point scale (not comprehensible; moderately comprehensible; highly comprehensible). A minimum of three respondents selecting for each item of moderately or highly comprehensible were regarded as comprehensive; (3) test-retest reliability conducted with a 2-wk interval. The intraclass correlation coefficients (ICCs) and their 95% CIs were calculated using a two-way random effects model. Wilcoxon Signed Rank test for related samples was adopted to compare the medians of test-retest scores. An ICC value of 0.85 or higher together with $P > 0.05$, was considered acceptable; and (4) internal consistency of the total items was measured and evaluated by Cronbach's coefficient alpha (α). A Cronbach's α of 0.7 or higher was considered to represent good internal consistency.

RESULTS: Eighteen Yin-deficiency and 14 Yang-deficiency presentation items were finalized from content validation. Five participants with type 2 diabetes mellitus (T2DM) performed the comprehensibility and test-retest reliability tests. Comprehensibility score level of each presentation item was found to be moderate or high in three out of the five participants. Test-retest reliability showed that the single measure ICC of the total Yin-deficiency presentation items was 0.99 (95%CI: 0.89-0.99) and the median scores on the first and 14th

days were 17 (IQR 6.5-27) and 21 (IQR 6-29) ($P = 0.144$) respectively. The single measure ICC of the total Yang-deficiency presentation items was 0.88 (95%CI: 0.79-0.99) and the median scores on the first and 14th days were 10 (IQR 6-18) and 14 (IQR 7-23) ($P = 0.144$) respectively. The results of a descriptive correlation study on 140 survey participants with T2DM using the YY-AQ showed that internal consistency of the total Yin-deficiency and Yang-deficiency presentation items was satisfactory, with Cronbach's α of 0.79 and 0.78 respectively.

CONCLUSION: The YY-AQ will be tested further for comprehensibility, test-retest and internal consistency reliabilities, scoring system validity, construct validity, convergent and discriminant validities, responsiveness and predictive validity.

Key words: Body constitution; Traditional Chinese medicine; Diabetes mellitus; Yin-deficiency; Yang-deficiency; Yin-Yang-deficiency; Yin-Yang assessment questionnaire; Initial validity and reliability

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Core tip: Unregulated “unhealthy” body constitution (BC) with an imbalanced Yin and Yang can induce chronic diseases. Past research findings support that food has natures that can regulate the “unhealthy” BC by balancing Yin and Yang. Yin-, Yang- and Yin-Yang-deficiency are the common “unhealthy” BC types in diabetes mellitus (DM). In order to identify the “unhealthy” BC presentations, it was necessary for dieticians to develop the Yin-Yang Assessment Questionnaire for DM. It has passed the initial validation and will be tested further for construct validity, convergent and discriminant validities, responsiveness and predictive validity; scoring system validity, comprehensibility, test-retest and internal consistency reliabilities.

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INTRODUCTION

From a perspective of traditional Chinese medicine (TCM), body constitution (BC) represents the health of an individual or a population in terms of the physical structure, physiological function, psychological reaction and metabolism^[1,2]. BC can be classified as “healthy” and “unhealthy”. “Healthy” BC occurs in the person with a balance of Yin (cold) and Yang (hot) while an unbalanced Yin and Yang leads to “unhealthy” BC, such

as Yin-deficiency and Yang-deficiency^[3]. In accordance with the theory of Yin-Yang interaction in TCM, the weaker the Yin, the weaker the Yang it will be and *vice versa*. That is, unregulated Yin-deficiency will weaken the Yang to further change to Yin-Yang-deficiency or *vice versa*. Different “unhealthy” BC types, such as Yin-deficiency and Yang-deficiency can be found in association with a single disease. Likewise, the same “unhealthy” BC type can be found to be associated with different diseases^[4]. Without a prompt and appropriate treatment, “unhealthy” BC will induce diseases. Studies showed that both Yin-deficiency and Yang-deficiency types of BC have a negative influence on nervous system^[5], long-term memory^[6], blood pressure^[7], heart health^[8], carcinoma^[9] and sleep quality^[10]. In addition, persons with Yang-deficiency are also found to have hormone abnormality^[11], organ dysfunction and decreasing metabolic rate^[12], accumulation of free radicals (destructive substances inside the body), declining immunity and sterility^[13].

Yin-deficiency, Yang-deficiency and Yin-Yang-deficiency are commonly recognized BC types in the population of diabetes mellitus (DM)^[14-17]. Empirical study found that persons with type 2 diabetes mellitus (T2DM) have the presentations of these “unhealthy” BC types (Table 1)^[18]. A validated Yin/Yang-deficiency assessment questionnaire will help provide prompt assessment of “unhealthy” BC presentations so that earlier and appropriate dietary therapy can be provided to regulate the “unhealthy” BC presentations by a balance of Yin and Yang. The ultimate goal of this treatment strategy is to prevent the development of other chronic diseases.

There are a few validated Yin-deficiency and Yang-deficiency assessment questionnaires available in the field of TCM, such as the Traditional Chinese Medical Yang-Xu Constitutional Questionnaire (TCMYCQ)^[19], the Yin-Deficiency Questionnaire 1 (Yin-DQ1)^[20] and the Cold-Heat Pattern Questionnaire^[21]. However, these questionnaires do not target those with DM. The TCMYCQ is applied for pregnant women while the Yin-DQ1 was developed for general patients and the Cold-Heat Pattern Questionnaire is mainly adopted in clinical trials only. In view of the unavailability of a Yin- and Yang-deficiency assessment questionnaire for persons with DM, it was necessary to develop one such instrument for three reasons. First, DM prevalence has been increasing worldwide^[22]. Second, research findings showed that Yin-deficiency, Yang-deficiency and Yin-Yang-deficiency are the common types of “unhealthy” BC in DM population^[14-17]. Third, if BC assessment is to be integrated into the current dietary practice in future, this specific assessment instrument would be helpful to healthcare professionals, such as dieticians, in the assessment of Yin-deficiency, Yang-deficiency or Yin-Yang-deficiency presentations for persons with DM before a Yin/Yang enhancing dietary therapy would be provided for regulating the “unhealthy” BC

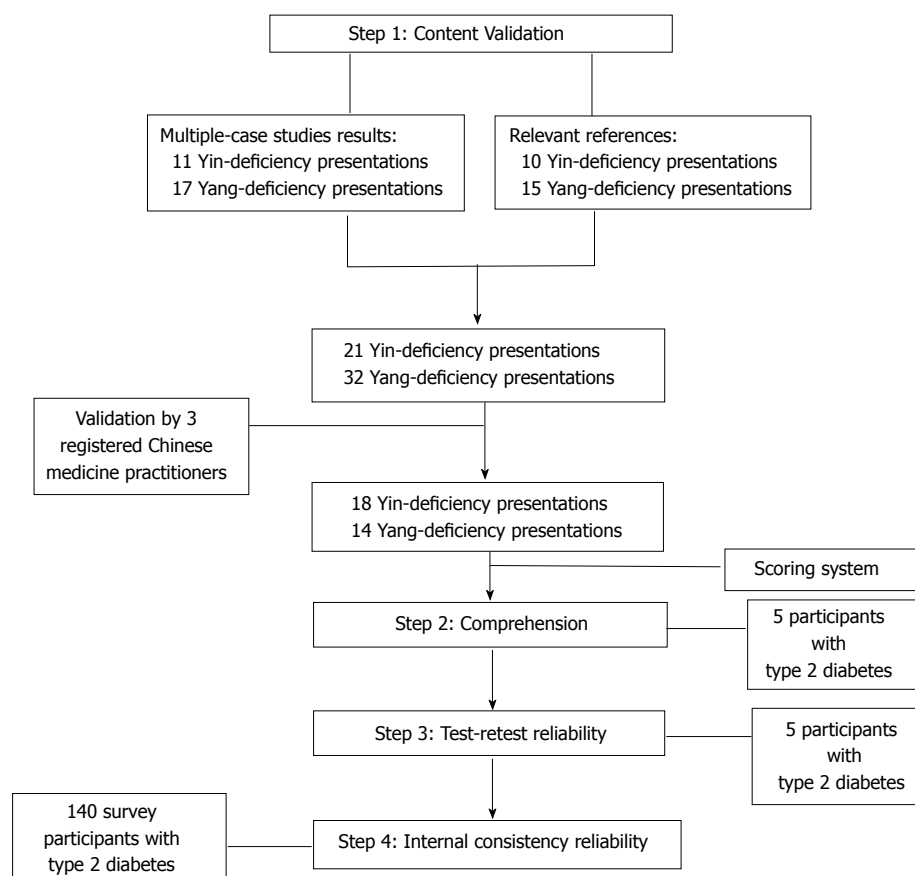


Figure 1 The process of developing the Yin-Yang Assessment Questionnaire.

presentations.

MATERIALS AND METHODS

The initial validation of the Yin-Yang Assessment Questionnaire (YY-AQ) for DM is summarized in four steps (Figure 1). They are content validation, comprehension, test-retest reliability and internal consistency reliability. Scoring system was set up in between the steps of content validation and comprehension.

Content validation

Content validation focuses on whether the full content of a conceptual definition is represented in the measure^[23]. The YY-AQ was performed using the findings from the multiple-case studies conducted by Wong *et al.*^[18], validated Yin-deficiency and Yang-deficiency assessment questionnaires and relevant literatures.

The multiple-case studies

Between May 2013 and June 2013, 18 persons with T2DM were recruited in the multiple-case studies^[18]. They were members of a non-profit organization for persons with DM. A specific characteristic of this participant group was that they all showed interests and faith in Chinese dietary therapy when asked why they liked to participate in this study. Apart from DM, all of them did not have other chronic diseases, non-diabetic

medications and other medical treatments, such as kidney dialysis, chemotherapy, radiotherapy or Chinese medicine tonification. About three quarters of the 18 cases was female. The majority was aged less than 60 years. Most of the participants had more than three years of known diagnosis and were overweight. Nearly all of them were taking oral diabetic medications and their blood glucose levels were within normal range.

Each participant was assessed for BC type and presentations by a registered Chinese medicine practitioner (RCMP), who has 25 years of clinical experience in TCM, performed the TCM diagnostic technique using "Four Examinations" to collect the data with an application of the "Eight Principles of Syndrome Differentiation" to diagnose the BC type. Inter-rater reliability test was performed by two other RCMPs on five cases with a consistency rate of 80%. Eleven Yin-deficiency presentations and 17 Yang-deficiency presentations were identified from the multiple-case studies.

Procedure of content validation

First, Yin-deficiency presentation items were compiled from the multiple-case studies findings, validated Yin-DQ1^[20] and two related studies^[10,24]. Twenty Yin-deficiency presentation items were compiled, of which 11 items were made reference to the findings of the 18 multiple-case studies^[18]. Apart from the Yin-deficiency

Table 1 Presentations of Yin-deficiency and Yang-deficiency in persons with type 2 diabetes mellitus

18 Yin-deficiency presentations	14 Yang-deficiency presentations
I have felt excessively warm in all seasons ¹	I have had an aversion to cold in all seasons ¹
I have worn thin clothes due to feeling excessively warm	I have worn thick clothes due to my aversion to cold
I have intermittent hot and cold spells	I have had an aversion to strong wind
My face has been flushed with crimson red	I have experienced heavy sweating ¹
I have been thin and slim ¹	My body looks puffy ¹
My palms or soles have felt hot	I have had pains on my knee, loin, shoulder, and back but feeling better with heat application ¹
I have had tinnitus ¹	I have running nose or and sneezing ¹
I have dry cough ¹	I have had clear sputum ¹
I have experienced hot flush especially in the afternoon	I have felt comfortable with hot drink ¹
I have experienced sweating at night ¹	I needed to wake up because of my diarrhea
My stools have been dry and hard ¹	My stools have been loose or watery ¹
I have felt hungry even after big meals	I have had diarrhea, itchy throat or cough after intake of cold food
I have passed minimal volumes of urine that were yellow colored ¹	I have passed large volumes of colorless urine ¹
I have always drunk water to quench my thirst	I have experienced bland taste in my mouth ¹
My thirst could not be relieved by frequent water intake ¹	
My skin has been very dry ¹	
My eyes have felt very dry	
My lips have felt very dry	

¹Presentations found in multiple-case studies^[18].

presentations, Yang-deficiency presentations were compiled from the multiple-case studies findings, validated TCMYQ^[19] and Wang^[10]'s study. Thirty-three Yang-deficiency presentation items were compiled, of which 17 items were found from the multiple-case studies findings^[18]. A total of 53 presentation items were finally compiled, of which 28 (52.8%) belonged to the findings of the multiple-case studies^[18]. Second, three RCMPs validated the contents of the 53 Yin-deficiency and Yang-deficiency presentation items. One of them has clinical experience for more than 25 years. The other two RCMPs, who are at PhD level in Chinese medicine, have been practising TCM for eight and 18 years respectively. The 53 presentation items were validated further into 32 items, of which 18 were Yin-deficiency type and 14 were Yang-deficiency type (Table 1). Of these 32 presentation items, 19 (53%) belonged to the findings of the multiple-case studies^[18].

Scoring system

With reference to the validated TCMYQ^[19], the presentation items of Yin-deficiency and Yang-deficiency were categorized into frequency scoring system including "never", "rare", "occasional", "often" and "always". Each score was set for the respondent on a scale from 0 score (never) to 1 score (rare), 2 scores (occasional), 3 scores (often) and 4 (always) (Table 2). The higher the presentation score level, the more severe the "unhealthy" BC presentations the person has experienced. For statistical purpose, the score levels of the Yin-deficiency, Yang-deficiency and Yin-Yang-deficiency presentations were defined into low, moderate, high and very high levels using median as the cut-off point.

Comprehension

Comprehension is an important procedure in the

development of an instrument. It requires respondent to be able to understand the contents presented and to have the opportunity to read, evaluate and consider the content presented^[25]. Five participants with T2DM were recruited and asked whether they understood the 32 presentation items from the YY-AQ. The levels of comprehensibility for each item were categorized on a 3-point scale (not comprehensible; moderately comprehensible; highly comprehensible). A minimum of three respondents selecting for each item of moderately or highly comprehensible was regarded as comprehensive.

Test-retest reliability

In order to ensure the reliability of the YY-AQ, it was necessary to ensure same score would be obtained when it would be given to the same person, under the same circumstances, but at a different time^[23]. The test-retest reliability requires two administrations of the measuring instrument so as to assure stability of measurement over time. The YY-AQ was administered by five participants with T2DM with a 2-wk interval. This interval was used because the participants considered this length of time to be reasonable for time convenience. The participants completed the first YY-AQ at the recruitment. The second YY-AQ was sent to them by mail. They were requested to complete this second questionnaire on the 14th day since the completion of the first YY-AQ.

Internal consistency reliability

A measurement with a high degree of internal consistency is able to ensure all items are consistent with each other, or all working in the same direction^[23]. Internal consistency estimation requires only one administration of the instrument. The YY-AQ was evaluated by a value of Cronbach's α ^[26] from a descriptive correlation

Table 2 The Yin-Yang Assessment Questionnaire

Your feelings (presentations)	Put a "tick" if applicable				
	No	Rare	Occasional	Often	Always
	0	(1 score)	(2 scores)	(3 scores)	(4 scores)
I have felt excessively warm in all seasons ¹					
I have worn thin clothes due to feeling excessively warm ¹					
I have had an aversion to cold in all seasons ²					
I have worn thick clothes due to my aversion to cold ²					
I have intermittent hot and cold spells ¹					
I have had an aversion to strong wind ²					
My face has been flushed with crimson red ¹					
I have experienced hot flush especially in the afternoon ¹					
I have experienced sweating at night ¹					
My skin has been very dry ¹					
My eyes have felt very dry ¹					
My lips have felt very dry ¹					
I have been thin and slim ¹					
My body looks puffy ²					
My palms or soles have felt hot ¹					
I have had pains on my knee, loin, shoulder, and back but feeling better with heat application ¹					
I have running nose or and sneezing ¹					
I have had tinnitus ²					
I have always drunk water to quench my thirst ¹					
I have experienced heavy sweating ²					
My thirst could not be relieved by frequent water intake ¹					
I have felt comfortable with hot drink ²					
I have dry cough ¹					
I have had clear sputum ²					
My stools have been dry and hard ¹					
My stools have been loose or watery ²					
I needed to wake up because of my diarrhea ²					
I have experienced bland taste in my mouth ²					
I have felt hungry even after big meals ¹					
I have had diarrhea, itchy throat or cough after intake of cold food ²					
I have passed minimal volumes of urine that were yellow colored ¹					
I have passed large volumes of colorless urine ²					

¹Yin-deficiency presentation; ²Yang-deficiency presentation.

study on BC presentations in a sample of persons with T2DM^[18].

The descriptive correlation study

One hundred and forty participants with T2DM were recruited to take part in the structured questionnaire survey between October 2013 and December 2013 after they had met the inclusion criteria: aged over 18 years; not on insulin injection and other medical treatments, such as kidney dialysis, chemotherapy or radiotherapy. Of the 140 survey participants, majority was the female. The mean age was about 65 years. Nearly half of the survey participants had more than 10 years of known diagnosis, taking either oral diabetic medications or non-diabetic medications. More than half of them had other chronic diseases, such as hypertension, heart disease, gout, cancer or liver disease. More than three quarters of the survey participants reported satisfactory blood glucose control. At the survey, each of them self-administered the YY-AQ. Completed questionnaires were then collected for data analysis of internal consistency reliability after data check by the researcher or trained helpers.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 20, SPSS Inc., United States) was used to analyze the questionnaire data. Intraclass correlation coefficient (ICC) was used to analyze the test-retest reliability of the YY-AQ. Wilcoxon Signed Rank test for related samples was adopted to compare the median of test-retest scores. If the ICC value of 0.85 or higher together with $P > 0.05$ for the related samples test, the test-retest reliability of the YY-AQ was considered acceptable^[27]. A Cronbach's α of 0.7 or higher was considered to represent good internal consistency^[23].

RESULTS

A total of 32 presentation items (18 Yin-deficiency items and 14 Yang-deficiency items) were validated for the YY-AQ (Table 2). Based on the cut-off point (median = 18), the score levels of the Yin-deficiency presentations questionnaire are categorized into low score (0-11 scores), moderate score (12-18 scores), high score (19-27 scores) and very high score (> 27 scores). The Yang-deficiency presentation score levels (median = 15)

Table 3 Categorization of the Yin-Yang Assessment Questionnaire score levels in research setting

Presentation	Presentation score levels			
	Low score	Moderate score	High score	Very high score
Yin-deficiency (median = 18)	0-11	12-18	19-27	> 27
Yang-deficiency (median = 15)	0-10	11-15	16-20	> 20
Yin-Yang-deficiency (median = 35)	0-23	24-35	36-48	> 48

Table 4 Comprehensibility of Yin-Yang Assessment Questionnaire (*n* = 5)

Your feelings (presentations)	Frequency of comprehensibility		
	Not	Moderately	Highly
I have felt excessively warm in all seasons	0	4	1
I have worn thin clothes due to feeling excessively warm	0	4	1
I have had an aversion to cold in all seasons	0	3	2
I have worn thick clothes due to my aversion to cold	0	3	2
I have intermittent hot and cold spells	1	4	0
I have had an aversion to strong wind	1	4	0
My face has been flushed with crimson red	0	5	0
I have experienced hot flush especially in the afternoon	1	4	0
I have experienced sweating at night	0	5	0
My skin has been very dry	0	5	0
My eyes have felt very dry	0	5	0
My lips have felt very dry	1	4	1
I have been thin and slim	0	0	5
My body looks puffy	1	4	0
My palms or soles have felt hot	0	5	0
I have had pains on my knee, loin, shoulder, and back but feeling better with heat application	0	4	1
I have running nose or and sneezing	0	5	0
I have had tinnitus	0	0	5
I have always drunk water to quench my thirst	0	0	5
I have experienced heavy sweating	0	5	0
My thirst could not be relieved by frequent water intake	0	3	2
I have felt comfortable with hot drink	0	0	5
I have dry cough	1	4	0
I have had clear sputum	0	5	0
My stools have been dry and hard	0	0	5
My stools have been loose or watery	0	0	5
I needed to wake up because of my diarrhea	1	4	0
I have experienced bland taste in my mouth	1	4	0
I have felt hungry even after big meals	0	0	5
I have had diarrhea, itchy throat or cough after intake of cold food	0	0	5
I have passed minimal volumes of urine that were yellow colored	0	4	1
I have passed large volumes of colorless urine	0	5	0

were set as: low score (0-10 scores), moderate score (11-15 scores), high score (16-20 scores) and very high score (> 20 scores). The Yin-Yang-deficiency scores were obtained by adding up the totals from the Yin- and Yang-deficiency scores. The Yin-Yang-deficiency score levels (median = 35) were classified as low score (0-23 scores), moderate score (24-35 scores), high score (36-48 scores) and very high score (> 48 scores) (Table 3). The results showed that more than three out of the five participants had selected each items of the YY-AQ as moderately or highly comprehensible (Table 4). Test-retest reliability showed that the single measure ICC of the total Yin-deficiency presentation items was 0.99 (95%CI: 0.89-0.99) and the median scores on the first and 14th days were 17 (IQR 6.5-27) and 21 (IQR 6-29) ($P = 0.144$) respectively. The single measure ICC of the total Yang-deficiency presentation items was 0.88 (95%CI: 0.79-0.99) and the median scores on the first

and 14th days were 10 (IQR 6-18) and 14 (IQR 7-23) ($P = 0.144$) respectively. Internal consistency of the total Yin-deficiency and Yang-deficiency presentation items showed Cronbach's α of 0.79 and 0.78 respectively.

DISCUSSION

Although the initial validation of the YY-AQ was performed in a sample of persons with T2DM, this questionnaire can also be applied to those with type 1 diabetes mellitus (T1DM) or impaired glucose tolerance (IGT). It is because DM in TCM is not treated like those in the Western medicine. Similar to other diseases, treatment variation for persons with DM is based on the syndrome differentiation and then treated accordingly with the consideration of BC^[28]. As such, the YY-AQ is not an instrument for diagnosing DM but serves to assess the presentations of Yin-deficiency, Yang-deficiency and

Table 5 Categorization of the Yin-Yang Assessment Questionnaire score levels in clinical setting

Presentation	Presentation score levels		
	Low score	Moderate score	High score
Yin-deficiency (median = 18)	0-11	12-18	> 18
Yang-deficiency (median = 15)	0-10	11-15	> 15
Yin-Yang-deficiency (median = 35)	0-23	24-35	> 35

Yin-Yang-deficiency in persons with DM. It is rather an assessment instrument that can help dietitians to make early identification of “unhealthy” BC presentations in people with DM so that an appropriate Yin/Yang enhancing dietary therapy could be provided to prevent them from development of other diseases.

In applying the YY-AQ to dietetic settings including clinical and community dietetics, it may be necessary to categorize the presentation scores into low, moderate and high levels (Table 5) because it is a common practice for dietitians to set an assessment instrument into three levels. A moderate score is the basic requirement for referral to a clinical dietitian. For those who get low scores, protocols for providing dietetic service can be set, such as issue of relevant information leaflet without referring to a clinical dietitian. The YY-AQ is designed for self-administration or by in-person interview. This is because some respondents might possibly be unable to understand all of the question contents for two reasons. First, some of them have poor vision due to the disease. Second, older age is a key factor in understanding, so there might be difficulties for those aged above 70 years or even 60 years. Assessment of the Yin- and Yang-deficiency presentations can be incorporated into conventional dietary therapy for DM care as well as community dietetic program so that Yin-deficiency and Yang-deficiency presentations can be identified, and persons thus identified can benefit from a Yin/Yang enhancing dietary program as early as possible.

We have found one limitation in the initial validation study. Some presentation items are similar in the meaning. First, “I have felt excessively warm in all seasons” and “I have worn thin clothes due to feeling excessively warm” mean persistent feelings of “burning hot” even though the temperatures were generally low. It was found from crosstabs statistics and Spearman’s correlation coefficient (ρ) that 55% of the 140 survey participants responded to both of these items ($\rho = 0.62$, $P = 0.000$). Second, “I have had an aversion to cold in all seasons” and “I have worn thick clothes due to my aversion to cold” suggest that the respondents had experienced persistent feelings of “chilling cold” although the temperatures were general high. Of the 140 survey participants, 62% reported having both of these complaints ($\rho = 0.63$, $P = 0.000$). Third, “My face has been flushed with crimson red” and “I have experienced hot flush especially in the afternoon” also mean the same thing. The results showed that 72.9% of the survey sample responded to both of the two items ($\rho = 0.63$, $P = 0.000$). Fourth, “My stools have been loose or watery” also has the similar meaning

to “I needed to wake up because of my diarrhea”. Of the 140 survey participants, 50% responded to both of the complaints ($\rho = 0.35$, $P = 0.008$). Finally, the results showed that only 39% of the survey participants responded to both “I have always drunk water to quench my thirst” and “My thirst could not be relieved by frequent water intake” ($\rho = 0.4$, $P = 0.000$). In view of the above statistical findings, the first three pairs of items could be tentatively considered for integration respectively due to being responded by over 50% of the survey participants. However, there is no “gold standard” of percentage requirement in considering the integration of items for measuring instruments. A larger sample size would be considered in the future validity and reliability study for the YY-AQ to find more substantial evidence.

In conclusion, the YY-AQ has initially passed the content validity, comprehensibility, test-retest and internal consistency reliabilities. Due to the small sample size in the tests for comprehensibility and test and retest reliability, need of a larger sample size for substantiating the need of items integration and other important validity and reliability for the YY-AQ, it is necessary to test it further for establishing its validity and reliability: comprehension if the contents are user friendly and easily understood by respondents; test-retest reliability for testing if the YY-AQ can give the same measurement to the same people with DM on different occasions; internal consistency reliability for testing the degree to which the items of the questionnaire are all measuring the same for DM; determination of the scoring system for its clinical significance; construct validity with factor analysis for testing if each item of the YY-AQ corresponds to one of the factors to be derived in the questionnaire; convergent and discriminant validities for testing the degree of the positive and negative correlation respectively between the total items and total factors to be derived in the YY-AQ; responsiveness and predictive validity; and the need of items integration.

This questionnaire is intended for use by dietitians in clinical or community settings. It will help them to early identify the Yin-deficiency or Yang-deficiency presentations in persons with DM so that a Yin/Yang enhancing dietary therapy or education program with a TCM approach can be provided for regulating the “unhealthy” BC presentations, apart from blood glucose stabilization using the nutrition approach in conventional dietary therapy. The goal of the integrated dietetic practice is to prevent the disease advancement by slowing down or preventing the development of other diseases in persons with DM, apart from stabilization

of blood glucose level. Ultimately, the disease burden, such as reduced DM-related quality of life, stress of healthcare professionals and the rising healthcare cost would probably be reduced.

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COMMENTS

Background

Conventional dietary therapy has its limitations in dealing with the “unhealthy” body constitution (BC) presentations by balancing one’s Yin and Yang in persons with diabetes mellitus (DM). It is because it controls blood glucose level with nutrition component only. An integration of current dietary practice and Chinese dietary approach may enhance the effectiveness on DM control as well as prevention of other diseases occurrence. In order to identify the “unhealthy” BC presentations, a validated Yin-Yang assessment questionnaire is required.

Research frontiers

The development of the Yin-Yang Assessment Questionnaire (YY-AQ) has completed its initial validation. However, it requires further test for validity and reliability.

Innovations and breakthroughs

It is comprehended that conventional dietary therapy has its limitations of regulating BC from a perspective of traditional Chinese medicine (TCM) while Chinese dietary therapy does not deal with nutrition component in the control of blood glucose. An integration of conventional dietary therapy and Chinese dietary therapy for DM care might be a future direction. The development of the YY-AQ has started a new page to the conventional dietary therapy for persons with DM.

Applications

The YY-AQ can be applied to persons with different types of DM in either clinical or community dietetic settings. In the future, this instrument could be further developed to be applied in different chronic diseases, such as hypertension.

Terminology

Yin-deficiency and Yang-deficiency are two common types of “unhealthy” BC in TCM. People with Yin-deficiency type of BC have the feelings of a “heat-dryness” due to an abnormally low level of humidity but extremely high level of temperature inside the body. Yang-deficiency is an indication of a “cold-dampness” nature of BC as a result of body humidity being at an extremely high above normal level but the body temperature is, however, extremely subnormal.

Peer-review

This study aimed to develop of a Yin/Yang assessment questionnaire for persons with type 2 diabetes from a Traditional Chinese Medicine perspective.

This questionnaire is intended for use by dieticians in clinical and community settings. It will help them to early make identification of the Yin- or Yang-deficiency presentations in persons with T2DM so that a Yin/Yang enhancing dietary therapy or program can be provided for regulating the unhealthy body constitution presentations.

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Treatment of prediabetes

Mustafa Kanat, Ralph A DeFronzo, Muhammad A Abdul-Ghani

Mustafa Kanat, Division of Diabetes, Department of Internal Medicine, Istanbul Medipol University, 34214 Istanbul, Turkey

Ralph A DeFronzo, Muhammad A Abdul-Ghani, Diabetes Division, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, United States

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Correspondence to: Dr. Mustafa Kanat, MD, Division of Diabetes, Department of Internal Medicine, Istanbul Medipol University, TEM Avrupa Otoyolu Göztepe Çıkışı No: 1, Bağcılar, 34214 Istanbul, Turkey. mustafa.kanat@gmail.com
 Telephone: +90-542-3131400

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Abstract

Progression of normal glucose tolerance (NGT) to overt diabetes is mediated by a transition state called impaired glucose tolerance (IGT). Beta cell dysfunction

and insulin resistance are the main defects in type 2 diabetes mellitus (type 2 DM) and even normoglycemic IGT patients manifest these defects. Beta cell dysfunction and insulin resistance also contribute to the progression of IGT to type 2 DM. Improving insulin sensitivity and/or preserving functions of beta-cells can be a rational way to normalize the GT and to control transition of IGT to type 2 DM. Losing weight, for example, improves whole body insulin sensitivity and preserves beta-cell function and its inhibitory effect on progression of IGT to type 2 DM had been proven. But interventions aiming weight loss usually not applicable in real life. Pharmacotherapy is another option to gain better insulin sensitivity and to maintain beta-cell function. In this review, two potential treatment options (lifestyle modification and pharmacologic agents) that limits the IGT-type 2 DM conversion in prediabetic subjects are discussed.

Key words: Prediabetes; Impaired fasting glucose; Impaired glucose tolerance; Diabetes prevention; Type 2 diabetes mellitus

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Core tip: Behavioral changes (dieting plus exercising) are effective in preventing impaired glucose tolerance (GT)-type 2 diabetes mellitus (type 2 DM) conversion as well as impaired fasting glucose (FG) - type 2 DM conversion but losing weight is hard and also difficult to maintain. Pharmacological interventions (plus dieting and exercising) improving and preserving beta-cell function and enhancing insulin sensitivity may be suitable choices for high-risk IGT patients. Troglitazone in Prevention of Diabetes Study, Pioglitazone in Prevention of Diabetes Study, Diabetes Reduction Assessment with ramipril and rosiglitazone Medication Trial, Actos Now for the prevention of diabetes study and Diabetes Prevention Program have proven that thiazolidinediones obviously prevent the development of type 2 DM in IGT subjects as well as IFG subjects. In Diabetes Prevention Program and Indian Diabetes Prevention Program, metformin slowed down the

progression of IGT to type 2 DM, and eventually American Diabetes Association Consensus Conference Statement proposed metformin usage in high-risk IGT individuals. However, the efficacy of pioglitazone and rosiglitazone efficacy in preventing IGT progression to type 2 DM nearly doubles metformin's efficacy (31% vs 72% and 62%, respectively). Rosiglitazone (low dose = 2 mg/d) together with metformin (850 mg/d) was proven to slow down IGT progression to type 2 DM as well as being more tolerable.

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INTRODUCTION

Impaired glucose tolerance (IGT) (second hour plasma glucose level 140-199 mg/dL) was first described in 1979 as "an intermediate stage in the transition from normal glucose tolerance (NGT) to overt type 2 diabetes mellitus (type 2 DM)"^[1]. Individuals with IGT possess higher risk for type 2 DM later in life^[2]. ADA-revised type 2 DM diagnostic criteria declared a new term called impaired fasting glucose (IFG) (glucose level 100-125 mg/dL) in 1997^[3]. IFG is an intermediate stage that GT changes from NGT to type 2 DM gradually and defined by fasting plasma glucose level. Subjects who have IFG are also candidates for developing type 2 DM later. But clinical and epidemiologic studies showed that IFG and IGT are different sorts of glucose intolerance^[4]. Both IGT and IFG are called "prediabetes" because of gradual progression to type 2 DM. Nearly 70 million prediabetics (IGT and/or IFG) live in America. Since prediabetes is so prevalent^[5], increase mortality, morbidity and healthcare costs (annually \$245 billion in 2012) it is accepted as an important public health problem. Thus, alleviating the progression of IGT and/or IFG to type 2 DM is a reasonable way to combat with diabetes epidemic and to lessen healthcare costs.

The Diabetes Control and Complications Trial^[6], the United Kingdom Prospective Diabetes Study (UKPDS)^[7,8] and the Kumamoto Study^[9] showed hyperglycemia is a risk factor for macrovascular and especially for microvascular complications^[10,11]. Latest evidence illuminated that strict glycemic control is more effective in controlling diabetic vascular complications in new-onset diabetes patients than in long-standing, poorly-controlled type 2 DM patients^[12,13]. Therefore, in new-onset type 2 DM, main target must be to achieve normoglycemic control^[14]. Early detection and effective intervention of type 2 DM diminishes long-term complications leading morbidity and mortality and eventually expected to provide social, medical, and economic benefits. Treatment should be initiated in IGT period in order to reverse the main pathophysiological defects in prediabetes^[4,15-18] because this is a hopeful

way of intervention to prevent hyperglycemia-related vascular complication development^[15-18].

TYPE 2 DM PATHOGENESIS

Recent proof favors dual-level emergence of type 2 DM^[19-24] (Figure 1). In individuals tended to progress type 2 DM, earliest metabolic abnormality is the insulin resistance. When insulin resistance appears, beta-cells increase their insulin secretion to maintain normoglycemia. Thus, hyperinsulinemia is the main sign of insulin resistance. If beta-cells can not overcome insulin resistance, GT aggravates. Eventually, IGT appears and followed by overt type 2 DM^[22-25].

Thus, IGT individuals' plasma insulin levels are high but their beta-cell function are extremely diminished^[22,23,25]. Therefore, noticing the difference between insulin secretion and beta-cell function is important.

Insulin resistance

The common defect in prediabetes and type 2 DM is insulin resistance^[26-29] and involves liver^[22,23,30], muscle^[22,23,28,31,32], and adipose tissue^[23]. Insulin resistance antecedents the glucose intolerance and type 2 DM^[22,23,33]. NGT offspring of two diabetic parents^[34,35] and people with IGT^[36] are markedly insulin resistant and develop hyperinsulinemia in order to compensate the pathologic state^[14,34,35]. Evidence supports that insulin resistance may have a genetic component that worsens by environmental factors such as sedentary lifestyle and gaining weight. Hence, interventions that ameliorate insulin resistance and limits the insulin secretory demand on beta-cells shown to stop or postpone IGT conversion to type 2 DM^[37-40].

Impairment of beta-cell function

Insulin resistance is the basic characteristics of IGT while deficiency of beta-cell function is the reason of IGT and its conversion to type 2 DM^[22,23,41]. Thus, interventions preserving beta-cell function may be a good idea to prevent the generation of type 2 DM. In order to estimate IGT progression to type 2 DM oral glucose tolerance test (OGTT) can be used and a low plasma insulin response is a clue for progression. Especially, reduction of insulin secretion in the first phase (0-10 min later following intravenous glucose challenge) is a good indicator for conversion to diabetes^[33,36,42,43]. The first phase insulin secretion deteriorates gradually when the fasting plasma glucose (PG) exceeds 90 mg/dL and is almost completely lost when the fasting PG reaches over 110 mg/dL^[22,23,44,45]. As previously described, it is crucial to discriminate insulin secretion from beta-cell function. Beta-cells respond unit glucose increase (ΔG) with unit insulin increase (ΔI), and this response is modulated by severity of insulin resistance^[46]. Pure plasma insulin response measurement can lead to confusing about the health of beta-cells. The gold standard for the estimation of beta-cell function is to calculate insulin secretion/insulin resistance (disposition) index ($\Delta I/\Delta G/IR$). Both genetic

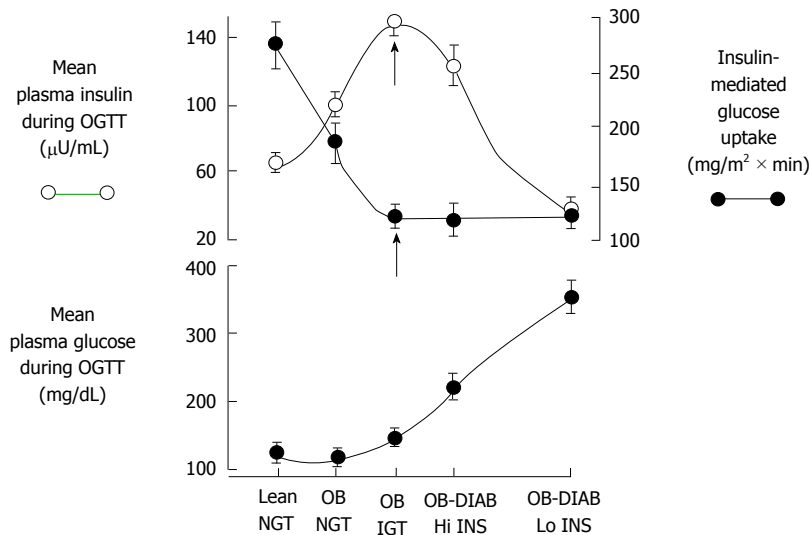


Figure 1 Natural history of type 2 diabetes mellitus. The plasma insulin response (open circles) depicts the classic Starling's curve of the pancreas. Closed circles = insulin-mediated glucose uptake (top panel). DIAB: Diabetes; Hi INS: High insulin secretion; IGT: Impaired glucose tolerance; Lo INS: Low insulin secretion; NGT: Normal glucose tolerance; OB: Obese; OGTT: Oral glucose tolerance test.

and acquired factors (glucotoxicity^[47] lipotoxicity^[48], incretin deficiency/resistance^[49-51]) effect loss of beta-cell function. Compared to normal glucose tolerant individuals, impaired glucose tolerant individuals have a 4-6 fold increment in type 2 DM risk^[52]. Prospective epidemiologic studies reveal that nearly 40% of subjects developing type 2 DM at follow-up had normal glucose tolerant initially. Beta-cell dysfunction is an optimal predictor for 2-h plasma glucose during OGTT in normal glucose tolerant individuals^[43,52]. Beta-cell dysfunction is also an optimal predictor for NGT conversion to IGT and thereby to type 2 DM^[23,24,52]. Individuals in the upper tertile of NGT have lost 50% of their beta-cell function, whereas subjects in the upper tertile of IGT 70%-80% (Figure 2). Individuals in the upper tertile of IGT are maximally insulin resistant and decline in beta-cell function is about 70%-80%. At this point, minimal extra reduction in insulin secretion causes a prominent increase in fasting and postprandial blood glucose levels. Once overt type 2 DM emerges, beta-cell function diminishes progressively^[53] despite therapies with metformin, sulfonylureas, and insulin to control glycemia. Genetics, insulin resistance leading insulin secretory demand increment, glucotoxicity, lipotoxicity, impaired incretin release/action, amylin accumulation, and decreased beta-cell mass are causative factors in the progression of beta-cell dysfunction. Interventions in order to postpone or preclude beta-cell failure are valuable tools in combatting with the conversion of IGT to type 2 DM.

BETA-CELL FUNCTION AND INSULIN RESISTANCE IN IFG AND IGT

IGT or IFG patients, and particularly people possessing both IGT and IFG^[54,55] carry high risk for type 2 DM^[56-58]. IGT and IFG are eventually end up with type 2 DM but

they exhibit different physiological and pathological processes and have distinct reflections on atherosclerotic cardiovascular disease emergence. In people with IFG hepatic insulin resistance is moderate and OGTT-early insulin response (0-30 min) is diminished^[59]. When hyperglycemic clamp and IVGTT techniques were used in OGTT, first phase insulin secretion is found to be blunted in IFG^[60,61] (Figure 3). But, late (60-120 min) plasma insulin response is unspoiled and muscle insulin sensitivity is near-normal in IFG patients; therefore two-hour plasma glucose levels returns to its initial fasting PG levels^[62-64]. Adversely, people with IGT have moderate to severe muscle insulin resistance and impaired plasma insulin responses (both early and late responses) during oral GT test^[63,64]. Even if fasting PG is relatively stable, it rises progressively during OGTT and not come back to normal levels for a long time while two-hour plasma glucose remains well above the fasting plasma glucose level. On the other hand, IGT and IFG share a characteristic impaired insulin secretion pattern in the first phase. However, insulin secretion in second-phase is intact in IFG states. Whereas, muscle insulin resistance is the dominant factor in IGT, in IFG tissue responsible for insulin resistance is that of liver. Also, IGT and IFG exhibit distinct characteristics for atherosclerotic cardiovascular disease. IGT seems to be related with metabolic syndrome and a good indicator of cardiovascular disease, while IFG predicts these events to a lesser extent^[65].

DETECTION OF HIGH RISK INDIVIDUAL BY HBA1C

ADA recommends considering HbA1c = 5.7%-6.4% level as an instrument to detect future diabetes risk. However, no previous study has adopted HbA1c level as a screening tool to identify subjects at high risk

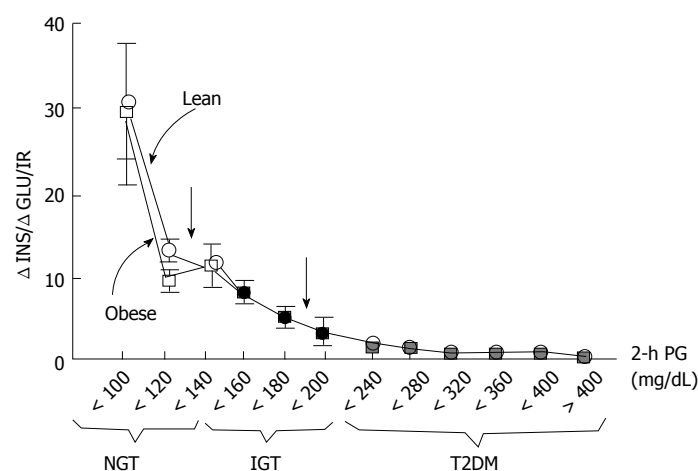


Figure 2 Insulin secretion/insulin resistance (disposition) index (defined as change in insulin/change in glucose/insulin resistance) in individuals with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes mellitus as a function of the 2-h plasma glucose concentration in lean (closed circles) and obese (open circles) subjects. IGT: Impaired glucose tolerance; NGT: Normal glucose tolerance; T2DM: Type 2 diabetes mellitus; PG: Plasma glucose; $\Delta\text{INS}/\Delta\text{GLU}/\text{IR}$: Change in insulin/change in glucose \div insulin resistance.

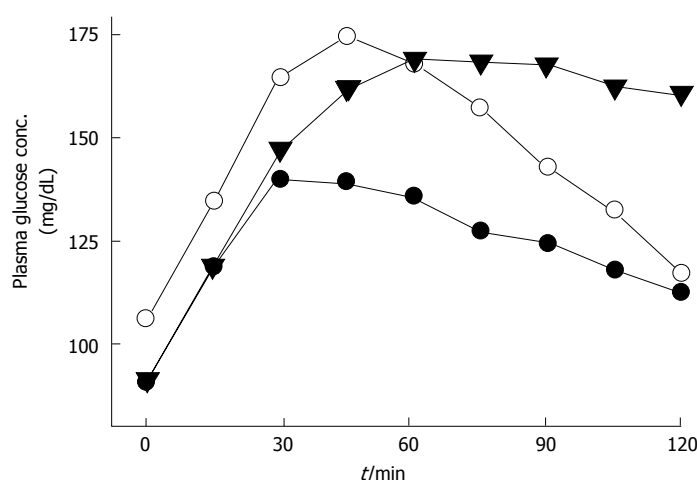


Figure 3 Plasma glucose concentration during the oral glucose tolerance test in normal glucose tolerant (close circles) individuals and in subjects with impaired glucose tolerance (closed triangles) and impaired fasting glucose (open circles).

(HbA1c = 5.7%-6.5%) and has examined the efficacy of interventions to reduce the risk of transition to type 2 DM. Kanat *et al.*^[66] and Færch *et al.*^[67] previously have demonstrated the concordance of HbA1c vs OGTT in high risk individuals and found only little overlap between them. Moreover, Kanat *et al.*^[66] have shown that HbA1c was a poor predictor of impaired beta cell function which is the principle factor mediating the process in which high risk individuals become overt diabetes. Discussion below is about how we should prevent diabetes among high risk individuals, namely individuals with IFG/IGT identified by OGTT results.

INTERVENTION TO PREVENT THE PROGRESSION OF IGT TO TYPE 2 DM

First step in the progression of NGT to type 2 DM is IGT and IFG^[22-24,33]. The IGT and IFG shares 2 features in common: Beta-cell function impairment and insulin resistance. Thereby, it seems logical to assume that efforts to preserve or increase functions of beta-cells and/or decrease insulin resistance may be a potent way to delay the conversion of IGT to DM.

Amelioration of insulin resistance: Loosing weight

The basic risk factor in the progression of IGT to

diabetes is obesity^[34,68]. The main reason of type 2 DM epidemic confronted during the last two decades may be the obesity epidemic itself. Sedantary lifestyle and eventually gaining weight triggers insulin resistance and force the capacity of beta-cell insulin secretion. On the other hand, loosing weight by means of lifestyle interventions, pharmacologic therapies or bariatric surgery augments insulin sensitivity, decreases beta-cell work overload, and gets GT better in IGT states^[69-71]. Four studies have shown that loosing weight through dieting and/or exercising improves insulin sensitivity and ameliorates beta-cell function, thus is a good way to limit IGT progression to type 2 DM^[72-74]. When individuals loose the 5% of their body weight, total body insulin sensitivity improves by 30%^[73] and decrease in their IGT to type 2 DM progression nearly by 58%^[37].

Finnish Diabetes Prevention Study, intervention individuals were given special advice to loose weight (> 5% of total body weight), to decrease total fat consumption (< 30% of total calories) as well as saturated fat consumption (< 10% of total fat), to increase fiber consumption (15 g for each 1000 kilocalories) and to increase physical activity (30 min/d). These individuals were followed up 3.2 years. Cumulative diabetes incidence was 58% lower in the intervention individuals compared to controls (HR = 0.4, $P < 0.001$). Individuals in the study were categorized

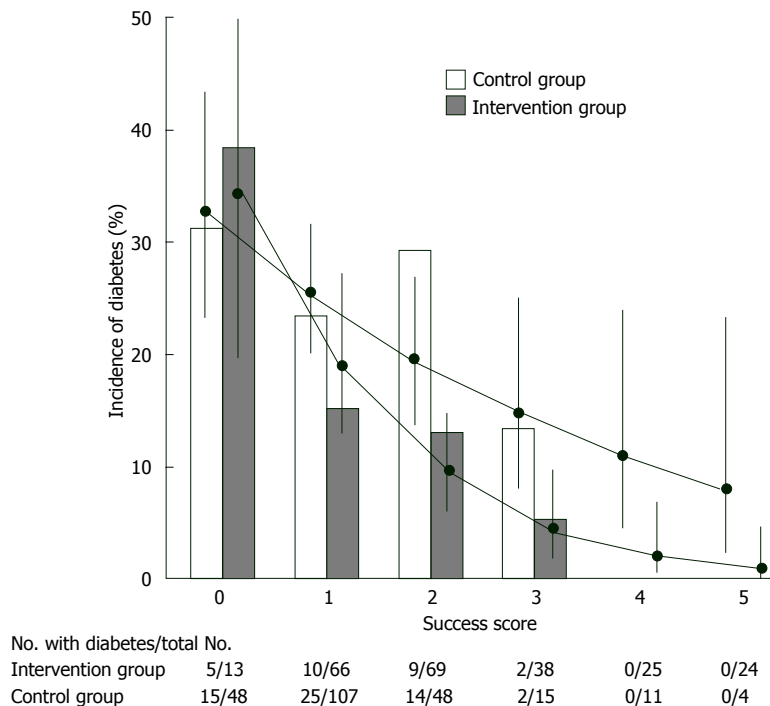


Figure 4 Incidence of diabetes during follow-up, according to the success score. At the one-year visit, each subject received grade of 0 for each intervention goal that had not been achieved and a grade 1 for each goal that had been achieved; the success score was computed as the sum of the grades (reproduced from J Tuomilehto and J Lindström).

considering whether they succeeded their initial targets at one year of assessment (Figure 4). Reciprocal relationship was determined between achievement score and new diabetes cases. If an individual succeeded 4–5 goals, diabetes did not develop^[72]. Another landmark clinical trial [Dipeptidyl peptidase (DPP)] assigned 3234 prediabetic patients (IFG + IGT) to placebo, metformin (2×850 mg per day), or a lifestyle modification program. In this program targets are losing 7% of body weight, taking 150 min-physical exercise every week and reducing (25% of total calories) total intake of fat. Individuals were followed up to 2.8 years. Lifestyle modifications (compared to placebo) decreased the new diabetes cases by 58%. However, in subjects who lost weight and who met physical exercise/dieting targets, risk of diabetes decreased > 90%. These results are consistent with the Finnish Diabetes Prevention Study in which participants met four or five of their goals. In post-hoc analyses of both studies, weight loss was the most important contributor to type 2 DM prevention. In the DPP trial, a 5-kg weight loss over time could account for the 55% reduction in the risk of diabetes over the mean of 3.2 years of follow-up in this high-risk population^[37].

Isolated IFG and isolated IGT individuals carry nearly the same risk about the progression of IFG to type 2 DM, but there is no major clinical trial assessing the lifestyle intervention efficacy on preventing IFG - type 2 DM conversion. A small study^[75] in Japanese subjects with IFG has reported that an intensive weight loss program is more effective in reducing the conversion rate from IFG to type 2 DM compared to less intensive intervention (HR = 0.56, 95%CI: 0.36–0.87). Subgroup analysis revealed that subjects who had IFG + IGT at baseline manifested greater reduction in the conversion to type 2 DM (HR = 0.41, 95%CI: 0.24–0.69) while it

was not statistically significant in subjects with isolated IFG (HR = 1.17, 95%CI: 0.50–2.74). A significant difference achieved by lifestyle intervention on diabetes conversion between two groups ($P = 0.03$).

Lifestyle intervention is the most effective approach to combat with progression of IGT to type 2 DM, but preserving the final weight and exercising is unsustainable^[76]; for example, when DPP trial ended, people gained weight again^[77] (Figure 5). Weight loss achieved by drugs is also a good way to diminish conversion of IGT to type 2 DM. Orlistat brings 5.8 kg loss while lifestyle changes brings 3.0 kg loss, while IGT - type 2 DM conversion limited by orlistat was about a 37% in XENDOS study^[78]. But, when placebo was given instead of the drug, individuals gained weight again although they continued their diets so weight loss provided by pharmacologic interventions is also unsustainable^[79]. Typically, most weight loss programs resulted in weight regain no matter what intervention type (lifestyle or pharmacologic) was used and when losing weight programme stopped, IGT - type 2 DM progression rate mimics control individuals^[80]. Thus, we can conclude that “legacy” effect *via* weight loss is not much in terms of slowing down the IGT - type 2 DM progression. In real-life, even maintaining 5% weight loss is unrealistic. In a study performed in Finland community^[81] a diabetes prevention program aiming 5%–7% weight loss applied 10149 registered subjects and 1/3 of these subjects lost more than 2.5% of their body weight. Moreover, in case of achievement of sustainable weight loss, diabetes incidence decrease was about 50%–60%. In other words, IGT - type 2 DM progression continued in 40% to 50% of subjects although they lost weight successfully. Therefore, changes in lifestyle are insufficient in preventing

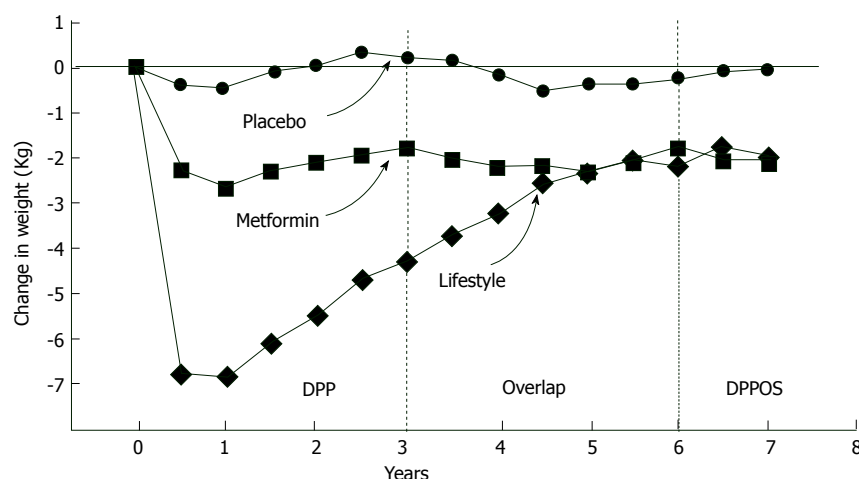


Figure 5 Change in body weight during the dipeptidyl peptidase, during the overlap period, and during the Dipeptidyl peptidase Outcomes Study (reproduced from Eriksson and Lindgärde). DPP: Dipeptidyl peptidase; DPPOS: Dipeptidyl peptidase Outcomes Study.

diabetes in prediabetic people. But opposite to behavioral interventions such as dieting and exercising, pharmacological interventions always limits IGT or IFG progression to type 2 DM.

Correction of insulin resistance: Pharmacotherapies

Lifestyle intervention is impractical and not satisfactory for insulin sensitivity improvement, pharmacologic agents used as an alternative way of enhancing insulin impact and limiting IGT - type 2 DM progression. In some clinical studies, pharmacotherapy getting insulin sensitivity better in adipocytes, in muscle-cells or liver-cells have found to diminish conversion of IGT - type 2 DM.

Metformin: Fasting PG concentration and hemoglobin A1c can be decreased by metformin in type 2 DM through inhibition of liver glucose production^[82-84] or through preserving beta-cell function^[85]. However, in some studies including UKPDS and ADOPT, it is shown that hemoglobin A1c decreases first and then rises again gradually^[7,8,85,86]. In DPP study, IGT conversion to type 2 DM by 31% when metformin was given at the dose of 1700 mg/d; also this therapy corrected insulin sensitivity and diminishes new metabolic syndrome cases. Again, metformin in Indian Diabetes Prevention Program limits the IGT - type 2 DM progression^[87]. Other minor studies^[88-90] show that metformin lowers the plasma glucose concentration in obese adolescents. However, there is no study investigating the efficacy of metformin on diminishing the conversion rate of IFG to type 2 DM. It is proven that metformin and weight loss has similar effectiveness on decreasing the progression of IGT to type 2 DM in younger than 65-year-old subjects, subjects with body mass index over 35 and subjects whose fasting plasma glucose exceeding 110 mg/dL^[37]. Thus, it is not unusual to claim that metformin would significantly lower the conversion rate from IFG to type 2 DM. A prospective randomized clinical trial illuminated the answer. Eventually, American Diabetes Association advices metformin usage in high-risk individuals (younger than 60-year-old, body mass

index over 30 kg/m² and HbA1c over 6.0%) with IGT or IFG, taking into account that metformin has been known as a safe generic drug^[91]. However, similar to sulfonylureas, metformin cannot stop beta-cell failure which is crucial for type 2 DM. While metformin response initially seems good, HbA1c begins to rise eventually.

Thiazolidinediones: Thiazolidinediones act on "peroxisome proliferator activator receptor gamma" (PPAR- γ) and eventually improve two main defects generated by IGT. Thiazolidinediones bring adipocytes as well as liver and muscle cells sensitivity to insulin^[92-94] and also support and protect beta-cells function^[95]. Hypothesis that defends "thiazolidinediones improve muscle insulin sensitivity by reducing plasma free fatty acid levels and intramyocellular lipid content, and redistributing fat from visceral to subcutaneous adipose depots" finds lots of evidence. Moreover, muscle and fat cell PPAR- γ receptors mediates insulin-sensitizing effect directly^[92-94]. There is no significant difference between troglitazone^[96], pioglitazone^[97], and rosiglitazone^[98] in controlling glycemia and increasing insulin sensitivity in type 2 DM. Troglitazone increase GT and insulin sensitivity as well as limits type 2 DM conversion in IGT individuals^[38,99,100] and in women developing diabetes during their pregnancies^[101]. In Diabetes Prevention Program, IGT - type 2 DM progression reduced by 23% by troglitazone within three years, even if the drug was stopped after 10 mo^[38]. After 1.5 years of follow-up diabetes incidence was markedly reduced for every 100 person-treatment years in IGT subjects taking troglitazone compared with placebo (3.0 vs 12.0 cases; $P < 0.001$), compared with metformin (3.0 vs 6.7 cases; $P = 0.02$) and compared with lifestyle changing activities (3.0 vs 5.1, $P = 0.18$) (Figure 6). IGT - type 2 DM conversion decrease attributed to rosiglitazone was 62% in DREAM trial^[39] and best indicator of diabetes prevention was recovery in insulin secretion/insulin resistance index. Pioglitazone and troglitazone slows down IGT progression to type 2 DM in women with gestational diabetes history^[101-103]. In Actos Now for the prevention of diabetes study, IGT -

type 2 DM conversion rate fall attributed to pioglitazone was 72% ($P < 0.00001$)^[40].

Beta-cell function sustainability

Because IGT - type 2 DM conversion and appearance of hyperglycemia led by gradual beta-cell failure, improving beta-cell function in IGT individuals are expected to be useful in lowering the new cases of type 2 DM. Although thiazolidinediones strikingly increase insulin sensitivity in IGT individuals, the best indicator of type 2 DM prevention is reinforcing beta-cell function. In diabetic human trials^[101,103] and animal studies^[104] troglitazone^[99-101], pioglitazone^[95,97,102], and rosiglitazone^[95,98] increased the function of beta-cells by: (1) unloading beta-cells *via* advancing insulin sensitivity; (2) decreasing plasma free fatty acid levels; (3) correcting lipotoxicity; in other words sending toxic lipid metabolites (diacylglycerol, ceramides and fatty acyl CoAs) away from beta-cells; and (4) exerting direct PPAR- γ receptor-mediated beta-cell effect^[48,94,95]. Thiazolidinediones both advance insulin sensitivity and protect beta-cell function so that they blocks IGT - type 2 DM conversion and create a longstanding HbA1c decrement in type 2 DM^[23]. Nevertheless, thiazolidinediones induce fluid retention plus fat weight gain and they have the disadvantage of being expensive^[39,105]. For that reason, American Diabetes Association declared metformin instead of thiazolidinediones for treatment of IGT or IFG^[91] even if thiazolidinediones doubles the effect of metformin in preventing IGT - type 2 DM conversion^[105,106] (Figure 6). In Actos Now for the prevention of diabetes study titrated pioglitazone dose was 45 mg per day. But, even 15 to 30 mg daily pioglitazone dose increased insulin secretion and sensitivity in type 2 DM^[107] while causing lesser fluid retention and lesser fat gain^[108]. Also, Canadian individuals with IGT were given 2 mg per day rosiglitazone plus 1000 mg per day metformin, and IGT - type 2 DM conversion reduction with this regimen was about 71% with no significant fluid retention and weight gain^[109].

In all of the 8 studies continued over 1.5 years, thiazolidinediones reduced HbA1c levels and maintained this decrement in type 2 DM subjects. In ADOPT, 5-year rosiglitazone-associated HbA1c decrease was obtained^[86]. Sustained reduction in HbA1c implicates that thiazolidinediones are long-acting drugs on beta-cell functionality. Parallely, in another study, insulin secretion/insulin resistance index which is the gold standart in the measurement of beta-cell function is calculated in 61 type 2 DM subjects and functions of beta-cells improved by rosiglitazone and pioglitazone in a similar way^[95]. Consequently, thiazolidinediones protect and augment beta-cell function, sensitize insulin as well as preserve long standing HbA1c reduction and delay IGT- type 2 DM progression.

Glucagon-like peptide-1 analogues: Oral glucose consumption provides 2-3-fold greater plasma insulin

response compared to same level of hyperglycemia enhanced by intravenous glucose and this is called "incretin effect"^[110-112]. Ninety percent of incretin effect derived from L cell-associated glucagon-like peptide-1 (GLP-1) release and K cell-associated GIP release. GIP and GLP-1 are strong stimuli for insulin secretion. GLP-1 also blocks secretion of glucagon, postpones emptying of stomach, diminishes appetite, limits food consumption and potentiates losing weight. Dipeptidyl peptidase-IV cleaves GLP-1 and GIP rapidly within one or two minutes, those peptides are not suitable for therapy of type 2 DM and/or IGT individuals. GLP-1 receptor agonists (namely liraglutide and exenatide) mimicing GLP-1 actions are resistant to degenarating effect of dipeptidyl peptidase-IV^[113,114]. Like endogenous GLP-1, liraglutide and exenatide are powerful insulin secretagogues, and they decrease secretion of glucagon, potentiate losing weight and effectively decrease plasma glucose levels in type 2 DM. A three-year prospective study showed exenatide reduced HbA1c for a long time, augmented functions of beta-cells and provided gradual weight loss^[115]. One favorable aspect of GLP-1 analogues is that hypoglycemia is uncommon during therapy because GLP-1 analogues merely increase secretion of insulin whenever there is hyperglycemia. Glucose physiologically triggers release of insulin. Glucose increases the ATP generation, eventually generated ATPs close the potassium channels. Consequently, membrane of beta-cells are depolarized, calcium influx occurs and exocytosis begins in insulin-containing vesicles^[116]. Eventually, glucose mediates insulin secretion. But effect of GIP and GLP-1 on beta-cells are totally independent from hyperglycemia. After they bind self receptors, adenylate cyclase is activated, ATP is converted to cAMP so they "amplifies" insulin secretion by means of hyperglycemia. If hyperglycemia does not exist, GLP-1 or GIP can not augment secretion of insulin^[117].

The typical signs in subjects with IGT and type 2 DM are severe decrease in functions of beta-cells and obvious decrease in incretin effect after meal or after glucose consumption^[110-112]. Studies have pointed out that in IGT and type 2 DM cases the main defect is the incapability of beta-cells to respond glucose. Incretin hormones partially overcome beta-cell "blindness" to glucose^[118]. In IGT cases GLP-1 response after meal usually is not changed or slight impairment is observed^[119-121] while GLP-1 response in the first 10 min is usually lessened (this implicates phasic defect in GLP-1 secretion) but GIP secretion is mildly elevated^[122]. On the contrary, in type 2 DM beta-cells are resistant to GLP-1-mediated insulin secretion^[123]. Also, beta-cells are resistant to GIP-mediated stimulation of insulin secretion. If insulin is given and glycemia reverted to normal, susceptibility of beta-cells to GIP can be improved, but this is not true for GLP-1^[50].

If hyperglycemia exists, NGT individuals give powerful insulin secretion response against the GLP-1 increase. Inversely, in type 2 DM the same

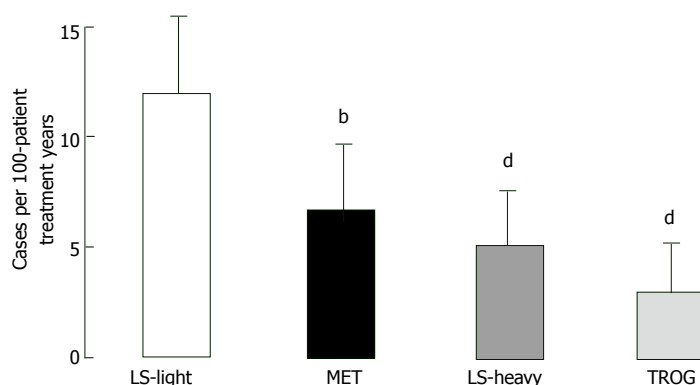


Figure 6 Effect of lifestyle intervention, metformin, and troglitazone on the conversion rate of impaired glucose tolerance to type 2 diabetes in the first 1.5 years of the dipeptidyl peptidase (i.e, before the discontinuation of troglitazone from the dipeptidyl peptidase). ^b $P < 0.01$ vs LS-light, ^d $P < 0.01$ vs LS-heavy. LS: Lifestyle; MET: Metformin; TROG: Troglitazone.

GLP-1 amount cannot increase insulin secretion even hyperglycemia exists^[50,51]. But whenever plasma GLP-1 levels increased pharmacologically, insulin response becomes normal in hyperglycemic states (Figure 7). Hence, pharmacological plasma GLP-1 levels may restore “beta-cell glucose blindness” in IGT and type 2 DM. Although GLP-1-analogue-mediated beta cell stimulation is only sustainable during wash-out period, a novel trial declared that 3-year exenatide therapy partially recovered responsiveness of beta-cell to glucose^[124].

Conversion of NGT to IGT and eventually to type 2 DM is mediated by nonstop failure of beta-cells (Figures 1 and 2). Exenatide: (1) increases responsiveness of beta cells to glucose and augments functions of beta-cells in type 2 DM; (2) facilitates losing weight; (3) does not induce hypoglycemia; and (4) is applied once a week (Bydureon). For that reason, exenatide could be a good choice to decrease the conversion of IFG/IGT to type 2 DM and to a guarantee for NGT. There is no study investigating GLP-1 analogue effect on IGT - type 2 DM conversion. On the other hand liraglutide was investigated in obese but nondiabetic individuals (31% had IGT)^[125]. In these IGT individuals, 84%-96% decrement was observed in type 2 DM progression. Five percent weight loss was achieved in 61% of individuals while ten percent weight loss achieved in 19% of individuals. New metabolic syndrome cases was decreased up to 60%. Therefore, long-acting GLP-1 analogues could be preferable drugs in order to prevent conversion of IGT to type 2 DM, because they carry additional effects such as weekly administration, beta cell function augmentation, and facilitation of losing weight^[126].

DPP-IV inhibitors: DPP-IV is the enzyme that cleaves GLP-1; DPP-IV inhibitors block this enzyme and therefore rise plasma GLP-1 concentrations. But, DPP-IV inhibitor-related increase in GLP-1 concentrations is uniquely dependent on endogenous GLP-1 secretion. Thus, DPP-IV inhibitor-related plasma GLP-1 rise usually is lower than GLP-1 analogue-related rise. DPP-IV inhibitors accomplish moderate increase in insulin secretion and have moderate inhibition on glucagon^[110]. Vildagliptin administration in IGT individuals reveals little augmentation on functionality of beta-cells. However,

vildagliptin effect totally disappeared after washout^[116]. There is no study calculating DPP-IV inhibitor-mediated conversion rate of impaired GT - type 2 DM switch. In contrary to GLP-1 analogues, DPP-IV inhibitors cannot help losing weight and they exert insufficient effect on beta-cells. Accordingly, GLP-1 analogues may be superior to DPP-IV inhibitors in IGT treatment.

Alpha-glucosidase inhibitors: IGT-type 2 DM conversion rate decreased about 25% by acarbose^[127] and voglibose^[128]. This effect was attributed to inhibition of carbohydrate absorption but increment in incretin secretion induced by alpha-glucosidase inhibitors may be the real reason of positive impact on glucose homeostasis^[129]. Alpha-glucosidase inhibitors changes microbial flora of gut, thus they may help to heal glucose intolerance^[130].

Pharmacotherapy cessation and emergence of diabetes: Pharmacological therapy applied to increase insulin sensitivity and beta-cell function have potent impact on prediabetes-diabetes conversion. But, we are not sure whether this effect is transient or sustained when the intervention is discontinued. Pharmacologic interventions prevents or delays diabetes onset by: (1) masking diabetes appearance by suppressing glucose; (2) preventing or delaying diabetes development only while it is being used; or (3) retaining their effects even after withdrawal.

Reassessing glycemic status after washing out the pharmacotherapy could clarify which possibility is relevant for the intervention^[131]. Several studies investigating wash out effect are conducted in order to answer these questions. After 2.8 years of intervention in DPP trial, the incidence of diabetes in individuals with IGT was reduced by 58% with lifestyle modifications while the reduction is only 31% with metformin therapy compared with placebo. At the end of the trial 11-d washout period applied, participants who were taking metformin or placebo and had not developed diabetes were tested with a repeat OGTT in order to assess whether the observed metformin effect was sustained after cessation of the drug. Washout control reveals metformin participants had a significant increase in fasting glucose levels. It is concluded that one-quarter of the beneficial effect of metformin to prevent type 2

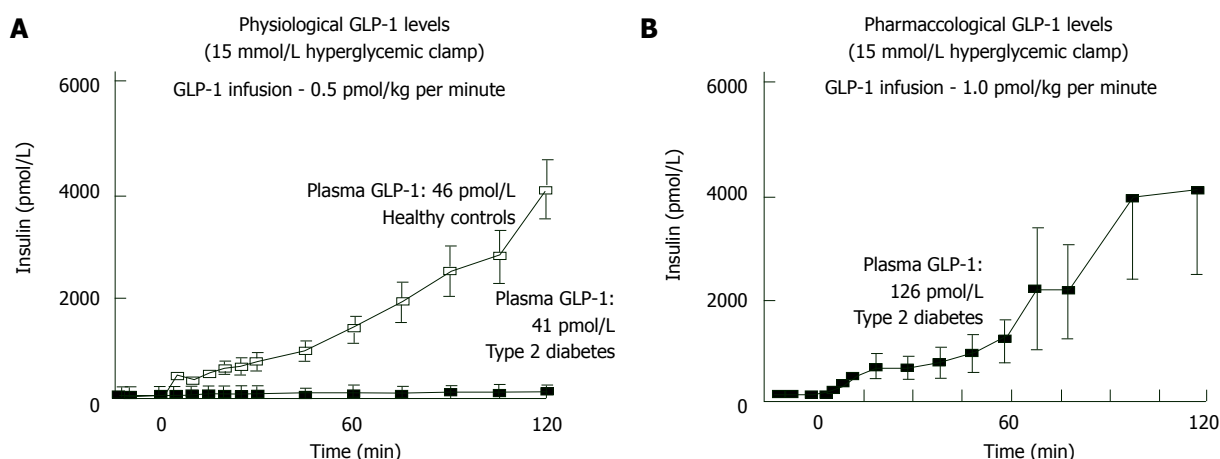


Figure 7 Effect of physiologic (A) and pharmacologic (B) doses of glucagon-like peptide-1 on insulin secretion in normal glucose tolerance individuals and in subjects with type 2 diabetes mellitus. GLP-1: Glucagon-like peptide-1.

DM was attributable to a pharmacological effect and this effect did not persist when the drug was withdrawn. However, the overall effect of metformin in preventing diabetes remained substantial at 25% after withdrawal of the intervention^[132].

In DREAM trial rosiglitazone slows down the new-onset diabetes in people with IGT \pm IFG significantly (HR = 0.40, $P < 0.0001$). After a median 71-d medication washout period, the incidence of diabetes is similar both in intervention and placebo groups. This evidence suggests rosiglitazone does not have a sustained effect on the underlying disease pathophysiology and effective as long as the therapy is being given^[131].

In STOP-NIDDM trial acarbose given to IGT patients delayed progression to Type 2 DM. The risk of progression to diabetes over 3.3 years was reduced by 25%. In the last 3 mo of the study placebo was given to all subjects. During this placebo treatment period, the incidence of diabetes was higher in the group originally assigned to acarbose than in the group first randomized to placebo (HR = 0.45, $P < 0.005$). On the other hand, STOP-NIDDM trial demonstrated that beneficial effect of acarbose preventing type 2 DM was partially attributable to its pharmacological effect and similar to metformin, the effect is not sustainable when drug use is stopped.

DETECTION OF HIGH RISK PERSONS FOR PHARMACOLOGICAL INTERVENTION

Prediabetics prone to develop type 2 DM plus atherosclerosis-induced cardiovascular complications are usually sub-maximally insulin resistant. In addition, these individuals have lost two thirds of their beta cell functions, their HbA1c levels usually are around 6% and at least 10% have diabetic retinopathy^[133,134], nearly the same percentage of individuals have peripheral neuropathy^[135]. Characteristic primers of diabetes are beta-cell dysfunction and insulin resistance. Gold standard measurement method for insulin sensitivity

is euglycemic insulin clamp technique while the gold standard measurement method for insulin secretion is hyperglycemic clamp technique. These techniques are not much applicable for screening in clinical practice. Other predictive models studied IGT - type 2 DM conversion^[136] and it is concluded that neither anthropometric criteria (waist-to-hip ratio or body mass index) nor metabolic syndrome components are superior to two-hour plasma glucose of OGTT. Another study illuminated two subgroups carrying high type 2 DM risk: First group consisted of IGT individuals whose total plasma glucose is in the upper fifth percentile during OGTT while the second group consisted of fasting plasma glucose over 95 mg/dL^[137]. Best predictive criterion for future type 2 DM in IGT subjects is one-hour plasma glucose over 155 mg/dL, independent of their GT status in the Botnia^[54] and San Antonio Heart^[138] studies. Some biomarkers such as fasting PG, ferritin, insulin, adiponectin, HbA1c, IL-2 receptor A, high-sensitivity C-reactive protein predict diabetes development in later life^[139]. Actos Now for the prevention of diabetes study and Diabetes Prevention Program gives inspiration to select IGT subjects carrying extra risks for type 2 DM, in order to discriminate people that take advantage from pharmacotherapy.

PREDIABETIC PATIENT ALGORITHM

The optimal strategy is to prevent development of hyperglycemia intervening at the stage of IGT and also to revert GT back to normal. Individuals with IGT are insulin resistant and lost 50%-80% of their beta-cell function. Also, in order to prevent vascular complications resumption of normoglycemia is crucial in type 2 DM. This algorithm is also cheaper in long run. Diabetes Prevention Program Research Group wrote "Over 3 years, metformin was clinically effective (in preventing diabetes in IGT subjects) and cost-effective from the perspective of a health system and society, especially if implemented with generic medication pricing"^[140,141].

When model simulations performed, similar results were reached^[142,143]. IGT - type 2 DM conversion blockage by pioglitazone^[40] is two fold that of metformin^[37], so it is logical to assume that pioglitazone also could be cost-effective. But, monitoring and side effect treatment costs of those drugs should be remembered. Two aspects should be taken into account while performing cost analysis of pioglitazone. First one is edema management (if occurs) and the second is monitoring and treating osteoporosis. Possible long bone fracture in postmenopausal women should also be evaluated in cost analysis. Some studies implies bladder cancer risk in individuals who are given 45 mg pioglitazone over two-year time. But FDA mandated a prospective study in order to clear the pioglitazone safety (Kaiser Permanente study) as after eight-year observation, in comparison to those who never used pioglitazone, hazard risk ratio of bladder cancer was 0.98 in diabetics receiving pioglitazone.

GLP-1 analogues are expensive and they may not be put on market in near future. For that reason, cost analysis of GLP-1 analogue use in prediabetes states should be done cautiously. From community perspective, different criteria are considered in drug usage. But from patient perspective any solution to postpone or avert hyperglycemia probably decreases new onset microvascular complications such as nephropathy, neuropathy and/or retinopathy. When the main argument is reducing new cases of blindness, amputations and/or end-stage renal disease, "cost" cannot be top criterion for the individual for ethical reasons.

Another option is to prefer waiting till diabetes emerges and initiate therapy at this stage rather than treating individuals with prediabetes. But there is several limitations for this option. First, it brings handicaps on detecting exact timing of diabetes onset, namely, prediabetic individuals should be regularly controlled during this period. Secondly, UKPDS results make us to realize that in initial stages of diabetes tight glucose control cannot prevent microvascular complications. Besides, progression of euglycemia to dysglycemia is a silent but secular process. Thus, defining diabetes initiation in the basis of plasma glucose (namely fasting plasma glucose or two-hour plasma glucose) levels or in the basis of HbA1c is controversial. In reality, one tenth of prediabetics already have evidence of diabetic microvascular complications. Thirdly, upper tertile of IGT group is insulin resistant, their beta cell function loss is nearly 70%-80% whereas volume loss is about 30%-40%. Fourthly, a major diminution in beta-cell mass in prediabetes accelerates the conversion process to type 2 DM^[144]. There is no remedy to increase human beta cell mass, today.

All pathophysiological events observed in type 2 DM also appears in prediabetic individuals and nearly 10% of prediabetics exhibit microvascular complications. Consequently, initiating lifestyle changes and pharmacotherapy in high-risk prediabetics instead of waiting till diabetes emerges seems reasonable. However

there is no study comparing prediabetic stage therapy vs the diabetic stage therapy. Because these studies necessitate large sample sizes and very long study periods in order to demonstrate incidence differences in terms of microvascular complications. Therefore, response to the question "when should we institute pharmacological therapy?" is unclear, yet.

Lastly, prediabetics carry high risk for cardiovascular complications (myocardial infarction, stroke, cardiovascular death) besides their type 2 DM risk. IGT individuals are highly insulin resistant and thereby, exhibit some typical metabolic abnormalities observed in insulin resistance. For example they become dysglycemic, dyslipidemic, hypertensive, obese, insulin resistant, prone to coagulation, vulnerable to inflammation and endothelial dysfunction. Those abnormalities are also the main risk factors for cardiovascular disease. Moreover, insulin resistance is an independent atherosclerotic risk factor irrespective of other associated risk factors^[94]. Thus cardiovascular disease risk of prediabetics is much more compared to normal individuals. Some measures diminishing diabetes risk also reduce cardiovascular risk. For instance, pioglitazone decreases triglyceride concentrations and increases HDL levels while losing weight decreases blood pressure and heals lipid profile^[37]. Eventually, in order to decrease cardiovascular disease risk of these individuals one should apply measures diminishing type 2 DM risk on one hand, while giving special attention on treating CVD risk factors (blood pressure and dyslipidemia) on the other hand.

"Diabetes prevention" or "reversal of prediabetes to normoglycemia"?

Restoration of normoglycemia in prediabetics obviously lessens diabetes risk. Diabetes Prevention Program Outcome Study (DPPOS) compared the 894 people who had at least one normal OGTT with the 1096 people who never regressed to normoglycemia in Diabetes Prevention Program. In follow-up period of the study relative risk of diabetes emergence was 56% lower in the first group (OR = 0.44)^[145]. Regression from prediabetes to normoglycemia not only reduces the risk of diabetes, but also the risk of cardiovascular disease. DPPOS has proven that if prediabetes can regress to normal glucose state, cardiovascular complications decrease^[146]. Because, nearly one tenth of prediabetics possess microvascular complications, it is likely that restoration of normoglycemia improves microvascular complications^[147].

SUMMARY

Behavioral changes (dieting plus exercising) are effective in preventing IGT-type 2 DM conversion as well as IFG - type 2 DM conversion but losing weight is hard and also difficult to maintain. Pharmacological interventions (plus dieting and exercising) improving and preserving beta-cell function and enhancing insulin sensitivity may be

Table 1 Summary of pharmacologic intervention trials in individuals with impaired glucose tolerance

Study	n	Duration (yr)	Incidence of DM in control (%)	Relative risk reduction (%)
IDPP	269	2.5	18.3	26
USDPP	2151	2.8	11	31
USDPP	1172	0.9	11	75
TRIPOD	236	2.5	13.1	55
PIPOD	89	3	13.1	55
DREAM	5269	3	6.5	60
ACT NOW	602	2.8	6	72
CANOE	207	3.9	10.1	66
STOP NIDDM	1368	3.2	8.1	36
XENDOS	3305	4	2.2	37

DM: Diabetes mellitus; IDPP: Indian Diabetes Prevention Programme; USDPP: United States Diabetes Prevention Programme; TRIPOD: Troglitazone in the prevention of Diabetes; PIPOD: The pioglitazone in prevention of diabetes; DREAM: Diabetes Reduction Assessment with ramipril and rosiglitazone Medication; ACT NOW: Actos Now for the prevention of diabetes; CANOE: Canadian normoglycaemia outcomes evaluation; STOP NIDDM: Study to Prevent Non-Insulin-Dependent Diabetes Mellitus; XENDOS: Xenical in the prevention of Diabetes in Obese Subjects.

suitable choices for high-risk IGT patients. Troglitazone in Prevention of Diabetes Study, Pioglitazone in Prevention of Diabetes Study, Diabetes Reduction Assessment with ramipril and rosiglitazone Medication Trial, Actos Now for the prevention of diabetes study and Diabetes Prevention Program proven that thiazolidinediones obviously prevent the development of type 2 DM in IGT subjects as well as IFG subjects (Table 1). In Diabetes Prevention Program and Indian Diabetes Prevention Program, metformin slowed down the progression of IGT to type 2 DM, eventually ADA Consensus Conference Statement proposed metformin usage in high-risk IGT individuals. However, pioglitazone and rosiglitazone efficacy in preventing IGT progression to type 2 DM nearly doubles metformin's efficacy (31% vs 72% and 62%, respectively). Rosiglitazone (low dose = 2 mg/d) together with metformin (850 mg/d) was proven to be slows down IGT progression to type 2 DM as well as to be more tolerable. GLP-1 analogues: (1) effectively treats type 2 DM; (2) blocks IGT - type 2 DM progression; (3) preserves and augments functions of beta-cells; (4) facilitates losing weight; (5) combat with cardiovascular risks; (6) do not cause hypoglycemia; and (7) can be used once a day (liraglutide) or once a week (Bydureon). For these reasons we speculate that this drug group, especially long-acting preparations^[127], be ideal for obese patients with IGT.

The benefits and disadvantages of pharmacotherapy must be evaluated simultaneously. Although rare, metformin can induce lactic acidosis. If serum creatinine levels exceeds 1.4 mg/dL in females and 1.5 mg/dL in males, metformin is contraindicated. Gastrointestinal side effects are often and one tenth of patients are metformin intolerable. On the other hand, pioglitazone users experience fluid retention, fat weight gain and congestive heart failure. Paradoxically, while fat weight

gain increases, reduction in HbA1c becomes more prevalent and much more insulin sensitivity/beta-cell function improvement is achieved. Easily detected clinical sign of fluid retention is peripheral edema and can be controlled easily with distally acting diuretics such as amiloride or spironolactone. Because these side effects are dose-related, restricting pioglitazone to 30 mg daily dose may decrease side effects. Trauma-related fracture cases were increased in postmenopausal women treated with pioglitazone. For that reason pioglitazone should be used carefully in postmenopausal women. Nausea/vomiting are main handicaps of GLP-1 receptor agonist usage; nearly one third of subjects experience nausea/vomiting. Though adverse effects are generally mild or temporary, liraglutide/exenatide intolerance is about 5%. Pancreatitis is also pronounced, but when large national databases were analysed retrospectively, there was no such increment in pancreatitis in GLP-1 receptor agonist users.

CONCLUSION

In conclusion, we recommend strict lifestyle modification for patients with IGT ± IFG. Another option is to initiate pharmacotherapy with metformin plus low-dose pioglitazone. In high risk IGT individuals long-acting GLP-1 analogue use as well as diet plus exercise may be another option. Each component of this approach is effective in type 2 DM prevention and turning IGT back to normal. Depending on evidence described earlier, we believe "combination therapy" would especially be preventive for microvascular complications and is associated with lower adverse effects. Also, pharmacotherapy with generic drugs may be cost effective.

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Molecular and biochemical trajectories from diabetes to Alzheimer's disease: A critical appraisal

Rajat Sandhir, Smriti Gupta

Rajat Sandhir, Smriti Gupta, Department of Biochemistry, Panjab University, Chandigarh 160014, India

Author contributions: Sandhir R and Gupta S contributed to this paper.

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Correspondence to: Rajat Sandhir, Professor, Department of Biochemistry, Panjab University, Sector 14, Chandigarh 160014, India. sandhir@pu.ac.in
 Telephone: +91-172-2534131-38

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Abstract

Diabetes mellitus (DM), a metabolic disorder is a major orchestra influencing brain and behavioral responses *via* direct or indirect mechanisms. Many lines of evidence suggest that diabetic patients apparently face severe brain complications, but the story is far from being fully understood. Type 2 diabetes, an ever increasing epidemic and its chronic brain complications are implicated in the development of Alzheimer's disease (AD). Evidences from clinical and experimental studies

suggest that insulin draws a clear trajectory from the peripheral system to the central nervous system. This review is a spot light on striking pathological, biochemical, molecular and behavioral commonalities of AD and DM. Incidence of cognitive decline in diabetic patients and diabetic symptoms in AD patients has brought the concept of brain diabetes to attention. Brain diabetes reflects insulin resistant brain state with oxidative stress, cognitive impairment, activation of various inflammatory cascade and mitochondrial vulnerability as a shared footprint of AD and DM. It has become extremely important for the investigators to understand the patho-physiology of brain complications in diabetes and put intensive pursuits for therapeutic interventions. Although, decades of research have yielded a range of molecules with potential beneficial effects, but they are yet to meet the expectations.

Key words: Diabetes mellitus; Alzheimer's disease; Insulin; Type 2 diabetes; Type 3 diabetes

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Core tip: This review provides a synopsis in which a metabolic disturbance becomes indispensable for life and emerges as a molecular signal defect leading to a syndrome with multiple complications. Insulin is a spotlight player which draws a trajectory from diabetes to Alzheimer's disease with multiple divergence and convergence. We have discussed their interplay to speculate their shared molecular footprints. These biochemical and molecular commonalities provide a clue to the investigators to look inside a therapy with a common experimental and clinical platform and also provide an insight for new interventions as future perspective to find a potential stone to kill two birds together.

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INTRODUCTION

Every human cell relies on a complex set of programs installed during ontogeny. These programs and commands over them are the two interfaces which need faultless execution for normal body physiology. Physiology, behavior and defense are three eventful networks, which are supposed to function in synchronicity. A defect in any of these three events alters rest of two without any delay. Diabetes is a complex disorder where a molecular compromise alters the physiology with significant changes in behavioral responses.

Diabetes is associated with the production of auto-antibodies against pancreatic β -cells, *i.e.*, type 1 diabetes (T1D) or with insulin resistance (IR), *i.e.*, T2D^[1,2]. T1D is a chronic hyperglycemic condition which affects multiple systems like brain, heart, eyes and kidneys^[3]. Diabetes is found to be one of the causes of brain atrophy, mild cognitive impairment and white matter abnormalities^[4-6]. In T2D insulin fails to stimulate utilization of glucose which gives rise to a phenomenon called IR. Chronic IR leads to several other complications such as lack of cellular energy, increased plasma lipids, cardiovascular problems and hypertension^[7-11]. Increased risk of developing dementia and Alzheimer's disease (AD) was suggested in T2D, which was further supported by clinical and epidemiological studies^[12,13]. Diabetes patients have two fold higher risk of AD as compare to non-diabetic patients^[12]. In central nervous system (CNS), presence and distribution of insulin, insulin receptors (IRs) and its substrate are region specific^[14,15]. Insulin is found to play important role in learning and memory by regulating the release of neurotransmitters and synaptic plasticity^[16]. It is inferred from the literature that defective insulin signaling in diabetic patients plays a crucial role in synaptic physiology^[17-19]. Many other molecules participating in insulin signaling pathway have been reported to be crucial for normal physiology^[20]. Remarkable presence of IRs in different brain areas has provided a clue of possible link between insulin signaling and synaptic plasticity. This fact is strongly supported by up-regulation of IRs in hippocampus after training for spatial memory task *via* Morris-Water Maze^[21].

In 1998, a possible link between insulin dysfunction and AD was established^[22,23]. The postmortem AD brains showed reduced insulin like growth factor (IGF) mRNA levels and its receptor as compared to controls^[24]. Impaired peripheral glucose sensitivity^[25] and elevated plasma and cerebro-spinal fluid (CSF) levels of insulin^[26-28] were also reported in AD patients. Thus, there are consistent reports showing involvement of impaired insulin signaling in cognitive decline in AD patients. The role of insulin in enhancing memory performance in AD patients was confirmed by rescuing

effect of intravenous and intranasal insulin administration^[29,30].

Attempts are being made to unscramble the cellular and molecular mechanisms connects diabetes and AD. Present review critically examines impaired insulin signaling in diabetes as well as in AD patients with emphasis on critical molecular players such as fork head box O-1 (FOXO1), mammalian target of rapamycin (mTOR) and glycogen synthase kinase 3 beta (GSK3 β) which can be potential therapeutic targets.

DIABETES MELLITUS

Diabetes mellitus (DM), a complex metabolic disorder is characterized by hyperglycemia with several macrovascular (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy)^[31]. Risk of developing any of microvascular complications of diabetes depends upon both the duration and the severity of hyperglycemia. Aldose reductase, initial enzyme in the intracellular polyol pathway is a key player involved in the development of diabetic complications. Polyol pathway converts glucose into sorbitol (glucose alcohol). Hyperglycemic condition increases the flux of glucose into this pathway and results in sorbitol accumulation which further leads to osmotic stress. Osmotic stress is reported to be most common underlying mechanism in the development of microvascular complications of diabetes^[31]. American Diabetes Association has categorized diabetes as T1D and T2D. T1D is characterized by autoimmune destruction of pancreatic beta cells resulting in absolute absence of insulin whereas T2D is identified by peripheral IR. According to WHO reports 2012, 90% cases of diabetes are from T2D. Clinical and experimental studies suggested strong association between diabetes and cognitive impairment^[32-35]. T2D is at the edge of several risk factors such as life style, obesity, physical inactivity, gestational diabetes history as well as genetic predispositions^[36,37]. The hallmark symptoms of the disease are polyurea, polydipsia, polyphagia and weight loss^[38]. Mechanisms of T2D involves lipid breakdown within fat cells, elevated plasma glucagon levels as well as an increase in electrolyte retention^[39].

AD

AD is an age dependent neurodegenerative disorder associated with deposits of plaques and tangles in brain^[40]. Only 1%-5% of the AD cases are found to have genetic differences and out of these cases, only 0.1% cases follow familial autosomal non-sex linked inheritance pattern^[41]. AD was for the first time reported in 1906 by Alois Alzheimer, a German psychiatrist and pathologist as a progressive neurodegenerative disorder of memory loss and confusion^[42]. Postmortem AD brains revealed intracellular accumulation of neurofibrillary tangles (NFTs) and extracellular deposition of amyloid

beta (A β) plaques as two major hallmarks of AD. NFTs are hyperphosphorylated form of tau protein, which are involved in microtubule dynamics while A β plaques are the cleavage product of amyloid precursor protein (APP) which is a transmembrane glycoprotein of unknown function. Mutation in three genes encoding APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2) contributes to genetic cases of AD^[43]. These loci are responsible for the familial type of disease while environmental factors influence sporadic form of AD with unclear etiology. Mutated form of these genes increase production of A β -42 protein product, a major component of senile plaques. ϵ 4 allele of the apolipoprotein E (APOE ϵ 4) is another risk factor for AD^[44,45] which is thought to contribute in neuronal lipid homeostasis, repairs injured neurons, maintains synapto-dendritic connections and scavenges neurotoxins. Loss of cholinergic system is a major cause of cognitive deficit in AD patients and the current therapies are targeted at improving cholinergic functions^[46].

TWIN MYSTERY OF AD AND DM: THE STORY SO FAR

In 1980, first line of evidence appeared when Adolfsson *et al.*^[47] performed glucose tolerance test on AD type dementia patients and hypothesized that hypoglycemic condition can ameliorate brain status. In 1994, Razay *et al.*^[48] spotted light on disturbed glucose metabolism and hyper-insulinemia in female AD patients and tried to establish a link between insulin dysfunction and dementia. In 1996, Messier *et al.*^[23] strengthened the evidences by uncovering the potential effects of glucose on memory and cognition of AD patients. In 2003, Messier^[49] further established a clear association between non-insulin dependent diabetes mellitus with neuropathy which is an incidence of vascular disease and retinopathy, he further suggested that DM is a probable risk factor for AD. Many more groups stepped ahead to address the fundamental question of whether the basic premise about the disease is true or not.

In 2005, Susanne de la Monte's group at Brown University introduced the concept of brain diabetes or type 3 diabetes (T3D) and observed that after blocking brain insulin supply, neurons get disoriented and develops AD pathology in rats. This provided a promising platform to investigators to touch insight into T3D or brain diabetes^[50]. In 2007, Li *et al.*^[50] published a review dedicated to common pathological process in AD and T2D which shared molecular degenerative cascades like dysfunction in insulin signaling pathway. In 2008, de la Monte *et al.*^[51] reappeared with some more set of explanations which were unclear in 2005. Diabetic brain was found to be compromised for acetylcholine homeostasis and cognitive impairment, whereas insulin sensitizers rescued these effects^[52]. In 2009, Gotz *et al.*^[52] described the molecular commonalities between T2D and AD with hallmark feature of amylin deposition

in pancreatic islets of T2D patients, whereas A β and NFTs deposition in AD brain which are characteristic fibrillar proteins leading to cell loss. In 2010, Saini *et al.*^[53] contributed a relevant publication to World Journal of Diabetes, establishing a molecular mechanism of IR in T2D. Crucial molecular players in these pathways came into the picture and provided new therapeutic targets. Streptozotocin induced diabetic rat model showed co-appearance of tau hyperphosphorylation and cognitive decline as an interesting evidence^[55]. On the basis of clinical and biochemical evidences, it was further suggested that both of these diseases promote each other's progression^[56]. It has recently been found that proinflammatory signals in the brain impair insulin signaling, mitochondrial dysfunction, synaptic crosstalk as well as cognitive impairment^[57].

Since 1980, many reports appeared in literature to describe the correlation between these two distinct problems with common molecular and cellular interface. Glucose metabolism and insulin signaling are major elements bridging AD and diabetes. Some relevant reports, unraveling the twin mystery of AD and DM are listed in Table 1.

THE CHICKEN OR EGG QUESTION

In spite of so many striking evidences, due to common interface of homeostatic mechanisms of AD and DM, the chicken or the egg question has remained unresolved. Citing all relevant findings, in 2005, first time Suzanne de la Monte has introduced insulin signaling dysfunction as a core of AD. To untangle this mystery, evidence of crosstalk between AD and DM, were put forward as crucial milestones. Patients with T2D were found to be at high risk of developing mild cognitive impairment (MCI), dementia and AD^[60,61]. Similar type of evidence for MCI, dementia and AD were found in experimental models of diabetes^[56,62-65]. AD brains have similar pathogenesis as observed during insulin deficiency^[24,66-68]. Studies with AD patients and animal model of AD showed that intranasal insulin therapy significantly improved cognitive performance^[69-71]. These clinical and experimental studies suggested that both of these disorders share common biochemical and molecular cascades^[60,72,73]. Some of these common bridging elements have been schematically represented in Figure 1. Interestingly, insulin has been found to regulate A β and tau metabolism, which are major hallmarks of AD^[74,75]. It is also evident that in T2D patients insulin signaling dysfunction accelerates A β PP (amyloid beta precursor protein)/A β trafficking from trans-Golgi network, a major site for A β generation and alters dynamicity of A β synthesis^[75]. Some studies report the presence of some downstream regulators of insulin signaling pathway which are involved in cleavage of A β PP at γ -secretase site, a determining site for A β amyloidogenicity^[76]. Although, investigators found many evidences of common features in both of these disorders, the chicken or the egg question is still valid

Table 1 Relevant reports bridging Alzheimer's disease and diabetes mellitus

Ref.	Key findings
Adolfsson <i>et al</i> ^[47]	Hypoglycemic condition can ameliorate brain status in AD
Razay <i>et al</i> ^[48]	Disturbances in glucose metabolism and hyper-insulinemia in female AD patients are responsible for cognitive decline
Ruigómez <i>et al</i> ^[58]	Documented a relationship between non-insulin dependent diabetes and neuropathy
Li <i>et al</i> ^[50]	Defective insulin signaling is a shared degenerative cascade in disease pathology of both AD and DM
Ke <i>et al</i> ^[59]	Amylin deposition in pancreatic islets of T2D patients whereas, A β and NFTs deposition in AD brain are common hallmarks feature of diabetes and Alzheimer's in terms of protein deposition
Saini ^[53]	Elucidated cellular and molecular mechanisms of insulin resistance and provided understanding for the molecular therapeutic targets
Park ^[54]	T2D and AD have some common pathogenic alterations like defects in insulin signaling, A β clearance, glucose metabolism, O-GlcNAcylation, A β aggregation by AGEs, inflammation, oxidative stress and circulating cortisol levels
Correia <i>et al</i> ^[55]	Amyloidogenesis and mitochondrial dysfunction are common denominators potentiating brain dysfunctions
Talbot <i>et al</i> ^[57]	Brain insulin signaling pathway including IGF-1R \rightarrow IRs-2 \rightarrow PI3K signaling is directly involved in AD and thus one of a causal factor in disease pathogenesis

AD: Alzheimer's disease; DM: Diabetes mellitus; T2D: Type 2 diabetes; NFTs: Neurofibrillary tangles; A β : Amyloid beta; AGEs: Advanced glycation end products; IGF-1: Insulin like growth factor-1; IRs: Insulin receptors; PI3K: Phosphatidylinositol 3-kinases.

Table 2 Symptoms of Alzheimer's disease symptoms in diabetes mellitus patients and symptoms of diabetes mellitus in Alzheimer's disease patients

Ref.	Key findings
Gasparini <i>et al</i> ^[75]	In T2D patients insulin metabolism dysfunction accelerates A β PP/A β trafficking from trans-Golgi network, a major site for A β generation
Phiel <i>et al</i> ^[76]	Some studies claim for the presence of downstream regulators of insulin signaling pathway which are involved in cleavage of A β PP at gamma-secretase site, a determining site for A β amyloidogenicity
Steen <i>et al</i> ^[24]	Extensive dysfunction of IGF-I and IGF II signaling mechanisms reported in AD brain
Rivera <i>et al</i> ^[66]	Insulin and IGF gene expression altered with abnormal receptor binding in AD brain

AD: Alzheimer's disease; T2D: Type 2 diabetes; A β : Amyloid beta; IGF: Insulin like growth factor.

and needs parsimonious explanations. Key reports supporting AD like symptoms in DM patients and DM like symptoms in AD patients are listed in Table 2.

AMYLOIDOGENESIS: A COMMON PATHOLOGY IN AD AND DM

Protein structure and function is crucial for maintenance of life, moreover its mishandling leads to diverse pathological conditions. Neurodegenerative disorders lie in a class of disorders associated with different types of abnormal fibrous, extracellular proteinaceous deposits which are referred as amyloid^[77]. β -sheet structured insoluble moieties play an important role in the pathology of many protein misfolding diseases^[77]. Globular proteins due to their tertiary structure constrain, undergo destabilization of their native structure and adopt partial folded and unfolded form while natively folded proteins are devoid of any ordered form so they passes through the stabilization process of fibrillogenesis and acquire a partially folded conformation^[78]. In a crowded cellular milieu when functional protein erroneously interacts with other components and transforms itself into ordered stable form, the phenomenon is known as amyloidogenesis.

Interestingly AD and DM both involve amyloidogenesis. Extracellular deposition of A β plaques is a feature of AD while amyloidogenic peptide deposition in pancreatic islets of Langerhans is a characteristic

feature of T2D^[79,80]. Amyloid deposits in islets consist of 37 amino acid peptide referred to as islet amyloid polypeptide (IAPP) amylin^[81,82]. A β and IAPP have same folding patterns and configuration^[83]. IAPP is reported to generate islet β -cells toxicity in the same way as A β do in neurons. Although we are far to understand the exact mechanism of amyloid formation, it can be speculated from the emerging data that amyloid formation is a basic cause of AD, DM and other disorders related to protein deposition^[84-86].

IR AS COMMON METABOLIC COMPROMISE IN AD AND DM

Glucose is the only required source of energy for neurons and any disruption in glucose metabolism leads to compromised neuronal functions^[39]. Presence of insulin is crucial for brain in terms of its peculiar CNS functions^[87] but any disturbance in its physiological level leads to CNS dysfunction. IRs are reported with low binding affinity with insulin in postmortem AD brain^[87,88]. Moreover many other insulin signaling markers were altered in AD brain^[24]. Elevated insulin plasma level in AD patients indicates a closed association of AD and IR^[26,28]. Animal model studies revealed that factors contributing to T2D also regulate A β dynamics^[89]. With this set of data it is clearly understood that IR or impaired IRs not only typify T2D but also orchestrate AD. Figure 2 depicts that how IR bridges peripheral and

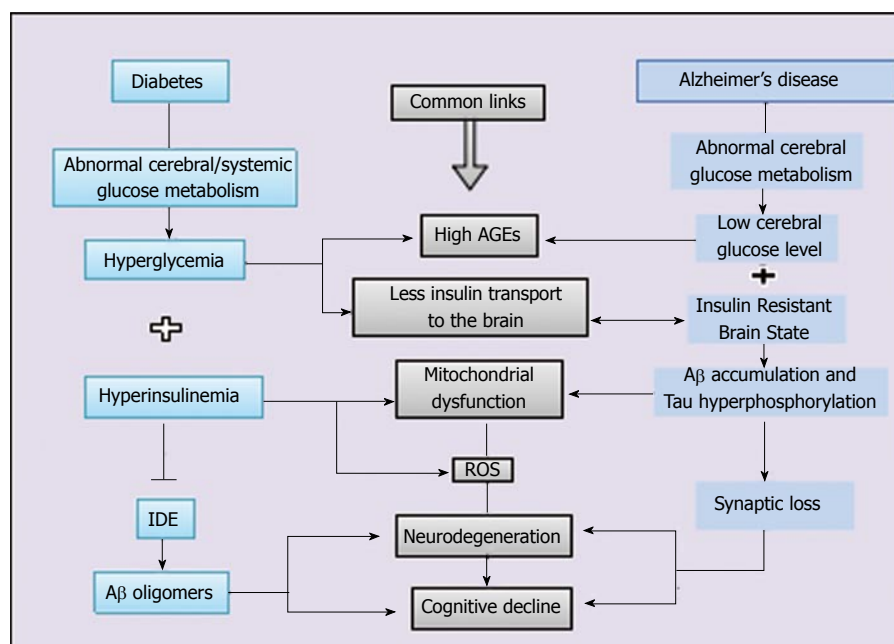


Figure 1 Schematic representation of commonalities between diabetes and Alzheimer's disease. Hyperglycemia and hyperinsulinemia are hallmark features of diabetes which leads to advanced glycation end product, reduced insulin supply to brain as well as mitochondrial dysfunction, which further leads to vicious cycle of oxidative stress. On the other side, any defect in glucose metabolism and insulin signaling in brain is one metabolic status of Alzheimer's disease brain which translates into insulin resistant brain status and converges to all common interfaces of mitochondrial dysfunction, oxidative stress and neurodegeneration. IDE: Insulin degrading enzyme; Aβ: Amyloid beta; AGEs: Advanced glycation end products; ROS: Reactive oxygen species.

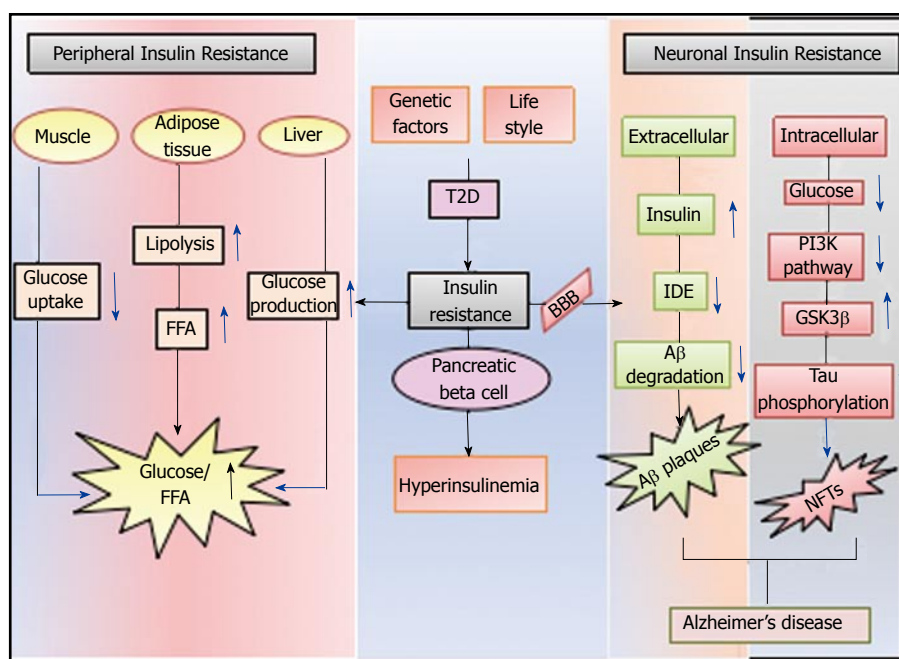


Figure 2 Diagrammatic representation of peripheral and neuronal complications of insulin resistance in case of type 2 diabetes. Insulin signaling dysfunction in peripheral system affect muscle, adipose tissue and liver (by decreasing glucose uptake, increasing free fatty acids) and by increasing glucose production respectively. When this dysfunction appears in CNS as a diabetes complication (by limited insulin supply to brain), it leads to deposition of Aβ plaques and NFTs in extracellular and intracellular milieu of neurons respectively and represents AD type brain status. AD: Alzheimer's disease; T2D: Type 2 diabetes; IDE: Insulin degrading enzyme; PI3K: Phosphoinositide 3-kinase; Aβ: Amyloid beta; NFTs: Neurofibrillary tangles; GSK3β: Glycogen synthase kinase 3 beta; FFA: Free fatty acids; BBB: Blood brain barrier.

neuronal IR and leads to AD.

How insulin modulates brain functions?

Insulin expression in brain remained a debated topic for

investigators and raised a question on its significance at ectopic site. Brain synthesizes insulin locally as well as receives through the blood brain barrier (BBB) mediated transfer^[90]. With curious attempts, scientists

documented its role in feeding behavior and energy homeostasis which integrate whole body physiology^[91]. The first article unpinning the relation between brain and insulin was reported in 1960, in which intracisternal injection of insulin in dogs reduced glucose levels, both in CSF and blood with its direct effects on the parasympathetic area of the brainstem^[92]. Later, brain-centered glucoregulatory system (BCGS) that is involved in maintenance of blood glucose levels was found to act *via* insulin dependent as well as independent mechanisms^[93]. The hypothesis of BCGS and its crosstalk with pancreatic islets gained experimental momentum by multiple supporting evidences that provided a clear understanding of BCGS^[93]. BCGS is recognized as mechanistic node present in CNS which is channeled through peripheral hormone status^[93]. Both of these regulatory nodes co-operate with each other and compensate the load of other's failure but when both are compromised, DM is an unavoidable issue.

Insulin as a synapto-dendritic player

Insulin has drawn a wide trajectory in brain molecular milieu from cognitive function to orchestrate functions like development of neurite outgrowth, modulation of catecholamine release and uptake, regulation and trafficking of ligand-gated ion channels, expression and localization gamma-aminobutyric acid (GABA), N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, synaptic plasticity regulation *via* NMDA, Phosphoinositide 3-kinase-Akt (PI3K-Akt)^[94] and maintenance of excitatory synapses^[95].

Presence of IRs at synapses rich in plasticity (hippocampus and cortex) reveals its involvement in cognition^[90]. This fact was further strengthened in 1999 when Zhao *et al.*^[21] reported that rat hippocampus IRs expression is up-regulated when they are subjected to spatial memory task in Morris water maze. IRs are enriched in synaptosomes^[96], co-localizes with axon terminal markers synaptophysin, synapsin, *etc.*^[97], and dominates in post-synaptic density (PSD) fractions to interact with scaffolding protein shank and PSD-95. Insulin is also involved in various neuromodulatory functions such as electrophysiological properties of neurons^[98,99], neurotransmitter receptors^[100,101], trafficking of ion channels^[102], neurotrophic effects^[103,104] and the neuroprotective role against a wide range of insults such as apoptosis^[105], oxidative stress^[106], β -amyloid toxicity^[107] and ischemia^[108] in animal models as well as human studies.

Hyperinsulinemia is reported to reduce cholinergic activity in mice brain and resulted in impaired retention of an inhibitory avoidance^[109]. It also alters membrane potential to affect the ion transport^[110,111]. In streptozotocin induced rat model of DM, long term memory potentiation was found to be impaired and insulin treatment rescued the effects^[112-114]. With these set of potential findings, it is evident that insulin is crucial synapto-dendritic player altering dendritic arbor

morphology as well physiology.

INSULIN RECEPTORS PLAYING DOWNSTREAM MOLECULAR ORCHESTRA: INSIGHT INTO THE MECHANISMS

Investigators unraveled the IRs downstream molecular orchestra and speculated that IRs activation further activates PI3K/protein kinase B (PI3K/PKB) pathway^[115]. GSK3 β is a major player of this pathway and involved in long term potentiation/long term depression (LTP/LTD) which is a sole mechanism of memory formation and synaptic plasticity^[116]. Other than insulin, PI3K can be activated by multiple growth factor ligands including nerve growth factor, brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), insulin like growth factor-1 (IGF-1)^[117].

After investigating for over two decades, it is safe to accept that PI3K/Akt signaling pathway is a potential window through which various ON/OFF switches of cognitive decline get operated. Protein kinase B (PKB), also known as Akt is a main downstream hub of various other pathways and exists with its widely expressed isoforms such as PKB- α , PKB- β and PKB- γ (predominates in CNS)^[118]. Akt pathway has its regulating arms over neuronal survival, glucose uptake, angiogenesis, metabolism and proliferation^[119]. Moreover Akt has a negative feedback regulation over these *via* phosphatase and tensin homolog, protein phosphatase 2A, c-jun N-terminal kinases (JNK) and forkhead box O (FOXO)^[119].

Loss of PI3K control is a central mechanism of neuro-degeneration in DM patients^[120]. Moreover, AD patients are reported with sustained PI3K/AKT signaling which is a primary response linking insulin, IGF resistance, tau pathogenesis and synaptic decline^[121]. GSK3, mammalian target of rapamycin (mTOR) and FOXO are three main downstream targets playing this whole orchestra (represented in Figure 3).

Glycogen synthase kinase3 β , a pivotal kinase in AD and diabetes

Extensive reports supporting pivotal role of GSK3 β proposed "GSK3 β hypothesis of AD"^[107], according to which GSK3 β over-expression leads to impaired memory, amyloid β accumulation, tau hyperphosphorylation, neuronal defects and microglial mediated inflammation cascades. Genetic studies established that insulin signaling genes are also loci of AD^[106]. Cholinergic system is one of the major regulating knob under GSK3 β control with choline acetyltransferase and acetylcholinesterase as regulating keys^[107,108]. GSK3 β leads to reduction of acetylcholine synthesis, which is in accordance with the cholinergic deficit observed in AD brain^[122]. GSK3 β negatively affects axonal transport, microtubule dynamics and destabilizes microtubule by

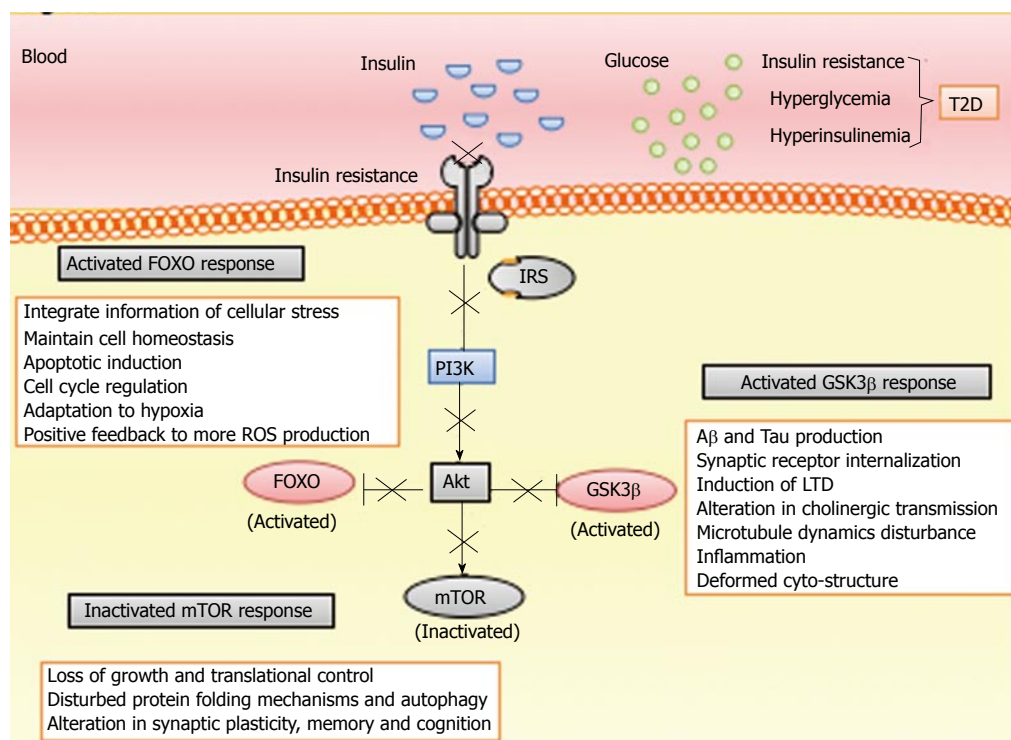


Figure 3 Diagrammatic representation of molecular orchestra downstream of insulin receptor. IRs activation leads to downstream PI3K signaling pathway with Akt as a central hub which diverges into three main branches including FOXO, GSK3 β and mTOR. Akt inhibition leads to activated FOXO and GSK3 β response while inactivated mTOR response. FOXO activation leads to cellular stress response, mTOR dysfunction leads to loss of translational control and altered cognition while GSK3 β activation leads to A β and tau production. T2D: Type 2 diabetes; PI3K: Phosphatidylinositol 3-kinases; A β : Amyloid beta; GSK3 β : Glycogen synthase kinase 3 beta; FOXO: Forkhead box O; mTOR: Mammalian target of rapamycin; LTD: Long term depression.

lowering its affinity with GSK3 β phosphorylated tau^[95-97] and contributes to AD pathology. Being a key mediator of apoptosis it may directly contribute to neuronal loss in AD^[105,123]. GSK3 β interestingly controls cell cycle in two way system by activating intrinsic pathway to trigger cell death and by inhibiting death receptors by extrinsic pathway^[124]. In 2002, Sun *et al.*^[125] and in 2003, Phiel *et al.*^[76] reported that GSK3 β increases A β production by regulating APP cleavage. On exposure of A β , neurons inhibit PI3K pathway and increase GSK3 β activity^[126]. GSK3 α as well as GSK3 β both are found to be an inducer of tau phosphorylation^[127-132]. Drastic alteration in dendritic arbor and post synaptic density, a common morphological feature of AD brain has been observed in GSK3 β deficient mice^[132]. GSK3 β is the only kinase involved in NMDAR-LTD^[124]. It also maintains a threshold of LTP and LTD, *i.e.*, maintenance of metaplasticity^[116,133,134]. Modulation in regulated/constituted expression of GSK3 β orchestrates neuronal plasticity^[84,116,134-140]. GSK3 β dramatically induces the internalization of AMPA and NMDA receptors^[141,142] and decreases the level of PSD proteins, a molecular marker of memory acquisition^[77]. GSK3 β phosphorylates CREB protein to inhibit its function which is a universal modulator of memory. It aids in cyto-architecture of cell by promoting actin and tubulin assembly for synaptic reorganization^[143]. GSK3 β is also a pivotal kinase involved in adult hippocampal neurogenesis

which negatively regulates it by reducing the number of proliferating neurons in the dentate gyrus region^[144,145]. GSK3 β is directly involved in the production of pro-inflammatory cytokines such as interleukin (IL) 6, IL-1 β , TNF- α which indicates its positive regulation towards inflammatory mechanisms^[146,147].

FOXO1 signaling: A mechanistic node for a vicious cycle of IR and A β up-regulation

FOXO1 signaling is a mechanistic node and regulates the fine balance of oxidative stress pathways (depicted in Figure 4). Before moving into the mechanism part, it has been briefly discussed about the dramatic story of its evolution in molecular series under discovery^[148]. Many lines of evidence suggest its role in AD as well as IR with major involvement in cell proliferation, differentiation, cell survival, apoptosis and development of proliferative late onset diseases^[148]. Short term activation of this player leads to protective mechanism of scavenging reactive oxygen species (ROS) which is a part of normal cell physiology but its persistent activation awakes the apoptosis pathway^[148]. Cellular milieu tends to maintain a balance oxidant and antioxidants concentration to cope up any environmental stress, but whenever this balance acquires any plane of inclination, it comes to the cell survival^[148].

Wnt and β catenin up-regulate FOXO signaling *via*

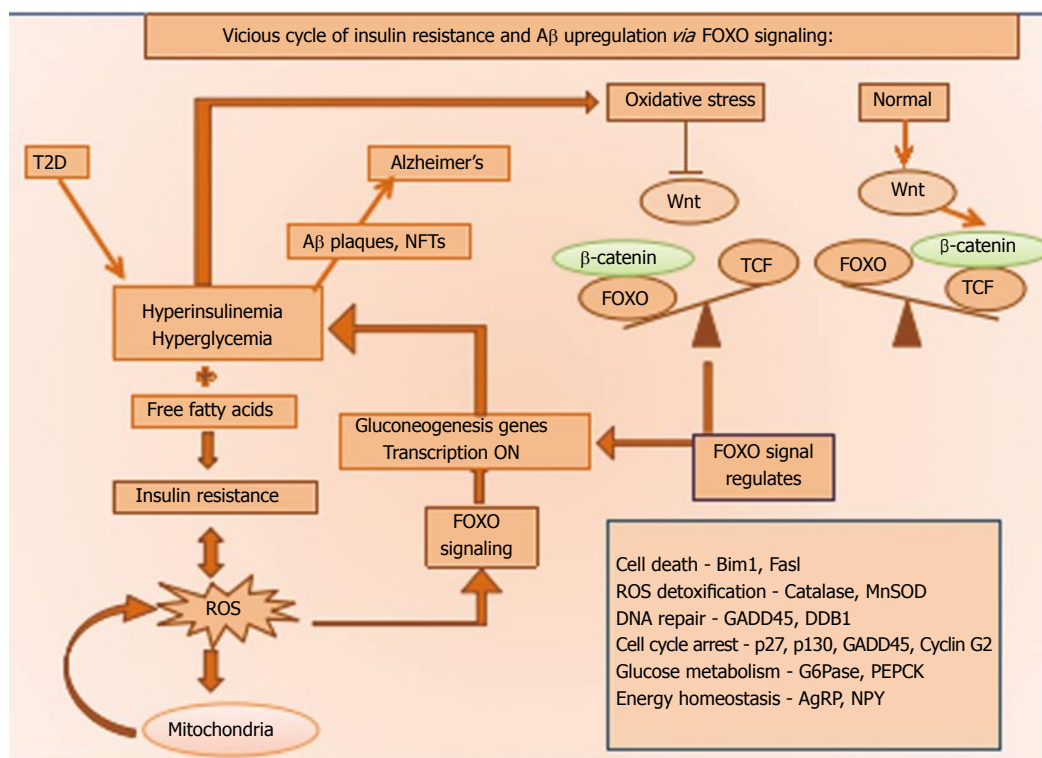


Figure 4 Diagrammatic representation of vicious cycle of insulin resistance and amyloid beta up regulation *via* forkhead box O signaling. FOXO signaling is a protective mechanism adopted by body to cope up with any cellular stress by ROS detoxification. In case of DM whenever there is any metabolic disturbance like hyperinsulinemia and hyperglycemia, it leads to further oxidative stress and ROS generation which promotes further trigger for FOXO signaling and promotes a vicious cycle of ROS generation leading the cell towards apoptosis. T2D: Type 2 diabetes; A β : Amyloid beta; FOXO: Forkhead box O; ROS: Reactive oxygen species; TCF: T cell factor; DM: Diabetes mellitus.

oxidative stress pathways^[148]. Wnt signaling inhibits GSK3 β expression and mediates β catenin transport into the nucleus and modulates transcription of T cell factor family gene, which has function opposite to FOXO1, this is known as the canonical pathway of Wnt signaling and involved in lipid and glucose metabolism^[149]. ROS production inhibits canonical pathway of Wnt signaling and guides β catenin towards FOXO which acts as a cofactor of FOXO and enhance its transcription. Foxo signaling promotes gluconeogenesis and leads to hyperglycemia and hyperinsulinemia which further increases NFTs and A β accumulation to gear up ROS production and drives the vicious cycle of Oxidative stress^[150].

When insulin is absent, FOXO1 is located in the nucleus and promotes transcription of respective enzymes for hepatic glucose production while in the presence of insulin; PKB is activated and leads to nuclear exclusion of FOXO 1 by phosphorylating it. State of IR in case of DM leads to impairment of PKB pathway and inhibition of FOXO activity resulting in hepatic glucose production triggering a vicious cycle of hyperglycemia and oxidative stress. FOXO, the downstream activator of PI3K/AKT controls energy homeostasis, locomotor behavior and leptin sensitivity^[151,152].

mTOR pathway: A crucial intersection of AD and DM

mTOR pathway has been evolved as environment

sensor and growth promoter in unicellular organisms but as multi-cellularity emerged it acquired its role in central growth and homeostasis mechanisms. Metabolism and cell growth are two basic requirements and their proper functioning depends upon each other. Since mTOR pathway is centered for growth processes, it is activated by nutrition as well as insulin^[136]. In evolutionary history from yeast to rodents, mTOR has evolved as key modulator of aging. Many investigators attempted to understand its basic role and decades of extensive pursuit revealed extensive network of mTOR. mTOR is found to accelerate growth but it has compromised some of metabolic signals by conflicting pathways and introduced a paradox or better to say insulin paradox^[137].

This paradox appeared from the evidences of compromised insulin signaling with good health and IR leading to compromised health while both of the cases are of poor insulin signaling^[138]. Parsimonious explanations are, compromised insulin signaling is unable to activate mTOR (good for health) while IR may be due to hyperactive mTOR which is bad. So in previous case compromised insulin signaling inhibits mTOR insurgence while active mTOR is promoting IR in the later case^[138]. Mechanistic node of this story, S6 kinase (S6K) is activated by mTOR to phosphorylate and degrade insulin receptor substrate-1 (IRS-1) which ultimately leads to insulin desensitization^[139,140].

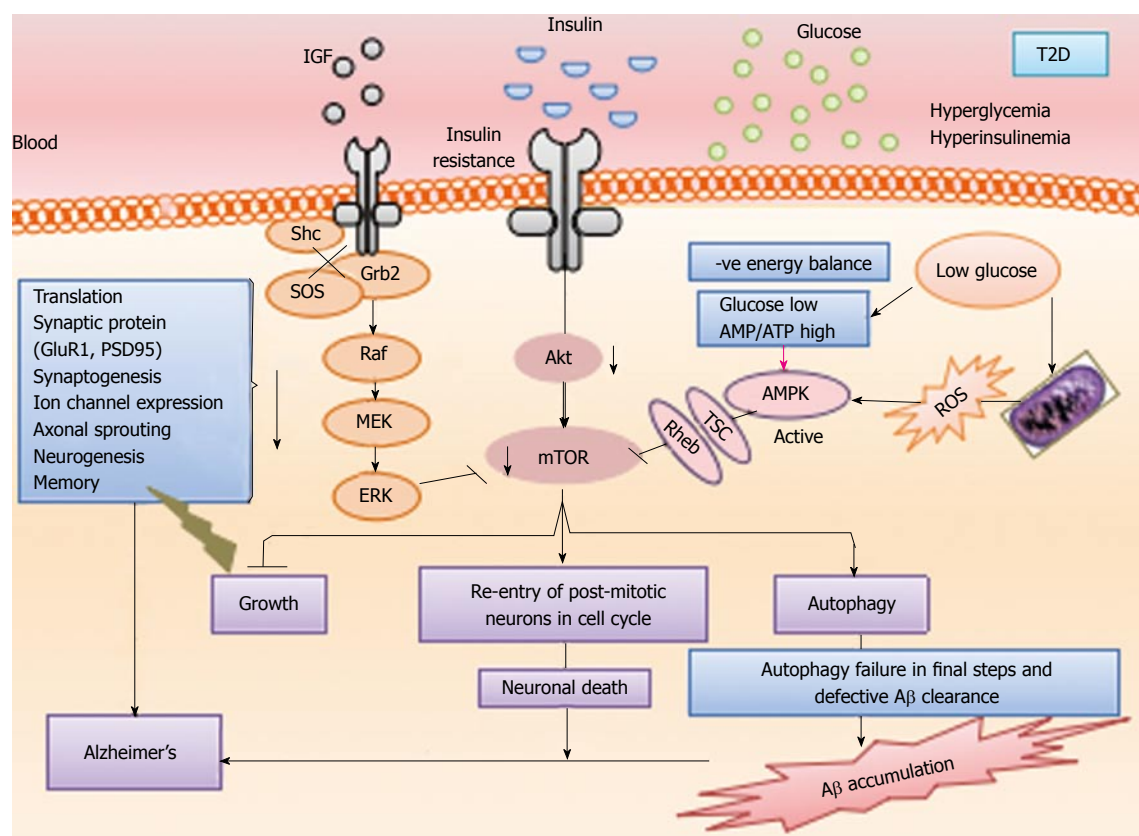


Figure 5 Diagrammatic representation of mammalian target of rapamycin pathway: A crucial intersection of type 2 diabetes and Alzheimer's disease. Diabetes results in dysfunctional insulin signaling which brings mTOR pathway down and ultimately results in autophagy failure to accumulate A β , inhibit re-entry of post mitotic neurons in cell cycle, stimulate aberrant growth pathways, lose of translational control and impaired neurogenesis, etc. T2D: Type 2 diabetes; IGF: Insulin like growth factor; mTOR: Mammalian target of rapamycin; A β : Amyloid beta; ROS: Reactive oxygen species.

mTOR signaling has a dramatic interplay with A β and tau proteins which are two hallmarks of AD in their aggregated forms. It was reported in 2012 that A β is an activator of PI3K/Akt pathway which further switches on mTOR cascade^[153]. *In vitro* studies suggest that A β application elevates the level of p70S6K, a downstream target of mTOR which contributes in development of NFTs^[154,155]. Consistent *in vitro* reports validated the fact that mTOR activity and activated p70S6K are either cause or consequence of the molecular cascade and hence are found with elevated levels in hippocampus and cortex of animal model of AD^[156,157]. mTOR suppression leads to induction of autophagy which is a cell cleaning process. In AD brain it is evident that neuronal autophagy is induced to end up with impaired steps and leads to massive accumulation of A β plaques^[158].

mTOR has characteristic property of maintenance of protein homeostasis, translational control and cellular maintenance, which plays an important role in the maintenance of synaptic plasticity. Figure 5 provides detailed information of mTOR domain. To execute these entire tasks mTOR pathway is operated under fine control of several surface receptors such as NMDA, dopaminergic and metabotropic glutamate receptors (mGluRs) and BDNF^[159-163]. mTORC1 is one of the downstream targets of PI3K/AKT pathway which is very important for synaptic plasticity, neuronal repair, protein

folding mechanism and autophagy^[164,165].

INFLAMMATION: A COMMON ALARM FOR AD AND DM

Inflammation is an exceedingly complex but equally fascinating and costly host defense system evolved with proximate set of mechanisms and exhibit phenotypic plasticity. It is crucial for life but once dysregulated, it can be detrimental. Emerging field of metabolic and aging syndromes spurred a renewed interest of scientists into inflammatory mechanisms. This is a compensatory mechanism for body to cope up with the hostile environment which involves many subtle factors and specialized cells to fight against any threat^[166]. It has very critical progressive role with analogous mechanism in diabetic patients showing IR and defective neuronal signaling in AD patients^[167]. Thus, DM and AD share inflammation as a common pathological feature.

Studies have reported elevated levels of proinflammatory cytokines such as TNF- α , IL-6, IL-1 β , etc., in AD patients^[168]. In diabetes patients, elevated TNF- α triggers various stress kinases to phosphorylate IRS-1 (at inhibitory serine residues) and disrupts insulin signaling^[169-171] (explained in Figure 6), while blocking TNF- α rescues its effects in obese mouse model^[172,173]. JNK and double-stranded RNA-dependent protein kinase

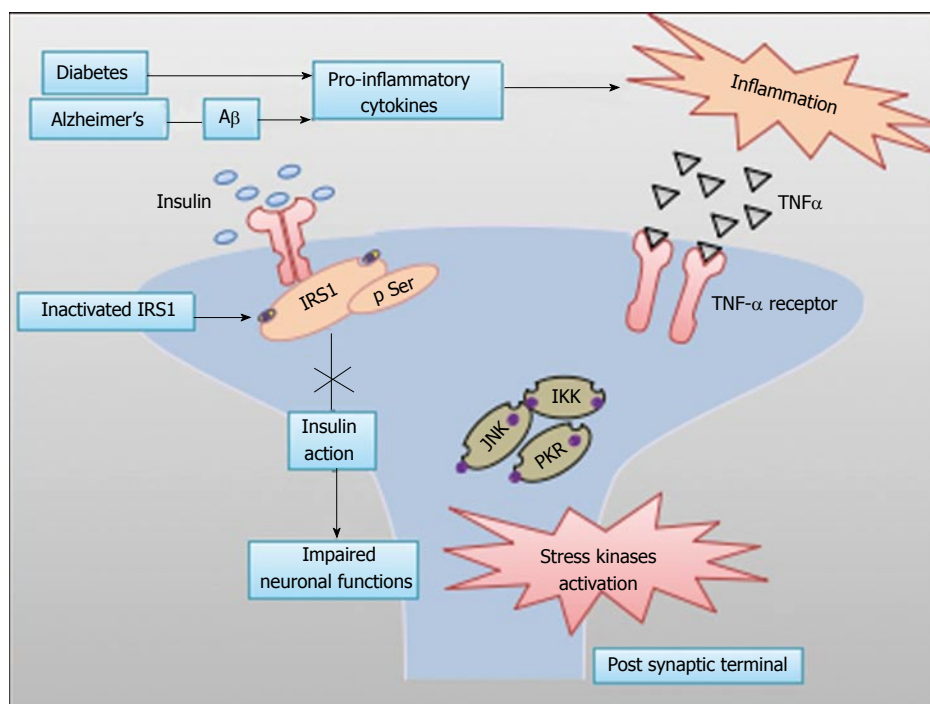


Figure 6 Diagrammatic representation of insulin signal dysfunction in Alzheimer's disease and diabetes mellitus *via* common inflammatory cascade. Diabetes and AD lead to production of pro-inflammatory cytokines in inflammatory response. These stress kinases inhibit IRS1, an adaptor protein for insulin receptor signaling and result into defective insulin signaling in brain. Aβ: Amyloid beta; IRs: Insulin receptors; JNK: c-Jun N-terminal kinases.

are major stress kinases which are common regulatory nodes between inflammation and metabolism^[174,175]. Since insulin signaling contributes to normal functioning of neurons, any inflammation mediated alteration in these, results into defective neuronal function^[95,176,177]. These evidences suggest that there is a common mechanistic pathway adopted by peripheral IR in T2D as well as impaired brain insulin signaling in AD.

OXIDATIVE STRESS: A COMMON BURDEN IN AD AND DM

Normal body physiology tends to maintain a balance between production of ROS and body's antioxidant defense system and any sort of imbalance altering this dynamic system leads to onset of metabolic disorder with cognitive dysfunction^[178]. Hydrogen peroxide, hydroxyl radical, superoxide ion and singlet oxygen are such reactive species which are abundantly produced in cellular respiration cycles and have very short half life^[179]. It is known that diabetic patients have more oxidative cellular environment as compared to healthy ones^[180-182]. Hyperglycemic condition has proportionality with sorbitol production which reduces NADPH, a cofactor for GSH production and hence decreases antioxidant levels in the body^[183-185]. One more prevalent mechanism of diabetes contributing towards ROS is insurgence of advanced glycation end products (AGEs) production^[183,184,186], which binds to cell surface receptors, *i.e.*, receptor for advanced glycation end products (RAGEs). RAGEs-AGEs interaction leads to ROS

production *via* NADPH oxidase system which in turn activates Ras-MAPK pathway and ultimately nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activation^[184,187]. Hyperglycemia also leads to flux of glucose or FFA into blood which turns hexosamine pathway on^[188] for further ROS production^[189]. Elevated levels of FFA have an adverse effect on mitochondrial functioning and uncouple oxidative phosphorylation to contribute in ROS production^[190,191]. ROS production worsens the status of insulin signaling and stress pathways which lead to further ROS production to turn a vicious cycle on.

High polyunsaturated fatty acid proportion with GSH content leave neurons vulnerable and make them prone to free radical attack^[192]. A noticeable increase in lipid peroxidation was observed in brain of AD patients^[193-195]. Oxidative stress and Aβ aggregation has both way relationships controlling each other's turnover. Oxidative stress channels regulate Aβ dynamicity from non-aggregated form to aggregated form^[196]. Aggregated Aβ acts like a source of free radical production and lipid peroxidation^[197] to drive brain towards neurodegeneration.

MITOCHONDRIAL VULNERABILITY IN CASE OF AD AND DM

Mitochondria, a result of 1.5 billion years of obligate endosymbiotic co-evolution is a sub-cellular niche to take care of cell survival as well as programmed cell death^[198]. Several decades of research has establis-

hed that fission-fusion dynamicity of mitochondria is critical in neurodegeneration^[198]. As the brain is offered with limited capacity of glycolysis, neuronal cells are highly dependent on aerobic oxidative phosphorylation for energy production which is an electron transfer event from lower redox potential to higher redox potential^[199-202]. Although, this electron chain transfer process is very efficient, still some ROS are produced which leads to oxidation of mitochondrial DNA, lipids and proteins further contributing to mitochondrial dysfunction which is a prominent feature of AD^[181,203].

Substantial data from diabetic patients and animal model systems revealed that brain faces several structural and functional deficits. Functional impairment of mitochondria leads to neurodegeneration and loss of control over neuronal metabolism. A study reflected that there is a significant decrease in coenzyme Q levels in diabetic animals which represents a marked deficit in antioxidant defense system^[204]. There are reports which are directly linking impairment in glucose utilization with mitochondrial dysfunction and metabolic disturbances^[205-208]. In 2003, clear evidence of oxidative phosphorylation uncoupling was found in rat model of T2D^[209]. Mitochondrial capacity of Ca^{2+} accumulation was also found to decrease in case of diabetes which is a favorable environment for mitochondrial permeability transition (MPT) opening and ultimately leads to cell death^[210,211].

AD animal models as well as human studies suggested that AD pathology leads to mitochondrial dysfunction and ROS production. Some crucial molecules such as $\text{A}\beta$ binding alcohol dehydrogenase are reported to aid to AD pathology by mediating $\text{A}\beta$ induced cell death *via* mitochondrial channel^[209,212]. In some reports it is mentioned that one of the insulin degrading enzymes isoform, a well established regulator of $\text{A}\beta$ dynamicity targets mitochondria and interfere with its normal functioning^[213]. $\text{A}\beta$ is also found to be a good inhibitor of respiratory chain complex and thus leads to marked decrease in cellular ATP levels^[214,215]. Importantly, $\text{A}\beta$ 40 and $\text{A}\beta$ 25-35 contribute in uncoupling of oxidative phosphorylation and impair respiratory chain as well as MPT opening^[204,210,211]. Moreover $\text{A}\beta$ induces H_2O_2 production which is rescued by CoQ10, a key enzyme of electron transport chain^[216]. Various tri-carboxylic acid (TCA) cycle enzymes such as pyruvate dehydrogenase, α -ketoglutarate dehydrogenase and ATP citrate lyase were also found to be dysregulated in case of AD^[217].

Mitochondrial morphology was found to be altered with some functional loss in neurodegenerative disorders such as AD^[218-221]. In brief it can be mentioned that a small metabolic compromise is sufficient to trigger a cascade and disrupt normal mitochondrial function which plays a vital role in neuronal survival, growth and plasticity.

predisposition are conspired forces responsible for worldwide epidemic of metabolic and aging syndrome. Discovered molecular trajectories from T2D to T3D gained experimental momentum for new therapeutic interventions. Elucidating role of anti-diabetic drug for the treatment of AD translated the disease information and added new armaments to the arsenal of putative therapies. It is unquestionable issue that both of these disorders share common pathologies including glucose metabolism defects, mitochondrial dysfunction, oxidative stress and abnormal deposition of amyloidogenic proteins^[55]. The reason why insulin got this recognition under frontier's of Alzheimer research is that its high level in CNS revealed its own crucial role in learning, memory, cognition and synaptic plasticity^[222]. Although, brain has potential pyramidal neurons involved in synthesis and secretion of insulin, majority of brain insulin is replenished by peripheral source from pancreatic β cells transported through blood across BBB^[223].

There are some well known potential oral drugs [such as biguanides, sulfonylureas (SUs), thiazolidinediones (TZDs), and dipeptidyl peptidase-IV (DPP-IV) inhibitors], injections (e.g., insulin and GLP-1 analogs), and some other molecules like glucokinase activators, amylin analogs, D2-dopamine agonists, bile acid chelators, and sodium/glucose-linked transporter-2 inhibitors *etc.*, established for T2D. Most of the anti-diabetic drugs act through the mechanism of maintenance of plasma glucose level, regulation of inflammatory cascades and establishing the balance between ROS and antioxidants. We will briefly provide an overview of experimental and clinical trials of some anti-diabetic drugs which are being tested in patients with AD and with low to moderate mild cognitive impairment.

Metformin

Metformin, a well known biguanide anti-diabetic drug is used to reduce IR. It sensitizes liver and skeletal muscle cell *via* AMP kinase cascade^[224,225]. Brain is most vulnerable vital organ for oxidative stress, because of high oxidative metabolism rate and limited antioxidant level. Under oxidative stress mitochondrial permeability pores open up to release cytochrome c and trigger apoptotic cascade. Metformin is reported to inhibit opening of these permeability pores in ectoposide-induced cell death model to inhibit apoptotic cascade^[226]. Metformin is also involved in neurogenesis by activation of protein kinase C-CREB binding pathway (PKC-CBP) pathway in neuronal cell culture study, in human and rodent model system^[227]. In neuronal cell lines (neuro2A), metformin promotes insulin action and attenuates molecular and pathological features observed in AD. Metformin treatment was found to reduce the risk of dementia in human aged subjects^[228]. AD patients taking calcium in diet supplemented with metformin were found to have better cognitive performance^[229]. Thus these evidences support the fact that metformin is not only a known anti-diabetic agent

THERAPEUTIC OPPORTUNITIES

Sedentary life style, dietary changes and genetic

but also an effective neuroprotective molecule.

Sulphonylurea

SUs is a class of anti-diabetic drugs which are used as mono or combined therapy to increase insulin secretion by enhancing pro-insulin level *via* voltage gated calcium channel but the actual mechanistic target is still under investigation^[230]. SUs limits liver glucose production and decreases insulin clearance by liver. Glipizide and Glyburide (glibenclamide) are the main SUs compounds which are investigated for memory and cognition in diabetic patients.

Experimental and clinical studies

In case of diabetes and AD, PI3K/mTOR is found to be aberrantly activated. Glyburide and glipizide are reported to have properties of mTOR antagonist^[231] but their efficacy to recover AD patients is yet to be determined. Inflammasomes are involved in the secretion of proinflammatory cytokines that results in inflammation and associates it to AD. Along with inhibiting mTOR pathway, gliburide is found to inhibit inflammasome and thus brain inflammation^[232]. Exalto *et al.*^[230] reported that SUs treated T2D patients shows improved AD type dementia symptoms but the precise mechanism is still unknown.

DM patients treated with glipizide are reported to have better learning efficiency^[233]. Some recent studies show that there is no alteration in the development of AD in population using SUs in long term^[234]. Metformin and SUs in combination are reported to reduce the risk of dementia upto 35% in a prospective cohort study^[228].

Intranasal insulin

Intranasal administration of insulin is reported to attenuate reduced insulin signaling in AD^[235]. Importantly, intranasal insulin does not adversely affect blood insulin or glucose levels.

Experimental and clinical studies

It is evident that AD patients have low insulin level and brain insulin resistant state which leads to impaired energy metabolism of neurons and make them vulnerable for survival. Insulin has been reported with its anti-amyloidogenic effect in human neuronal cell lines^[236]. Some reports have shown that A β induced neuronal IR is attenuated by insulin treatment^[237].

In a study it is found that 20 IU insulin twice a day over a period of 21 d in early AD or MCI subject's helps to retain verbal information more effectively^[30]. In 2006 Reger *et al.*^[30] showed that 10 IU intranasal insulin improves cognition in APOE4 AD/MCI subjects.

TZDs

TZDs (also represented as glitazones) are a potential class of drug used for T2D which includes rosiglitazone (avandia), pioglitazone (actos) and troglitazone (rezulin). Mechanism of this group lies in activation of peroxisome proliferator-activated receptors by mimicking as a po-

tential agonist of it and involved in transcription of lipid and glucose metabolism genes^[238,239]. Since TZDs are anti-amyloidogenic and anti-inflammatory compounds with insulin sensitizing role, these delay neurodegeneration^[240]. It also improves glycemic control in diabetic patients by inhibiting hepatic gluconeogenesis. Moreover, TZDs (mainly Troglitazone) are supposed to have their involvement in rescuing memory loss and decreasing plasma A β 40 and A β 42 levels^[241,242] but again it needs to be investigated further.

Experimental and clinical studies

Rosiglitazone is reported to attenuate neuronal IR induced by A β oligomers^[237]. Pioglitazone is found to improve cognitive performance in a rodent dementia model induced by intracerebroventricular (ICV) injection of streptozotocin^[243].

In a randomized trial rosiglitazone (8 mg) is reported to improve cognitive function in mild to moderate AD patients (non APOE4 carrier^[244]). In contrast, a recent phase III trial of the same drug has failed to show similar effects in AD subjects^[245]. Moreover, long term use of TZDs, in general has no effect on risk of AD development^[234].

Glucagon like peptide 1

Glucagon like peptide 1 (GLP1) analogs are "incretin mimetics", used to treat T2D. Exenatide, a 39 amino acid long peptide is analogous to human GLP1 which stimulates insulin secretion in a glucose dependent fashion. In brain these analogues bind to GLP receptors and mediate various functions like suppression of glucagon production, slow down gastric emptying, increase satiety and reduce food intake with lower risk of hypoglycemia.

Experimental and clinical studies

In an animal study, GLP1 is reported to protect neurons from oxidative stress with reduced apoptosis, plaque formation and inflammatory response. Moreover, it strengthens synaptic plasticity in AD mouse brain^[246]. It is shown to improve spatial memory in transgenic AD mice model^[247]. Liraglutide and lixisenatide are GLP1 receptor agonists which are reported to activate cAMP in the brain and induce neurogenesis^[248]. In addition, liraglutide attenuates memory impairments in a mouse model of AD^[249]. Subcutaneous administration of liraglutide is reported to restore both peripheral and brain insulin sensitivity and ameliorates tau hyperphosphorylation in rat model of T2D^[250]. Clinical research on the effect of liraglutide on AD patients is still going to evaluate the changes in cognition using a neuropsychological test battery^[251].

DPP IV inhibitors: Oral hypoglycemic

DPP-IV, pharmacological inhibitors are oral hypoglycemic. These compounds reduce blood glucose levels by increasing incretin (GLP-1 and GIP levels) and attenuating glucagon effects. Sitagliptin, Vildagliptin,

Saxagliptin, Linagliptin, Teneligliptin, Gemigliptin and Dutogliptin are major members of gliptins, out of which Dutogliptin is under Phase III clinical trial^[252]. Effect of sitagliptin administration is studied double transgenic mice model of AD and reported to significantly delay AD pathology including amyloid deposition and taupathies^[253].

Insulin and oral anti-diabetics: A combined therapy

Combination of insulin and other oral anti-diabetic drugs are reported to lower neuritic plaque density by 20% in AD brains^[253]. Metformin in combination with rosiglitazone or glyburide is reported to improve working memory very significantly^[253]. In a prospective cohort study, metformin and SUs are reported to reduce risk of dementia by 35%^[228]. Although, a number of anti-diabetic drugs are reported to improve cognitive effect, it is still not well understood whether these effects are due to glucose lowering effects or adopt different pathways of neuroprotection. A broad range of anti diabetic therapies are undergoing clinical trials including those involving stimulation of the pancreatic beta-cell with the gut-derived insulinotropic hormones (incretins), GIP and GLP-1^[254]. Some drugs have good glycemic control but have no history to improve cognitive functions^[255]. In a study diabetes patients were maintained at normoglycemia over 3 mo but no significant improvement in cognitive performance was observed^[256]. Other than glycemic control, anti-diabetic drugs improve cognitive function. Although various clinical trials are underway to evaluate the role of anti-diabetic drugs in treatment of neurodegenerative disorders such as dementia and AD but the search is still not over.

CONCLUSION

This review provides a synopsis in which a metabolic disturbance becomes indispensable for life. This is a talk of a metabolic problem which emerges as a molecular signal defect and takes a form of syndrome with multiple complications. Spotlighted player, insulin draws a trajectory from diabetes to AD with multiple divergence and convergence.

AD and DM are two devastating syndromes with complex molecular interplay. Evidences of their shared molecular and biochemical footprints shed light on.

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**EDITORIAL**

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Editorial Board Member of *World Journal of Diabetes*, Nikolaos Papanas, MD, Assistant Professor in Internal Medicine, Assistant Professor in Internal Medicine, Democritus University of Thrace, 68100 Alexandroupolis, Greece

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

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In treating diabetes, what is important? Glucose levels or outcome measures?

Anil K Mandal

Anil K Mandal, Mandal Diabetes Research Foundation, Saint Augustine, FL 32084, United States

Anil K Mandal, Nephrology, North East Florida Area Hospitals, Saint Augustine, Palatka, FL 32177, United States

Anil K Mandal, Medicine, University of Florida, Gainesville, FL 32608, United States

Anil K Mandal, Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

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Correspondence to: Anil K Mandal, MB, BS, Consultant in Nephrology, Courtesy Clinical Professor of Medicine, Adjunct Professor of Pathology and Laboratory Medicine, Mandal Diabetes Research Foundation, 665 SR 207, Suite 102, Saint Augustine, FL 32084, United States. amandal@med-spec.com
 Telephone: +1-904-8248158
 Fax: +1-904-8231284

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Abstract

Gaps in knowledge prevail in recognizing which glycemic parameters to order and in determining glycemic control. However glycosylated hemoglobin (HbA1c) is most commonly ordered to determine glycemic control. HbA1c provides information of overtime glycemic control but does not inform post meal glycemic excursions. The latter may be significant in outcome measure such as cardiovascular disorder (CVD), renal failure or amputation in diabetes. In order to obviate the dilemma in the importance between fasting blood glucose (FBG) and 2-h post prandial glucose (2hPPG), we innovated delta (d) which is the difference between 2hPPG minus FBG. There is much information available relating 2hPPG or postprandial hyperglycemia to CVD and some information relating 2hPPG to renal failure or amputation. Thus much emphasis is laid upon glycemic control with little or no emphasis on the complications of diabetes or the outcome measures. The focus of this editorial is to draw attention to outcome measures by ordering fasting and 2-h postprandial (2hPP) basic metabolic panel (BMP) which provides glucose levels, renal function test and electrolytes. HbA1c significantly relates to 2hPPG, thus by ordering F and 2hPP BMP instead of HbA1c alone will serve both purposes: Glycemic control and outcome measure. Delta (d) glucose (dhPPG-FBG) is a stronger predictor than 2hPPG of renal function deterioration.

Key words: Diabetes; Outcome measures; Amputation; Renal failure; Glycosylated hemoglobin; Postprandial hyperglycemia; 2-h postprandial glucose

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Core tip: Postprandial glucose level (2-h after major meal: Breakfast or lunch) is the cornerstone of laboratory test for diabetes to monitor glycemic control and prognosticate development or progression of diabetic

complications.

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INTRODUCTION

Lowering of blood glucose levels to normal or near normal levels in diabetes mellitus is a legitimate consideration. But why and which glycemic parameters are to follow in therapeutic strategy. There are three glycemic parameters to consider: Glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG) and 2-h postprandial blood glucose (2hPPG). The latter is obtained after a major meal or by oral glucose tolerance test. There are valid reports in the literature to suggest that lowering of blood glucose to normal levels with intensive insulin therapy will prevent microvascular complications^[1,2]. The pitfalls of previously published reports are that no information is provided which glycemic parameters were used to determine outcome. However FBG and HbA1c were most commonly used in outcome studies. There is no indication that 2hPPG was used to monitor prevention or progression of microvascular complications. Author orders FBG and 2hPPG in all patients with diabetes prior to their office visits. HbA1c is ordered quarterly which is permitted by health insurance. 2hPPG is the pivotal glycemic marker for author's studies. We initially observed that elevation of blood glucose ≥ 200 mg/dL (≥ 11.1 mmol/L) or even ≥ 50 mg/dL above FBG at 2-h postprandial (2hPP) period is associated with a discerning increase of serum creatinine (Scr) and a proportionate decrease of estimated glomerular filtration rate (eGFR) when sampled on the same day. The above renal function changes are less noticeable when 2hPPG is less than 200 mg/dL or difference between 2hPPG-FBG called dglucose is less than 50 mg/dL. Renal function change is easily noticeable when d glucose is above 100 mg/dL. Here is a brief example to that effect (Table 1).

Thus with delta (d) glucose of 121 mg/dL, increase of Scr and decrease of eGFR are very noticeable. He was being treated with metformin and Lisinopril. These medication were discontinued and he was placed on Glargine insulin (Lantus®), subcutaneously 15 units after breakfast and 15 units after dinner. He is also hypertensive; hypertension is kept under control with spironolactone and chlorthalidone. His 24 h Urine total protein was less than 111 mg. Close to two years later his blood pressure is 120/60 mmHg and his 2hPPG is decreased to 191 mg/dL (10.8 mmol/L) and renal function improved with decrease of Scr from 1.28 mg/dL to 1.17 mg/dL and increase of eGFR from 58 to 59 mL/min. In his subsequent office visit, renal function is stable or better.

The greatest pitfall in Advance Trial and many similar trials using oral anti diabetic agents is the renal outcome defined by diabetic nephropathy. This is an unmeaningful way to determine the renal outcome. Nephropathy defined clinically as the presence of microalbuminuria is a common complication of type 2 diabetes. There was no mention whether any renal function tests were done in the assessment of nephropathy in Advance trial or other clinical trials. Thus the serious deficiency in the assessment of significant risk reduction of nephropathy in Advance Trial is the lack of use of renal function test such as Scr or GFR in defining nephropathy^[3]. It should also be noted that many subjects with diabetes are also hypertensive; hence proteinuria can result from diabetic or hypertensive nephropathy. Thus, without kidney biopsy, it would be most difficult to determine cause of proteinuria whether due to diabetes or hypertension. Renal biopsy was seldom done in outcome studies.

In our studies, renal function test as already defined is the mirror of glycemic control. Our goal is to determine the staging of diabetes-related chronic kidney disease (CKD) by the available eGFR and treat them with a combination of insulin therapy to determine if progression of CKD into end stage renal disease can be halted.

In Advance trial, intensive glucose control had considerable renoprotective effects compared with standard control, with 21% risk reduction ratio for new or worsening nephropathy. The component of nephropathy that was clearly reduced was macroalbuminuria (risk reduction ratio of 30%; $0 < 0.001$).

The purpose of this editorial is to reveal which glycemic parameters are most predictive of renal function changes.

We already reported that delta (d) glucose (2hPPG-FBG) relates significantly to renal function changes. For every 100 mg/dL increase in dglucose, dScr increases by 0.11 mg/dL and d eGFR decreases by 3.73 mL/min. Thus dglucose is a stronger predictor of renal function than 2hPPG^[4].

Our current study is an expanded study and for a longer duration. All patients are treated with a combination of Glargine (Lantus®) or detemir insulin twice daily after breakfast and dinner and one of the regular or fast acting insulin before each meal and at bedtime. This is similar to what Frederick G. Banting used for his patients at University of Toronto^[5]. We have noted essentially no change in renal function in a period of 26 mo. Although FBG or 2hPPG did not decrease between the two periods, dglucose was significantly reduced from baseline 63.5 ± 68.1 to 36.6 ± 65.6 mg/dL. We have noted that as dglucose increases above 50 mg/dL (2.7 mmol/L), serum creatinine increases in step wise fashion^[6].

We have found in our previous study (unpublished) that although glucose levels did not decrease despite insulin therapy, renal function remained unchanged during the two periods of 14.2 mo. This indicates that insulin therapy is important for renal protection which

Table 1 A 78-year white male with established diabetes showed the following results in his first office visit

Glucose (mg/dL)		Scr (mg/dL)		eGFR (mL/min)	
F	2hPP	F	2hPP	F	2hPP
114 (6.3 mmol/L)	235 (13 mmol/L)	1.18	1.28	> 60	58
Dglucose (2hPPG-FBG) 121 mg/dL					

2hPP: 2-h postprandial; 2hPPG-FBG: 2-h postprandial blood glucose - fasting blood glucose; eGFR: Estimated glomerular filtration rate; Scr: Serum creatinine; F: Fasting.

may not be entirely dependent on tight glycemic control.

Hypertension control is achieved as always in author's patients by beta blockers, calcium channel blocker either alone or in combination, sympathetic inhibitor and in resistant cases, chlorthalidone. Angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) is excluded to reduce the risk of acute or chronic renal failure in diabetes^[6,7].

We have previously reported that use of ACEI/ARB drug is associated with high risk of recurrent attack of acute renal failure or development of CKD in diabetes^[7]. Other authors characterized acute kidney injury as a significant risk factor for CKD independent of other risk factors of progression in diabetes^[8].

The pearl of wisdom of this editorial is the first step to establish the diagnosis of diabetes. The most sensitive test to establish the diagnosis is to order a post challenge glucose 2-h after a major meal. Blood glucose greater than 200 mg/dL, establishes the diagnosis of diabetes^[9]. In order to monitor outcome measures in particular renal failure, it is important to order fasting and 2hPP basic metabolic panel which will provide glucose and renal function tests. The cornerstone of therapy of established diabetes is insulin therapy. Although evidence is tenuous for prevention of many of the complications of diabetes, author's studies confirm that insulin therapy is conducive to protection against renal failure and dialysis. Equally

important in author's studies is to exclude use of renin-angiotensin inhibitors drugs to treat diabetes as a complimentary measure of protection for renal failure.

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Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research

Benjamin M Leon, Thomas M Maddox

Benjamin M Leon, Department of Education, University of Colorado School of Medicine, Aurora, CO 80045, United States

Thomas M Maddox, Cardiology 111b, VA Eastern Colorado HCS, Denver, CO 80220, United States

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Correspondence to: Thomas M Maddox, MD, MSc, Cardiology 111b, VA Eastern Colorado HCS, 1055 Clermont St, Denver, CO 80220, United States. thomas.maddox@va.gov
Telephone: +1-303-3932826
Fax: +1-303-3935054

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Abstract

The incidence of diabetes mellitus (DM) continues to rise and has quickly become one of the most prevalent and costly chronic diseases worldwide. A close link exists between DM and cardiovascular disease (CVD), which is the most prevalent cause of morbidity and mortality in diabetic patients. Cardiovascular (CV) risk factors such as obesity, hypertension and dyslipidemia are common in patients with DM, placing them at increased risk for cardiac events. In addition, many studies have found biological mechanisms associated with DM that independently increase the risk of CVD in diabetic patients. Therefore, targeting CV risk factors in patients with DM is critical to minimize the long-term CV complications of the disease. This paper summarizes the relationship between diabetes and CVD, examines possible mechanisms of disease progression, discusses current treatment recommendations, and outlines future research directions.

Key words: Diabetes mellitus; Cardiovascular disease; Mechanism; Treatment

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Core tip: The link between diabetes and cardiovascular disease (CVD) is summarized and discussed in detail with a focus on growing prevalence, mechanisms of disease progression and current treatment of CVD in diabetic patients. Directions of future research are also examined.

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INTRODUCTION

The incidence of diabetes mellitus (DM) is increasing substantially worldwide. Over the past three decades, the global burden of DM has swelled from 30 million in 1985 to 382 million in 2014, with current trends indicating that these rates will only continue to rise^[1]. The latest estimates by the international diabetes federation project that 592 million (1 in 10 persons) worldwide will have DM by 2035^[2]. While the rates of both type 1 DM (T1DM) and T2DM are growing, T2DM has a disproportionately greater contribution to the rising prevalence of DM globally compared to T1DM^[1]. One consequence of the growing rates of DM is a considerable economic burden both for the patient and the healthcare system. In the United States, the total cost of DM averages \$2108/patient per year, which is nearly twice that of non-diabetic patients^[3]. The economic burden associated with DM is substantial both in terms of the direct costs of medical care as well as indirect costs of diminished productivity tied to diabetes related morbidity and mortality^[4]. The direct costs of DM are primarily attributed to both macrovascular and microvascular complications such as coronary artery disease, myocardial infarction, hypertension, peripheral vascular disease, retinopathy, end-stage renal disease and neuropathy^[3,4].

A close link exists between DM and cardiovascular disease (CVD). CVD is the most prevalent cause of mortality and morbidity in diabetic populations^[5]. CVD death rates in the United States are 1.7 times higher among adults (> 18 years) with DM than those without diagnosed DM, largely due to an increased risk of stroke and myocardial infarction (MI)^[6]. This increased risk of CVD mortality in diabetic patients is found in both men and women. The relative risk for CVD morbidity and mortality in adults with diabetes ranges from 1 to 3 in men and from 2 to 5 in women compared to those without DM^[7].

Proper control and treatment of DM is critical as both the prevalence and economic burden of the disease continue to mount. As CVD is the most prevalent cause of mortality and morbidity in patients with DM, a primary goal of diabetes treatment should be to improve the cardiovascular (CV) risk of diabetic patients. However, one challenge associated with treating DM and reducing CV events is the complex and multifaceted nature of the relationship linking DM to CVD. CV risk factors including obesity, hypertension and dyslipidemia are common in patients with DM, particularly those with T2DM. In addition, studies have reported that several factors including increased oxidative stress, increased coagulability, endothelial dysfunction and autonomic neuropathy are often present in patients with DM and

may directly contribute to the development of CVD^[5]. Collectively, the high rates of CV risk factors and direct biological effects of diabetes on the CV system place diabetic patients at increased risk of developing CVD, and contribute to the increased prevalence of MI, revascularization, stroke and CHF^[5,8]. Due to the complexity and numerous mechanisms linking DM to CVD, it is crucial to focus treatment to what will have the greatest clinical impact on improving CV outcomes. This paper examines the mechanisms linking DM to CVD as well as current treatment recommendations and future research in diabetes management.

CV RISK FACTORS AND CVD

Obesity

Obesity is common in patients with DM, particularly T2DM, and is associated with an increased risk of CVD. One possible mechanism linking DM and obesity with subsequent CVD is low-grade inflammation^[9]. DM and insulin resistance are associated with the overexpression of many cytokines by adipose tissue including tumor necrosis factor- α , interleukin (IL)-1, IL-6, leptin, resistin MCP-1, PAI-1, fibrinogen and angiotensin^[10]. The overexpression of these cytokines contributes to increased inflammation and lipid accumulation, which have a deleterious effect on blood vessels and can lead to the development of endothelial dysfunction, MI and cardiomyopathy (CMP)^[5,11-14]. Diabetic patients also have increased amounts of C-reactive protein (CRP), which may contribute to endothelial dysfunction. Many studies have demonstrated that CRP impairs endothelial production of nitric oxide (NO) and prostacyclin, which are vital to vessel compliance. CRP has also been shown to increase the uptake of oxidized low-density lipoprotein (LDL) in coronary vasculature walls, which can contribute to endothelial dysfunction as well as the development of atherosclerotic plaques^[14]. Patients with DM also have decreased adiponectin production, which may result in diminished endothelial function^[10]. Adiponectin helps limit endothelial dysfunction by increasing NO production and reducing the expression of adhesion molecules. Adiponectin is also protective in the atherosclerotic process by inhibiting LDL oxidation^[15]. This increase in atherosclerotic plaque can place diabetic patients at a heightened risk of MI. In particular, increased levels in the inflammatory cytokine IL-1, as seen in patients with DM, can contribute to the destabilization of atheromatous plaques and subsequent MI^[11]. Insulin resistance is also associated with an elevation of plasma free fatty acids, leading to increases in muscular triglycerides stores, hepatic glucose production, and increased insulin production in patients with T2DM^[16]. Insulin resistance has also been linked to CMP in diabetics *via* cardiomyocyte hypertrophy and contractile dysfunction^[16,17].

Hypertension

Hypertension is very common among patients with

T1DM and T2DM, with prevalence rates of 30% and 60%, respectively^[5]. Hypertension among diabetic patients is closely tied to the development of diabetic nephropathy (DN)^[18]. With DN, renal cells are stimulated by hyperglycemia, leading to the production of humoral mediators, cytokines, and growth factors. The production of these factors is often responsible for structural alterations seen in the glomeruli of diabetic patients including hyaline arteriosclerosis (primarily of the efferent arteriole), increased collagen deposition of the extracellular matrix, and increased permeability of the glomerular basement membrane^[19]. These structural changes increase filtration pressure and often lead to microalbuminemia with a compensatory activation of the renin-angiotensin system (RAAS). Chronic activation of the RAAS often progresses to hypertension, placing added stress on the glomeruli and causing additional damage to the nephrons of diabetic patients. If left untreated, DN can progress to a nephrotic syndrome, characterized by proteinuria, a hypercoagulable state (due to loss of ATIII) and hyperlipidemia, which may contribute to the increased risk of CVD seen in diabetic patients with renal dysfunction^[20,21].

Dyslipidemia

Diabetic patients are at increased risk of developing dyslipidemia^[22]. One mechanism underlying this connection is increased free fatty-acid release present in insulin-resistant fat cells. High levels of free-fatty acids promote triglyceride production, which in turn stimulates the secretion of apolipoprotein B (ApoB) and very LDL (VLDL) cholesterol. High levels of ApoB and VLDL have both been tied to increased risk of CVD^[23-26]. In addition to high ApoB and VLDL, hyperinsulinemia is associated with low high-density lipoprotein (HDL) cholesterol levels^[27]. Hyperglycemia may also negatively impact lipoproteins (particularly LDL and VLDL) through increased glycosylation and oxidation, decreasing vascular compliance and facilitating the development of aggressive atherosclerosis^[28]. High circulating FFA's and triglycerides, increased stimulation of ApoB and VLDL cholesterol, decreased HDL levels and lipoprotein modification have all been appreciated in patients with DM and likely contributes to the high prevalence of CVD in diabetic patients.

Diabetic cardiomyopathy

DM appears to contribute directly to the development of CMP, rather than solely *via* coronary atherosclerosis and hypertension^[29]. This diabetic CMP has been described in many noninvasive studies and includes changes that occur in LV structure and cardiac function of diabetics. Specifically, diabetics tend to have greater cardiac mass, particularly LV mass, than those without DM^[30,31]. This may be related to an increased adipocyte release of cytokines such as leptin and resistin which have hypertrophic effects on cardiomyocytes^[12,13]. One study looking at a multi-ethnic population found that the likelihood of having LV mass that exceeds the 75th

percentile is greater in patients with T2DM, even after adjusting for covariates^[32]. Patients with DM also tend to have a slightly diminished diastolic function compared to nondiabetics^[33-35]. One possible mechanism could be that increased triglyceride synthesis in patients with DM leads to increased myocardial triglyceride content^[36]. Increased cardiac triglyceride accumulation is associated with lipotoxicity and altered calcium hemostasis in myocardium, both of which negatively impact diastolic function^[37-39]. This could help explain the finding that 40%-75% of individuals with DM and no signs of overt coronary artery disease (CAD) suffer from diastolic dysfunction^[34,35]. Subtle abnormalities in systolic function have also been observed in patients with DM using tissue Doppler imaging and Doppler strain analysis of peak systolic velocity^[40-44]. This systolic dysfunction may be related to impaired myocardial sympathetic innervation and impaired contractile reserve^[45]. In addition, interstitial fibrosis with increased collagen deposition has been observed in patients with DM and may negatively contribute to the diminished cardiac function seen in diabetics^[46]. It is likely that many of the mechanisms that contribute to reductions in systolic and diastolic function seen in diabetic patients also place them at an increased risk of heart failure (HF)^[47,48]. The prevalence of HF, particularly heart failure and preserved ejection fraction, is higher in diabetic patients (16%-31%) than the general population (4%-6%)^[49]. While some of the difference may be accounted for by traditional CV risk factors, DM may independently alter cardiac structure and function by promoting hypertrophy and fibrosis^[50].

Cardiovascular autonomic neuropathy

Cardiovascular autonomic neuropathy (CAN) is common among patients with DM and is correlated with an increased 5-year mortality rate from CVD^[51]. The clinical manifestations of CAN are resting tachycardia, postural hypotension, exercise intolerance, abnormal coronary vasomotor regulation, increased QT interval, and perioperative instability. Collectively, the clinical manifestations of CAN are related to an increased risk of renal disease, stroke, CVD and sudden death^[52]. The development and progression of CAN is likely related to dysregulation of the autonomic nervous system (ANS) with increased sympathetic activity and elevated inflammatory markers. As the ANS is responsible for maintaining the activity of the sinus node, end diastolic volume, end systolic volume and systemic vascular resistance, ANS dysfunction can lead to arterial stiffness, left ventricular hypertrophy and ventricular diastolic dysfunction^[53]. Incidence of CAN increases with age and inadequate glycemic control, which places patients with DM at higher risk of developing both CAN and CVD^[54].

Myocardial infarction and DM

Diabetes is a major risk factor for the development of CAD with a higher incidence of MI in patients with DM than those without^[55,56]. In addition, following a MI, diabetic patients have higher rates of morbidity,

mortality and re-infarction than non-diabetics, with one-year mortality rates of nearly 50%^[57]. Although the exact pathophysiology of CAD progression in patients with DM has not yet been determined, the most recent studies postulate that the underlying atherosclerotic process is similar between those with and without DM. It is thought that the higher incidence of myocardial infarction in patients with DM is attributable to increased coagulability^[58]. Many studies have found that diabetics have increased expression of glycoprotein II B/III A receptors and vWF, which are responsible for platelet activation^[59,60]. Patients with DM also have increased plasminogen activator inhibitor type 1 which could decrease fibrinolysis, increase thrombus formation and accelerate plaque formation^[61]. Finally, diabetic patients also tend to have decreased circulating anti-coagulants such as protein c and antithrombin III due in a large part to the proteinuria present with DN^[62]. Collectively, these factors place patients with DM in a prothrombotic and procoagulant state, which may account for the higher rates of MI seen in diabetic patients.

Silent myocardial ischemia may also contribute to the higher rates of MI seen in diabetic patients. Ischemia and subsequent angina often serves as an early warning system to patients developing obstructive CAD^[63]. However, those with silent ischemia are often asymptomatic and diagnosed later into the progression of CAD, which is associated with higher rates of MI-related mortality and morbidity^[64]. Silent ischemia is far more prevalent in patients with DM (10%-20%) than those without DM (1%-4%). This disparity may be responsible for the observation seen in some angiographic studies where CAD was usually more advanced at the time of diagnosis in diabetic patients^[65,66]. Diabetic neuropathy is one factor that may explain the increased incidence of silent ischemia in patients with DM^[67,68].

TREATMENT

As CVD is the most prevalent cause of mortality and morbidity in patients with DM, effective treatment is critical to lower the subsequent risk of CV events, particularly MI, CAD, stroke and CHF in diabetics. Suboptimal glycemic control, obesity, hypertension, dyslipidemia and autonomic dysfunction are common CV risk factors among diabetic patients, placing them at heightened risk of CV complications. Therapy that is targeted to modify these risk factors can improve CV outcomes, but this can be a challenging to achieve. The guidelines pertaining to these risk factors typically vary from the guidelines for non-diabetic patients and the recommendations often change or differ depending on what organization publishes them. In addition, the research on how these different risk factors affect the CV risk profile of diabetics can be unclear, and at times, contradictory. The purpose of this section is to provide the most recent guidelines for the treatment of glycemic control, hypertension, dyslipidemia and autonomic dysfunction in patients with DM, and also describe the

research that pertains to each of these topics.

GLYCEMIC CONTROL

As many studies have linked poor glycemic control to worse CV outcomes, current treatment recommendations for patients with DM place a heavy emphasis on closely monitoring and controlling glycemic levels in an effort to improve cardiac outcomes. The exact glycemic level that should be targeted for diabetics, however, is controversial and varies depending on which organization is making the guideline. For example, the current recommendation by the American Association of Clinical Endocrinologists Guidelines has a goal hemoglobin A1c (HbA1c) of less than or equal to 6.5%, and encourages providers to treat patients with an A1c value greater than 6.5% with a combination of lifestyle modification, weight loss and pharmacological agents^[69]. The ACC/AHA have a slightly more relaxed A1c goal of less than 7% for non-pregnant patients with T1DM or T2DM in order to reduce the risk of microvascular or macrovascular complications. In addition, ACC/AHA also qualifies their recommendation by including a recommendation that an A1c goal of greater than 7 may be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbidities, or for those with long-standing diabetes. The recommendation also states that an A1c goal lower than the general goal of less than 7.0% may be beneficial for certain diabetic patient populations including those with a short duration of diabetes, long life expectancy, and no CVD^[70]. The VA/DoD guidelines use a more individualized algorithm for determining an appropriate A1c goal for diabetic patients. This guideline range from a target A1c of < 7 to < 9 depending on the patient's current health status, comorbid conditions, life expectancy, risk of hypoglycemia and duration of diabetes status^[71].

CV OUTCOMES

There have been many studies that have investigated the effect of intensive treatment of hyperglycemia on CV outcomes in patients with diabetes. The UKPDS trial was one of the first multi-center, randomized control trials to investigate the effect of intensive glycemic control in patients with recently diagnosed T2DM. Patients were either randomized to "conventional" or "intensive" glycemia-lowering therapy and were followed for 10 years. The intensive glycemic group reduced HbA1c by 11% over 10 years (median 7.0%) as compared to the group treated with conventional therapy who did not have a significant change in their HbA1c (median 7.9%). The primary effect seen in the group with tighter glycemic control was a 12% reduction in all diabetes-related endpoints and a 25% reduction in microvascular disease (primarily through decreased retinopathy). In addition, the intensive therapy group trended towards a

decrease in macrovascular disease although it was not statistically significant^[72].

Another large study that investigated the effect of tight glycemic control in patients with T2DM was the VADT trial. The population for this study consisted primarily of older (mean age 60.4 years) adult men with poorly controlled T2DM (average HbA1c of 9.4%) and an average duration of disease of 11.5 years. The subjects were randomized to either "intensive" or "conventional" glycemia-lowering therapy and were followed for 5.6 years. The group with the tighter glycemic control did have a significantly greater decrease in A1c levels over the course of the study (6.9% vs 8.4%), but there was no significant decrease in MI or all cause mortality in the "intensive" therapy group as compared to the "conventional" therapy group^[73].

The ADVANCE trial placed a focus on the vascular effects of intensive glycemic therapy in adults with T2DM. This large multi-center randomized control trial recruited T2DM patients with a history of major macrovascular or microvascular disease from 215 collaborating centers in 20 countries. Subjects were randomized to either an "intensive" or "standard" glycemia-lowering strategy and followed for 5 years. The intensive glycemic therapy group was treated to an HbA1c of less than or equal to 6.5%. The group randomized to the tighter glycemic control did have a significantly greater reduction in HbA1c (6.5% vs 7.3%) and experienced a 23% reduction in microvascular events (primarily nephropathy). However, there was no difference between the groups in MI or all cause mortality and the group with 'intensive' therapy had increased rates of severe hypoglycemia hospitalization^[74].

The ACCORD trial was conducted concurrently to the ADVANCE trial and focused primarily on whether intensive glycemic control reduced to risk of CV events. This multi-center randomized control trial investigated if very tight glycemic control (less than or equal to an HbA1c of 6%) had lower rates of nonfatal MI, nonfatal stroke and CV death than standard glycemic control (HbA1c of 7%-7.9%) in older adults. The subjects were followed for an average of 3.4 years and the group with the tighter glycemic control did achieve a significantly lower HbA1c than those with standard treatment (7.3% vs 6.5%). The intensive glycemic control group had slightly lower rates of nonfatal MI, but after 3.7 years the trial was stopped early because the intensive treatment group had increased rates of all-cause and CV mortality. The group with tight glycemic control also had increased weight gain, and risk of hypoglycemia as seen in the ADVANCE trial^[75].

DCCT and the long-term follow-up trial EDIC investigated how strict glycemic control with intensive therapy effected CV outcomes in patients with T1DM. These trials randomized young (ages 13-39 years) patients with T1DM to either "intensive" or "conventional" glycemic therapy with an HbA1c goal of 7% in the group for those in the "intensive" treatment group. The primary finding of the DCCT trial was that after 10 years of follow-up, the

group with strict glycemic control had a 70% decrease in the number of microvascular complications, particularly retinopathy. In addition, the long-term follow-up study, EDIC, found a 42% reduction in CV events in the group with intensive glycemic treatment as compared to the conventional glycemic therapy^[18,76].

While it does appear that a link exists between glycemic control and CV outcomes in diabetic patients, the findings thus far on the effect of tight glycemic control on CVD are conflicting. Current studies fail to show that intensive glycemic control (HbA1c \leq 6.5%) has a significant CV benefit compared to standard glycemic control targets (HbA1c of 7%-7.9%) in patients with T2DM. While there may be a small reduction in the number of microvascular events in T2D patients with the tighter glycemic control, there does not seem to be a sizeable benefit in the rates of all-cause and CV-specific mortality. Furthermore, very tight glycemic control (HbA1c \leq 6%), as seen in the ACCORD trial, may place patients at additional risk of hypoglycemia, weight gain and all cause mortality^[75]. In patients with T1DM, tighter glycemic control does appear to be beneficial. The DCCT and EDIC trials do suggest that intensive glycemic therapy (goal HbA1c \geq 7%) can help reduce rates of microvascular and macrovascular disease in T1D^[18,76].

One potential interpretation of the studies thus far is that the concurrent CV risk factors present in diabetics may overwhelm any benefit that intensive treatment of hyperglycemia can provide in reducing risk. Thus, diabetic patients who achieve tighter glycemic control earlier during their disease course and prior to the development of other CV risk factors may see the greatest benefit from more intensive therapy in terms of CV outcomes. For this reason, many of the new recommendations look to tailor A1c goals to the individual patient as opposed to a single A1c cutoff for all diabetic patients. The ACC/AHA and VA/DoD, for example, adjust their glycemic goals based on factors such as age, years with the disease and CV risk^[70,71]. While further studies are needed to determine what the best glycemic treatment goal is for these different patient populations, adjusting the target A1c depending on the individual's current level of CVD risk may provide benefit to diabetic patients.

Obesity

Obesity is a common comorbidity of DM, particularly T2DM, and is linked with higher rates of CV morbidity and mortality. Thus, current treatment recommendations encourage weight loss in overweight and obese patients with DM to improve their CV risk profile and decrease the risk of CVD. The recommendation is for 5% weight loss over 4 years in diabetic patients that are overweight or obese. A "moderate" amount of evidence suggests that 5% weight loss by lifestyle intervention is associated with an increase in HDL-c, a reduction in triglycerides and a decrease in newly prescribed lipid lowering medications in diabetic patients. In addition, there is a "high" level of evidence suggesting that orlistat results in

2-3 kg of weight loss in overweight and obese diabetic patients at 1 and 2 years, and is associated with greater reductions in fasting blood glucose and HbA1c. These recommendations were graded as high, moderate, or low on the basis of scientific methodology, scientific strength, and consistency of results^[77].

As obesity is a major risk factor both for CVD and T2DM, many studies have investigated the efficacy of weight loss in reducing the development and severity of DM. Some studies have focused on body weight reduction in pre-diabetic patients in order to decrease the incidence of subsequent DM. Of note, the diabetes prevention program (DPP) and finnish diabetes prevention studies evaluated the effect of behavior modification on weight loss and consequent risk of developing diabetes in pre-diabetic adults. Both studies yielded similar results in that those randomized to the lifestyle intervention group had significantly greater weight loss and reduced risk of developing diabetes as compared to the control group^[78,79]. Other studies have looked at methods for attaining weight loss and improving the CV risk profile of patients who are already diabetic. A variety of techniques including intensive lifestyle intervention, weight loss medications and bariatric surgery were effective in achieving weight loss and improving the CV risk profile of diabetic patients through improved glycemic control, blood pressure and cholesterol levels^[80-82].

Although many studies have shown that weight loss can be achieved in diabetic patients, there is mixed evidence as to whether weight loss in these patients actually reduces subsequent CV morbidity and mortality. Thus far, there has been mixed evidence if modest weight loss in patients with DM does improve their CV risk. While the SCOUT trial found that modest weight loss could improve 5-year CV mortality rates among diabetic patients, the Look AHEAD trial did not find that weight loss had any effect on CV mortality, MI, stroke, or angina hospitalization after 9.6 years of follow-up^[83,84].

The current recommendation for overweight and obese patients with DM is a goal weight loss of 5%^[77]. Studies thus far have demonstrated that this goal is attainable both in pre-diabetic and diabetic patients through a variety of techniques including intensive behavioral modification therapy, pharmacological agents and bariatric surgery. In addition, all of these methods of weight loss appear to either decrease the rates of incident DM in pre-diabetic patients, or improve the CV risk profile of diabetic patients^[78-82]. However, it is unclear whether modest weight loss in diabetic patients translates to a decrease in CVD^[83,84].

It is possible that the CV risk profile is too high in older adults with DM for modest weight loss to make a significant improvement in CV outcomes. It might be more advantageous to focus obesity treatment efforts on pre-diabetics before they develop DM. Programs such as the DPP have demonstrated that weight loss can decrease the rate of incident diabetes, but further

research is needed to determine if modest weight loss in pre-diabetic patients results in improved CV morbidity and mortality^[78]. It is also possible that while modest weight loss does seem to improve the CV risk profile of patients with DM, even greater weight loss is necessary to see more definitive improvements in the rates of CV events. Further investigation into the effects of weight loss greater than 5% on CVD in diabetic patients may help identify the existence of a dose effect with weight loss and CV health.

Hypertension

Since hypertension is a common comorbidity of patients with DM and a major risk factor for CVD, the current treatment recommendations strongly encourage providers to lower BP in hypertensive diabetics. There are many studies that have investigated the effect of lowering blood pressure in patients with diabetes on CV outcomes. The UKPDS 38 trial examined the effect of tight control of blood pressure control (< 150/85) compared to less tight control (< 180/105) on macrovascular and microvascular complications in patients with T2DM. After 9 years follow-up, mean blood pressure was significantly lower in the tightly controlled BP group (144/82 mmHg) compared to the patients in the less tightly controlled group (154/87 mmHg). In addition, the group with tighter BP control had a 34% reduction in macrovascular disease risk (myocardial infarction, sudden death, stroke, and peripheral vascular disease) and a 37% reduction in risk of microvascular disease (retinopathy requiring photocoagulation, vitreous haemorrhage, and fatal or non-fatal renal failure) compared with the less tightly controlled BP group^[85].

While many studies have shown that lowering BP in diabetics does improve CV outcomes, the ACCORD-BP trial investigated the effect of intensive BP control (systolic BP < 120 mmHg) compared to standard BP control (systolic BP < 140 mmHg) on the risk of fatal or nonfatal major CV events in patients with T2DM. After 4.7 years of follow-up, the group with intensive BP control did not have a reduction in fatal and nonfatal major CV events as compared to the standard BP control group (1.87% vs 2.09% per year). In addition, the intensive BP group had increased adverse events including hypotension, syncope, bradycardia or arrhythmia, hyperkalemia, angioedema and renal failure^[86].

Given the results of these trials, recent treatment recommendations indicate that, pharmacologic treatment should be initiated at a SBP of > 140 mmHg or a DBP of > 90 mmHg for diabetic adults between 18 and 60 years of age. For patients older than 60, the threshold to initiate treatment is a SBP of < 150 mmHg or a DBP of < 90 mmHg. The recommendation on the type of pharmacological therapy that should be used varies in the general nonblack vs black population. For nonblack patients with DM and hypertension, initial treatment should include a thiazide-type diuretic, calcium channel blocker (CCB), angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker

(ARB). For black patients with DM and hypertension, the initial treatment should include a thiazide-type diuretic or a CCB. In addition, hypertensive patients with DM and CKD should be treated with an ACE inhibitor or an ARB to improve kidney outcomes^[87]. While different antihypertensive agents used to treat hypertension have varying metabolic effects, many studies, including the ALLHAT trial, found no significant difference in the risk of coronary heart disease, nonfatal myocardial infarction, total mortality, or other clinical complications attributable to the initial antihypertensive drug therapy used to treat diabetic patients^[88,89]. This would suggest that metabolic differences between the various antihypertensive agents do not play a major role in the subsequent development of CVD in patients with DM. It should be noted that these recommendations have been controversial and several authors have argued that the guideline is too relaxed in the treatment of certain at-risk groups including African Americans, women and the elderly based on previous studies evaluating blood pressure control and subsequent CVD in these populations^[90]. There is likely a therapeutic BP range that provides diabetic patients with a lower CV risk but also protects them from adverse events associated with hypotension. Whether the new guidelines, particularly with the increased systolic BP threshold in adults over 60 years, match this therapeutic BP range is yet to be determined. There is also little evidence as to what the proper treatment range should be for different age groups. In addition, hypertension in different racial subgroups may have different effects on CV health. Further research is needed to investigate the ideal BP range for adults of different age groups as well as different racial groups.

Dyslipidemia

Dyslipidemia is both common in patients with DM and associated with increased risk of CVD^[91,92]. Health providers are encouraged to identify and aggressively treat patients with dyslipidemia to help diminish their risk of subsequent CV events. The current recommendation for treating dyslipidemia in diabetic patients varies by age and is in line with recognition that treatment with fixed-dose statins, rather to specific LDL target levels, is the validated approach from clinical trials. Accordingly, diabetic patients who are under the age of 40 are recommended to take a high-intensity statin if they have clinical evidence of atherosclerotic CVD or a LDL-c greater than 189 mg/dL. All diabetic patients over the age of 40 are encouraged to begin statin therapy. Patients over 40 with an estimated 10-year ASCVD risk greater than 7.5% are treated with a high-intensity statin, and patients with a 10-year ASCVD risk less than 7.5% are treated with a moderate-intensity statin^[93].

There have been many studies conducted to determine the effect of treating dyslipidemia in diabetic patients as a means to lower CV risk. The CARDS study was the first multicenter randomized controlled trial to evaluate statin therapy prospectively in patients with T2DM. Adult patients with T2DM were randomized to

either receive a placebo or 10 mg/d of atorvastatin. The median follow-up time was 3.9 years and the group treated with atorvastatin had an average 26% reduction in total cholesterol and a 40% reduction in LDL-c. In addition, the statin therapy group had a 37% reduction in CV events, a 27% reduction in all-cause mortality and a 48% reduction in stroke as compared to the group treated with the placebo. The CARDS trial was stopped early to due the significant benefit demonstrated with statin therapy^[94].

After the CARDS trial found that statin therapy provided a significant CV benefit to diabetic patients, the TNT trial examined the effect of high-dose statins on CAD mortality, non-fatal MI, and fatal or nonfatal stroke in diabetic patients with T2DM. Adult patients with T2DM were randomized to receive either a high dose (80 mg/d) or low dose (10 mg/d) statin and followed on average for 4.9 years. The high dose statin group achieved a greater reduction in LDL-c (77 mg/dL vs 101 mg/dL) and had a greater reduction in combined CAD mortality, non-fatal MI, or fatal or nonfatal stroke (8.7% vs 10.9%) compared to the lower dose group. However, it was noted that the higher dose group did have a higher rate of adverse events (myalgia, persistent elevation in alanine aminotransferase, aspartate aminotransferase, or rhabdomyolysis)^[95].

As many studies had demonstrated that statins, particularly high-dose statins, had CV benefit in diabetic patients, the 4D study examined the effect of statins in diabetic patients receiving hemodialysis. In the 4D trial, diabetic patients receiving hemodialysis were randomly assigned either 20 mg of atorvastatin per day or a placebo. The purpose of the study was to determine if a low-dose statin in diabetic patients with end stage renal disease lowered the rates of death from cardiac causes, nonfatal myocardial infarction, and stroke as compared to the placebo group. The group randomized to the statin therapy did have a significant reduction in their LDL-c compared to the placebo group (-42.0% vs -1.3%), but there was no significant difference between the groups in CV outcomes after 3.96 years of follow-up. In addition, there were significantly more cases of fatal stroke in the statin therapy group than those treated with a placebo^[96].

While the previous studies had focused on reducing cholesterol in diabetic patients using statin therapy, other research groups have investigated the effect of non-statin lipid-lowering therapies on CVD in diabetic patients. For example, the FIELD trial evaluated if lowering cholesterol *via* fenofibrate therapy could improve CV outcomes in patients with DM. In the FIELD trial, diabetic patients (mean age 62 years; 63% men) were randomized to either receive a fenofibrate (200 mg/d) or a placebo and then assessed for subsequent rates of fatal coronary heart disease (CHD) or nonfatal MIs. While the group randomized to the fenofibrate therapy did reduce their cholesterol compared to the placebo group at 4 mo (total cholesterol, LDL-cholesterol, and triglycerides by 11%, 12%, and 29%, respectively),

the differences decreased between the groups as the trial continued due in a large part to patients starting additional cholesterol lowering therapies outside of the study. After a median of 5 years, the group randomized to the fenofibrate group had a combined 11% reduction in fatal CHD or nonfatal MIs, but this difference was non-significant. The fenofibrate group did however have a statistically significant reduction (24%) in nonfatal MI's compared to the placebo group^[97]. In addition, since HDL has been identified in many large prospective studies to be associated with improved CV health, some research groups have investigated whether raising HDL through pharmaceutical agents reduces the risk of CV events. The HATS trial was the first to investigate the effect of increasing HDL with Niacin therapy and generated promising results on improving CV outcomes in adult patients (16% with DM). After 38 mo of follow-up, the group randomized to the niacin therapy did have a significant increase in HDL and patients with T2DM had a 13% decrease in absolute risk of CV disease^[98]. Recently however, the AIM-HIGH trial found no significant clinical benefit in adding Niacin therapy to patients with atherosclerotic CVD as compared to a placebo. The trial was stopped after 3 years due to lack of efficacy; the group randomized to the niacin therapy (34% with DM) did not have a significant reduction in composite coronary heart disease, nonfatal myocardial infarction, ischemic stroke, hospitalization for an acute coronary syndrome, or symptom-driven coronary or cerebral revascularization (16.4% vs 16.2%) despite significant improvements in HDL (25% vs 11.8%). These findings were similar between diabetics and nondiabetics^[99].

Dyslipidemia is prevalent among diabetic patients and a major risk factor for CVD^[91,92]. Current treatment recommendations encourage providers to lower lipid levels in diabetic patients, primarily through the use of statins, with a dose dependent on the patient's level of risk. Some trials have also investigated if additional CV benefit can be achieved in patients with DM by combining a statin with other lipid-lowering therapies. For example, the IMPROVE-IT trial found that the combination of ezetimibe (a cholesterol absorption inhibitor) with simvastatin was superior to simvastatin alone in reducing CV events for diabetic patients with acute coronary syndrome^[100]. The evidence thus far suggests that statin therapy in patients with DM is advantageous for CV health and that higher doses, as well as combined lipid-lowering therapy, can provide additional CV protection^[93]. While some meta-analyses have suggested that statin therapy could be associated with increased incidence of DM, the absolute benefit of the therapy in diabetic patients largely outweighs the risk^[101]. Other lipid lowering agents, such as fenofibrates, have not demonstrated the same level of efficacy and reductions in CV events as statins^[97]. Pharmacological agents that raise HDL also appear to provide minimal, if any, CV benefit^[98,99]. Further studies are necessary to better understand the role of HDL in CV health.

CAN

CAN is a common complication of diabetes and places patients with DM at increased risk of CV related morbidity and mortality. The autonomic dysfunction commonly found in diabetic patients is associated with a high risk of cardiac arrhythmias and sudden death, as well as other serious CV sequelae including silent myocardial ischemia, diabetic cardiomyopathy, stroke, and both intraoperative and perioperative CV instability. Some of the most common clinical manifestations of CAN include heart rate variability (variability in the instantaneous beat-to-beat intervals), resting tachycardia, exercise intolerance, orthostatic hypotension and abnormal blood pressure regulation^[102].

Early treatment of autonomic dysfunction can slow the pathogenesis and complications of CAN^[102]. Some studies have shown that tight glycemic control may play an important role in reducing the incidence of CAN in patients with DM. For example, the DCCT demonstrated that patients with better glycemic control, as measured by HbA1c, had significantly lower risk of developing autonomic dysfunction according to a CAN index^[103]. While the effect of glycemic control on CAN in patients with T2DM have been less conclusive, some trials, including the Steno-2 study found that improving glucose control and other CV risk factors reduced the prevalence of CAN in T2DM patients^[104]. Lifestyle interventions that focus on improving exercise endurance and promote weight loss have also improved autonomic dysfunction. Pharmacological therapy including ACE inhibitors, angiotensin receptor blockers and aldose reductase inhibitors also appear to help slow the progression of CAN^[54]. In addition, IGF-1, ACE inhibitors and beta-blockers appear to be beneficial in the treatment of diabetic cardiomyopathy by slowing ventricular hypertrophy and normalizing the calcium homeostasis in diabetic cardiomyocytes^[105-109]. Further studies are necessary, however, to validate what the best pharmacological treatment is for diabetic patients with CAN.

FUTURE DIRECTIONS IN THE TREATMENT OF DM

While there have been many trials that have helped further the understanding of DM as it relates to CVD, further research is required to better identify and quantify CV risk in patients with DM. Determining how glycemic control relates to CVD is one another area where additional research is needed. There is some evidence that improved glycemic control does in fact improve CV outcomes patients with DM^[72,73]. One study even found that HbA1c in non-diabetic patients is an independent predictor of coronary artery disease and its severity which would suggest that glycemic control is critical to managing CV health in all patient populations^[110]. While this observational trial suggests an independent association may exist between glycemic

levels and CVD, large randomized control trials such as ADVANCE and ACCORD have shown that the effect of tight glycemic control on subsequent CVD is modest and largely attributable to coexistent traditional risk factors^[73-75,110].

One possible explanation for the conflicting results surrounding the relationship between glycemic control and CVD is due to poor measurement tools. For example, fasting plasma glucose (FPG) is often used as a measure of glycemia, but studies have found a day-to-day within-person variance of 12%-15% in FPG levels of diabetic patients^[111]. While the day-to-day within-person variance for HbA1c is far better (< 2%), there is evidence that HbA1c does not accurately reflect glycemic control due to biological variations and differences in RBC survival among patients^[111-113]. If glycemic control does matter, properly measuring glycemia and correlating it to CV risk is essential in order to set clinically meaningful goals for patients with DM.

The duration and onset of improved glycemic control may also contribute to the progression and severity of CVD. The UKPDS demonstrated that tight glycemic control was associated with reductions in CV outcomes in middle-aged adults (median 54 years) who were recently diagnosed with DM^[72]. Conversely, the ADVANCE and ACCORD trials reported that tight glycemic control may not provide any reduction in subsequent CVD and may actually be harmful in patients that were slightly older and with a longer duration of diabetes^[74,75]. This might reveal that treating hyperglycemia aggressively in high-risk patients with longer-standing DM is too late to have a clinically significant impact, and that earlier, aggressive treatment among patients shortly after DM diagnosis may be more beneficial. More studies are needed to better understand the relationship between glycemic control and the development of CVD and determine if the onset and duration of treatment matters in the reduction of CV events in patients with DM.

Further research is also necessary to determine what the best treatment is to decrease the risk and severity of cardiomyopathy and CAN in patients with DM. Many studies have demonstrated that autonomic dysfunction and diabetic cardiomyopathy are disease processes that are not only common in patients with DM, but also place them at increased risk of subsequent CV complications^[102]. Lifestyle modification, tighter glycemic control and pharmacological agents appear to provide some benefit in slowing the progression of CAN and diabetic cardiomyopathy^[54,102-109]. However, few studies have investigated what specific therapy is most effective in treating these conditions, as well as what might be done to prevent the development of these disease processes altogether.

Additional research is also needed to better understand how traditional CV risk factors including dyslipidemia, obesity and blood pressure should be monitored and managed in diabetic patients. For example, combination therapy may be the best way to treat

dyslipidemia, contrary to the current recommendation that focuses primarily on statin mono-therapy. More studies like IMPROVE-IT could help determine what therapy is most effective to manage dyslipidemia in diabetic patients^[100]. In addition, the role of HDL on CV health is complicated, and further investigation is necessary to determine if pharmacological agents designed to increase HDL can provide clinical benefit in diabetic patients. The effect of weight loss in patients with DM is also somewhat unclear as to if, and how much, weight loss is necessary to achieve clinically significant improvements in CV outcomes. Five percent weight loss may not be sufficient for diabetic patients with other CV risk factors and comorbidities. Further studies are needed to determine what amount of weight loss is needed to reach that weight loss goal. Finally, follow-up regarding the new blood pressure guidelines, particularly in adults over 60 years who now fall under the higher systolic BP threshold, will need to be closely monitored moving forward.

CONCLUSION

As the prevalence of DM continues to rise, associated CVD - through both traditional CV risk factors and the direct effects of DM on CVD - can also be expected to rise. Accordingly, proper control and treatment of DM, along with aggressive treatment of associated CV risk factors is central to curbing the growing prevalence and progression of DM and CVD. Additional research is needed to better understand the disease process and its effects on CV health in order to improve medical management and CV outcomes in diabetic patients.

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Editorial Board Member of *World Journal of Diabetes*, Charlotte Brøns, MSc, PhD, Steno Diabetes Center, Niels Steensens Vej 1, 2820 Gentofte, Denmark

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World Journal of Diabetes

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

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Erythropoietin and diabetes mellitus

Kenneth Maiese

Kenneth Maiese, Cellular and Molecular Signaling, Newark, NJ 07101, United States

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Correspondence to: Kenneth Maiese, MD, Cellular and Molecular Signaling, 125 Main Street, Newark, NJ 07101, United States. wntin75@yahoo.com

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Abstract

Erythropoietin (EPO) is a 30.4 kDa growth factor and cytokine that governs cell proliferation, immune modulation, metabolic homeostasis, vascular function, and cytoprotection. EPO is under investigation for the treatment of variety of diseases, but appears especially suited for the treatment of disorders of metabolism that include diabetes mellitus (DM). DM and the com-

plications of this disease impact a significant portion of the global population leading to disability and death with currently limited therapeutic options. In addition to its utility for the treatment of anemia, EPO can improve cardiac function, reduce fatigue, and improve cognition in patients with DM as well as regulate cellular energy metabolism, obesity, tissue repair and regeneration, apoptosis, and autophagy in experimental models of DM. Yet, EPO can have adverse effects that involve the vasculature system and unchecked cellular proliferation. Critical to the cytoprotective capacity and the potential for a positive clinical outcome with EPO are the control of signal transduction pathways that include protein kinase B, the mechanistic target of rapamycin, Wnt signaling, mammalian forkhead transcription factors of the O class, silent mating type information regulation 2 homolog 1 (*Saccharomyces cerevisiae*), and AMP activated protein kinase. Therapeutic strategies that can specifically target and control EPO and its signaling pathways hold great promise for the development of new and effective clinical treatments for DM and the complications of this disorder.

Key words: Protein kinase B; AMP activated protein kinase; Apoptosis; Autophagy; Forkhead; Metabolism; Factors of the O class; Diabetes mellitus; Erythropoietin; Stem cells; Silent mating type information regulation 2 homolog 1; Oxidative stress; Wnt1 inducible signaling pathway protein 1; Wnt

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Core tip: Erythropoietin and the downstream signaling pathways of this cytokine that include protein kinase B, mechanistic target of rapamycin, Wnt signaling, Factors of the O class proteins, silent mating type information regulation 2 homolog 1 (*Saccharomyces cerevisiae*), and AMP activated protein kinase offer new avenues for the development of novel treatments for diabetes mellitus and the complications of this disease.

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ERYTHROPOIETIN: DISCOVERY AND BIOLOGY

The concept of circulating agents that travel throughout the body may have initially originated from Ernest Starling^[1]. In 1905 at the Royal College of Surgeons, Sterling introduced the term "hormones", a term with Greek origins meaning to "excite" or "arouse", to depict the action of chemicals that are dispersed in the body and can target specific organs. Earlier work prior to the presentation by Sterling also described processes that could come under the description as being defined as "hormonal". Claude Bernard described the chemical release of glucose that was processed from glycogen in the liver^[2]. Arnold Adolphe Berthold, another pioneer, also described messenger signals that could communicate among the different bodily organs^[3].

Interestingly, almost as a counterpart to the discussions provided by Starling, Carnot *et al*^[4] in 1906 presented the agent "hemopoietine". This agent was detected in the blood of rabbits after prompted by bleeding that led to the production of immature erythrocytes in untreated rabbits. Subsequent work by other investigators also showed that bled animals could result in prominent reticulocytosis in the plasma^[5-7]. Later, the agent responsible for reticulocytosis was termed erythropoietin (EPO). EPO was linked to depressed oxygen levels and was shown to increase hemoglobin levels in parabiotic rat experiments when one of the two rats experienced hypoxia^[8]. Subsequently, purification of the EPO protein in humans was achieved and cloning of the EPO gene fostered recombinant EPO (rhEPO) production for clinical treatments^[9,10].

EPO is located on chromosome 7 and is a single copy in a 5.4 kb region of the genomic DNA^[11]. The EPO gene encodes for a polypeptide chain that has initially 193 amino acids. A 27 amino acid hydrophobic secretory leader at the amino-terminal to result in a 166 amino acid peptide in the EPO protein is then cleaved^[12]. Additional post-translational processing occurs with the removal of a carboxy-terminal arginine¹⁶⁶ in the mature human and rhEPO to lead to a protein of 30.4 kDa with 165 amino acids^[13-16].

EPO has four glycosylated chains that include three N-linked and one O-linked acidic oligosaccharide side chains^[17]. The N-linked glycosylation sites are at aspartate²⁴, aspartate³⁸, and aspartate⁸³ and the O-linked glycosylation site is at serine¹²⁶. Both the production and secretion of the mature EPO protein is dependent upon N- and O-linked chain integrity^[18]. Replacement of asparagine³⁸ and asparagine⁸³ by glutamate or the replacement of serine¹²⁶ by glycine can impair EPO

production and secretion^[19].

Several factors determine the biological activity of EPO^[20]. The two disulfide bonds formed between cysteine⁷ and cysteine¹⁶⁰ as well as cysteine²⁹ and cysteine³³ control the function of EPO^[21]. EPO biological activity is lost with reduction of these disulfide bonds and with alkylation of the sulfhydryl groups. Almost 85% of EPO biological activity is restored with re-oxidization of EPO after reduction by guanidine^[22]. In addition, EPO biological activity is maintained by the by the glycosylated chains^[23] and EPO stability is fostered by the carbohydrate chains^[24]. Free radical degradation of EPO is limited by both the glycosylated chains^[23] and the oligosaccharides^[25].

Currently, erythropoiesis-stimulating agents including EPO are approved for the treatment of anemia that results from chronic kidney failure, chemotherapy, human immunodeficiency virus, and to limit the number of blood transfusions for surgery^[21,26]. The principal source for the production and secretion of EPO are the kidney peritubular interstitial cells^[27]. Other organs that include the brain, uterus, and liver are also responsible for EPO production and secretion^[17,27-30]. Expression of EPO is controlled by changes in oxygen tension and not by the concentration of red blood cells^[28,31,32]. Hypoxia-inducible factor 1 (HIF-1) can control EPO expression and the EPO receptor (EPOR) to increase the production of EPO^[11,28,33,34]. EPO and EPOR gene transcription occurs following HIF-1 activation. This gene transcription is governed by the transcription enhancer region in the 3'-flanking region of the EPO gene that binds to HIF-1^[11,14]. HIF-1 also can foster pathways that provide cellular protection against injury^[35-37]. Of note, EPO also can be generated from stimuli that may not directly involve hypoxia. During maturation of the brain that may be exposed to various toxic elements, EPO blood levels may be elevated and associated with greater disability^[38]. Elevated EPO serum concentrations have been reported following xenon anesthesia in cardiac surgery^[39]. Agents that decrease inflammation in cerebral microglia have been recently shown to lead to the release of EPO^[40] and infection with malaria can result in significant serum levels of EPO^[41]. Under some conditions during chronic hyperglycemia in adults, EPO levels may be depressed^[42]. Conversely, EPO in the amniotic fluid of diabetic patients can be elevated and be suggestive of perinatal complications^[43]. Furthermore, trophic factors such as insulin can stimulate EPO production in specific cells such as astrocytes^[44].

EPO, OXIDATIVE STRESS, AND CELL SURVIVAL

As a cytoprotective agent, EPO promotes cellular survival, at least in part, through the control of oxidative stress mediated cell injury^[45,46]. Reactive oxygen species (ROS) are released during oxidative stress^[47]. This in turn can cause mitochondrial injury, DNA damage, and

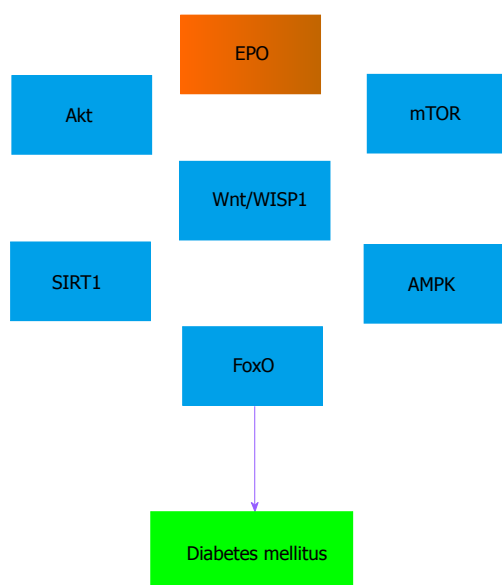


Figure 1 Erythropoietin signal transduction pathways that can lead to clinical benefit during diabetes mellitus. EPO governs a number of signal transduction pathways that involve protein kinase B (Akt), the mechanistic target of rapamycin (mTOR), Wnt and WISP1 signaling, mammalian forkhead transcription factors of the O class (FoxO), silent mating type information regulation 2 homolog 1 (*Saccharomyces cerevisiae*) (SIRT1), and AMP activated protein kinase (AMPK). EPO: Erythropoietin; Akt: Protein kinase B; mTOR: Mechanistic target of rapamycin; FoxO: Factors of the O class; SIRT1: Silent mating type information regulation 2 homolog 1 (*Saccharomyces cerevisiae*); AMPK: AMP activated protein kinase.

protein misfolding^[48-52].

Following the generation of ROS, cell death pathways of programmed cell death can ultimately determine cell survival^[53-62]. Two particular pathways of programmed cell death involve autophagy^[50,63-65] and apoptosis^[15,55,57,66,67]. EPO prevents autophagic cell injury in glomerular mesangial cells during lipopolysaccharide exposure^[68]. Administration of EPO also limits excessive autophagy that precedes apoptosis during experimental neonatal necrotizing enterocolitis^[69]. During hyperoxia exposure and oxygen toxicity to the developing rodent brain, EPO has been shown to modify the activity of autophagy and limit neonatal brain damage^[70].

In regards to apoptotic cell death, EPO prevents apoptotic injury during oxidative stress in endothelial progenitor cells^[71] and attenuates neuroinflammation that can result in apoptosis^[72]. EPO can assist with erythroid differentiation and prevent cellular apoptosis^[73] as well as promote ventricular-subventricular zone neurogenesis and oligodendrogenesis^[74]. Derivatives of EPO, such as glutaraldehyde-EPO, can protect renal cells from apoptosis during ischemia/re-perfusion injury and oxidative stress^[75]. Administration of EPO also can block apoptotic cell death during neuronal kainate-induced oxidative stress^[76], wound injury^[77], vascular oxygen-glucose deprivation^[78-80], loss of protective zinc finger transcription factors^[81], anoxia^[82-84], astroglial glutamate toxicity^[85], beta-amyloid (A β) toxicity^[86-90], renal adriamycin-induced nephropathy^[91], ischemic brain injury^[92], and multi-organ dysfunction induced by

thermal injury^[93]. In addition, EPO is protective against retinal disease^[94], sepsis^[95,96], advanced glycation endproducts (AGEs) exposure in Schwann cells^[97], elevated glucose^[78,98-102], free radicals^[103-108], and toxins that lead to microglial injury^[30,40,90,94,109].

SIGNAL TRANSDUCTION PATHWAYS FOR EPO

EPO cytoprotection is tied to a number of cell pathways^[3]. In particular, phosphoinositide 3-kinase (PI 3-K) and protein kinase B (Akt) can lead to increased cellular survival with EPO (Figure 1). PI 3-K phosphorylates membrane lipids and controls Akt transition from the cytosol to the plasma membrane. Phosphorylation of Akt occurs at serine⁴⁷³ and threonine³⁰⁸ by phosphoinositide dependent kinase (PDK) PDK1 and PDK2^[110-112]. EPO leads to Akt phosphorylation on serine⁴⁷³ to activate this kinase. EPO uses the Akt pathway to protect against autophagy and apoptosis injury in gastrointestinal disease^[69], maintain vascular integrity and reduce inflammation^[113], limit A β toxicity in microglia and neurons^[90,114-116], reduce injury from sepsis^[95,117], increase survival in cardiomyocytes during cardiac hypoxic/re-oxygenation injury^[118], and block oxidative stress injury^[78,82,104,105,119-122]. Akt in conjunction with EPO also improves the function of cells. For example, EPO activates Akt to increase the adhesive properties of endothelial cells and improve the vasculogenic potential of peripheral blood mononuclear cells^[123].

The mechanistic target of rapamycin (mTOR) is closely linked to PI 3-K and Akt^[124] (Figure 1). mTOR is a 289-kDa serine/threonine protein kinase that is encoded by a single gene *FRAP1*^[124,125]. mTOR is important for the function of mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2)^[126-129]. Neurons are protected against sepsis during exposure to EPO and activation of mTOR^[95]. EPO prevents microglial cell injury through mTOR activation during oxidative stress^[109] and A β toxicity^[90]. During oxygen-glucose exposure in neurons, EPO affects multiple pathways of mTOR signaling^[130] to include Akt and proline rich Akt substrate 40 kDa (PRAS40) to increase neuronal survival^[79]. EPO and mTOR are required for the differentiation of neural precursor cells^[131] and to control bone homeostasis with osteoblastogenesis and osteoclastogenesis^[132]. EPO through mTOR can mediate resistance to hypoxia and oxidative stress in retinal progenitor cells^[133] and also protect against increased activity of autophagy in epithelial cells^[69]. Activation of mTOR prevents the induction of autophagy by phosphorylating autophagic related genes (*Atg*) and proteins that include Atg13 and ULKs to inhibit the UNC like kinase complex ULK-Atg13-FIP200^[128]. Under some conditions, the concentration of EPO and activity of mTOR may be important for the degree of cellular protection that can be achieved. Elevated concentrations of EPO have been reported to lead to decreased phosphorylation and activity of mTOR

with increased apoptotic cell death^[134]. Increased mTOR activity also is tied to tumor cell growth^[135-138].

Closely associated to the protective pathways of Akt and mTOR are the wingless pathways of Wnt proteins^[139] (Figure 1). Crosstalk occurs among Wnt signaling pathways, Akt, and mTOR^[140] to foster cellular survival during A β toxicity^[141,142], reduce cerebral ischemia^[143,144], promote progenitor cell activation during intestinal inflammation^[145], prevent neuronal cell loss^[146], limit 6-hydroxydopamine toxicity^[147], enhance microglial and macrophage survival and function^[148,149], and increase tissue fibrosis^[150]. EPO employs the Wnt pathway to lead to cellular protection. During renal ischemia and reperfusion, EPO limits tubular cell apoptosis by increasing the expression of Wnt7b and β -catenin as well as by down-regulating specific micro-RNAs (miRNA)^[151,152]. Through Wnt1, EPO protects against elevated glucose exposure in cerebral endothelial cells and maintains the expression of Wnt1^[100]. In addition, EPO uses Wnt signaling to prevent immune cell loss during oxidative stress^[109], prevent A β toxicity in microglia^[90], limit the activity of forkhead transcription factors that result in apoptosis^[99,153], and maintain the survival of mesenchymal stem cells^[154]. Of note, both EPO and the pathways of Wnt signaling are proliferative in nature and have the potential to lead to tumorigenesis. For example, prolonged exposure of growth factors such as EPO that rely upon Wnt signaling can result in inflammation, blood-brain barrier injury^[155], and tumor growth^[156-158].

Cellular protection with EPO that relies upon Wnt signaling also can be associated with the modulation of mammalian forkhead transcription factors^[159]. Mammalian FOXO proteins are assigned to the O class of the forkhead box class transcription factors^[160,161] (Figure 1). These transcription factors consist of FOXO1, FOXO3, FOXO4, and FOXO6 and exist throughout the body^[162]. FoxO proteins can impact cellular survival^[163] and are homologous to DAUER Formation-16 (DAF-16), a transcription factor in *Caenorhabditis elegans*, that leads to lifespan extension and affects insulin signaling^[164,165]. Under many circumstances, the activation of FoxO proteins results in apoptotic cell death^[153]. FoxO3a expression increases in the hippocampus during cerebral ischemia^[166] and FoxO3a may lead to cell cycle induction that can promote neuronal apoptotic cell death^[167]. Loss of FoxO3a expression and prevention of nuclear shuttling of FoxO3a in microglial cells and neurons results in increased survival during oxidative stress^[146,148]. Inhibitory phosphorylation of FoxO3a and the nuclear export of FoxO3a during periods of elevated glucose also protects vascular cells^[80,99,168,169] and neuronal cells^[170].

In endothelial cells, EPO uses Wnt1 to block FoxO3a activity and maintain cerebral endothelial survival during elevated glucose^[99]. Without Wnt signaling, EPO also has been shown to phosphorylate FoxO3a and lead to its inactivation to block apoptosis in neuronal cells^[73]. EPO can prevent endothelial cell injury during

oxygen-glucose deprivation by preventing FoxO3a nuclear subcellular trafficking that would lead to "pro-apoptotic" protein transcription and translation^[20,80]. EPO can oversee stem cell proliferation through FoxO protein regulation. Through the control of FoxO3a activity, EPO promotes the development of erythroid progenitor cells^[57,73,171,172].

FoxO protein activity is controlled by post-translation protein modifications that involve phosphorylation, ubiquitylation, and acetylation^[162,173]. In regards to acetylation, FoxO proteins are deacetylated by histone deacetylases that includes the silent mating type information regulation 2 homolog 1 (*Saccharomyces cerevisiae*) (SIRT1)^[54] (Figure 1). SIRT1 deacetylation of FoxO proteins can influence autophagic pathways such that glucose deprivation leads to increases in autophagic flux that maintain left ventricular function during periods of starvation^[174]. SIRT1 may be required to promote cortical bone formation with osteoblast progenitors by deacetylation of FoxOs and preventing FoxO protein binding to β -catenin to inhibit Wnt signaling^[175]. However, the degree of SIRT1 expression in relation to FoxO protein activity may be a significant determinant for cellular survival^[160,161]. For example, during exercise a controlled up-regulation of FoxO3a and SIRT1 expression in cardiac tissue may be important to improve cell survival^[176]. During oxidative stress, cell injury may be reduced with catalase expression regulated by FoxO1a expression and SIRT1 levels less than 7.5-fold. However, decreased cardiac function and apoptotic cell death in cardiomyocytes can ensue with elevated SIRT1 levels of 12.5-fold^[177]. FoxO proteins, such as FoxO1, also can control SIRT1 transcription and increase SIRT1 expression^[178]. Under some circumstances, SIRT1 and FoxO proteins may function synergistically to promote cell survival. Loss of the forkhead transcription factors FoxO1 and FoxO3 in combination with decreased SIRT1 activity during oxidative stress leads to a reduction in autophagy with chondrocyte cell death, demonstrating that SIRT1 with FoxO proteins may be required for cellular protection^[179]. SIRT1 also has been shown to increase lifespan in higher organisms and offer protection against oxidative stress^[180]. EPO relies upon SIRT1 activity to prevent cell injury during oxidative stress and elevated glucose^[181]. EPO can raise cellular activity of SIRT1 and promote the subcellular trafficking of SIRT1 to the nucleus to protect endothelial cells during oxidative stress^[80]. EPO is able to maintain adipose cell energy homeostasis and protect against metabolic disorders through SIRT1^[101]. Pathways that involve Wnt signaling with the CCN family member Wnt1 inducible signaling pathway protein 1 (WISP1)^[139] also require up-regulation of SIRT1 activity to block apoptotic pathways controlled by FoxO proteins^[182] (Figure 1). WISP1 can increase neuronal survival by limiting FoxO3a activity and FoxO3a deacetylation, blocking caspase 1 and 3 activation, and promoting SIRT1 activity and trafficking to the cell nucleus^[146].

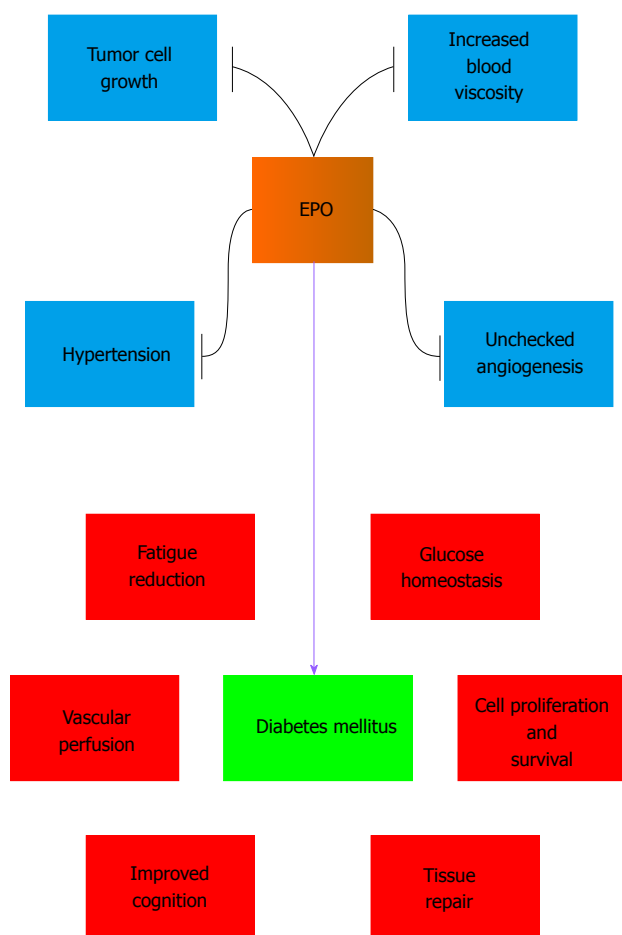


Figure 2 Targeting erythropoietin involves a balance that fosters clinical improvement over clinical disability. EPO can play a significant role in reducing disability and fostering clinical benefit during diabetes mellitus. Through its signal transduction pathways, EPO may improve organ and tissue function, reduce fatigue, improve vascular perfusion, maintain glucose homeostasis, assist with wound and tissue repair, and promote cellular proliferation, differentiation, and survival. However, the detrimental effects of EPO that can include tumor cell growth, hypertension, increased blood viscosity, and unchecked angiogenesis must be considered and eliminated for successful therapeutic treatments against diabetes mellitus. EPO: Erythropoietin.

NOVEL AVENUES FOR EPO AND METABOLIC DISEASE

Growth factors such as EPO offer potentially new treatment approaches for numerous disorders, but given the signal transduction pathways that are regulated by EPO, this agent provides exciting prospects for the treatment of diabetes mellitus (DM)^[16,45]. DM affects at least 350 million individuals worldwide^[182] and is increasing in incidence^[183]. Of potentially greater concern are the numbers of undiagnosed individuals that just in the United States alone may exceed 8 million individuals who are believed to suffer from metabolic disorders^[32,184,185]. DM can affect the entire body and involve the immune system^[63,77,181,186-190], liver^[55,191-196], musculoskeletal function^[197-201], kidney^[202-206], and cardiovascular system^[163,188,207-213] to result in endothelial cell dysfunction^[115,16,99,100,168,214,215] and atherosclerosis^[45,67,199,216]. These

disorders can easily affect other regions of the body such as the nervous system to lead to cognitive loss^[14,217-219], visual deterioration^[32,119,220,221], peripheral nerve disease^[55], and ischemic disease of the brain^[23,49,67,222-224].

EPO as well as its downstream pathways have been shown to have a high potential to treat multiple complications of DM^[32] (Figure 2). In earlier work that examined diabetics and non-diabetics with severe congestive heart failure, EPO increased left ventricular ejection fraction, reduced fatigue, and lessened duration of hospital stay^[225]. In patients with Type 1 DM and cognitive impairment related to hypoglycemia, administration of EPO leads to improvement in complex reaction time task assessing associated with attention and working memory^[226]. EPO also could provide a small improvement to treat fatigue in patients with Type 2 DM and chronic kidney disease^[227].

In experimental models of DM, EPO can reduce blood glucose levels in animal models of DM and obesity^[228], protect against the detrimental effects of obesity in animal models^[16], treat diabetic peripheral neuropathy^[229], and block apoptosis in Schwann cells mediated by AGEs^[97]. EPO has been shown to limit high glucose-induced oxidative stress in renal tubular cells^[230], control cellular mitochondrial function^[76,80,103,109,118], and maintain energy metabolism^[15]. Through anti-inflammatory mechanisms and the blockade of apoptosis, EPO can protect pancreatic islet cells in models of type 1 DM and Type 2 DM^[98]. Intravitreal administration of EPO in rodent models of DM can normalize gene expression that can lead to apoptotic and inflammatory cell death^[231]. EPO is cardioprotective in DM models with the inhibition of glycogen synthase kinase-3 β (GSK-3 β)^[232] that can limit Wnt signaling pathways^[233]. Through increased angiogenesis and decreased apoptotic cell death, EPO can improve wound healing and wound closure in diabetic mice^[77,234]. In vascular disease, EPO has been reported to protect the neuroglialvascular unit in a model of retinal neurodegeneration and secondary vasoregression^[119]. EPO can directly protect against endothelial cell apoptosis during elevated glucose through activation of Wnt1^[100] and the inhibition of GSK-3 β and FoxO3a^[99]. Improvement in vascular perfusion by EPO^[123] also may afford indirect protection to assist with cognitive repair^[235] and decrease peripheral nerve injury during DM^[102].

Not all studies demonstrate a beneficial effect with EPO during DM, suggesting that focus upon the downstream signaling pathways of EPO with mTOR, Wnt signaling, FoxO proteins, and SIRT1 may yield greater utility for some clinical populations with complications of DM. In patients with DM and renal disease, EPO administration results in a two-fold increase in stroke that is not attributed to any baseline characteristic or to blood pressure, hemoglobin, platelet count, or treatment dose of EPO^[236]. In mice that overexpress EPO, blood viscosity has been reported to be increased with a reduction in cerebral blood flow^[237]. As a result, EPO may increase the risk for stroke through increased blood viscosity. Although

systemic administration of EPO may block retinopathy in animal models^[94], elevated EPO concentrations in patients with DM also may lead to proliferative diabetic retinopathy^[238] that could be associated with excessive vascular growth. EPO can increase vascular responsiveness^[239] and may lead to hypertension^[26,57,240]. Sustained erythrocytosis with agents such as EPO may result in the activation of inflammatory pathways and blood-brain barrier dysfunction^[155]. As a proliferative agent, EPO also can lead to new tumor growth as well as foster the progression of existing tumors^[156-158,241].

The potential adverse effects of EPO may be avoided by targeting more specific pathways controlled by EPO such as mTOR and AMP activated protein kinase (AMPK)^[40,208] (Figure 2). AMPK oversees the activity of the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2) complex that is an inhibitor of mTORC1^[135]. Metformin, an agent that controls hyperglycemia in DM, can reduce cardiomyopathy in experimental models of DM through AMPK activation^[242]. EPO as well may dependent upon AMPK to promote antioxidant gene expression^[243]. Furthermore, other EPO signaling pathways play a role in controlling AMPK. AMPK can increase nicotinamide phosphoribosyltransferase levels during glucose limitation resulting in elevated nicotinamide adenine dinucleotide^[244] and lower levels of the SIRT1 inhibitor nicotinamide^[245]. SIRT1 and AMPK activation promotes autophagy that offers endothelial cell protection during exposure to oxidized low density lipoproteins that can lead to atherosclerosis^[246]. WISP1, a component of Wnt signaling, also controls the post-translational phosphorylation of AMPK that is involved in glucose homeostasis^[124,247-249]. WISP1 regulates AMPK activation by decreasing phosphorylation of TSC2 at serine¹³⁸⁷, a target of AMPK, and increasing phosphorylation of TSC2 at threonine¹⁴⁶², a target of Akt^[142]. The ability of WISP1 to modulate AMPK activity is vital for the regulation of cellular metabolism during DM^[249]. AMPK activity is able to reduce insulin resistance and lessen oxidative stress through activation of autophagy^[200]. AMPK can prevent myocardial ischemia in experimental models of DM^[250], assist with proper metabolic function of cells^[251], and limit adipocyte differentiation, lipid accumulation, and obesity^[252]. Yet, similar to SIRT1, the degree of AMPK activity is a significant consideration in DM. AMPK activation can lead to apoptosis in pancreatic islet cells in some experimental models of Type 2 DM^[253].

CONCLUSIONS AND FUTURE PERSPECTIVES

In the global population, DM is a significant cause of disability and death. Treatment options to limit the onset and progression of this disease are insufficient and warrant the development of novel treatments. EPO, as a cytoprotective agent that controls a broad array of signal transduction pathways offers exceptional

promise for the treatment of DM and pathways of oxidative stress. EPO has been shown in diabetic patients to improve cardiac function, reduce fatigue, and improve cognition. In experimental models of DM, EPO can reduce blood glucose levels, limit peripheral neuropathy, maintain mitochondrial function and energy metabolism, and block programmed cell death in many cell types such as Schwann cells, endothelial cells, neurons, pancreatic islet cells, and cardiomyocytes.

However, several challenges exist to move EPO forward as an effective treatment for DM. EPO has been reported to increase the risk of stroke in patients with DM and renal disease and has been demonstrated to increase blood viscosity in animal studies. EPO may be contraindicated in hypertensive patients and may contribute to elevated mean arterial blood pressure. Elevated concentrations of EPO have been linked to proliferative diabetic retinopathy that may be associated with excessive microvascular angiogenesis. Finally, EPO, as a growth factor and proliferative agent, may lead to new tumor growth and also promote the growth of existing tumors, especially in the treatment of patients with cancer and anemia.

Further investigations that assess the protective capacity of EPO and limit any potential detrimental clinical outcomes are warranted. New work has been directed to improving the molecular stability, solubility, and immunogenicity of EPO for improved therapeutic strategies to treat the complications of DM. Glycoengineering, a method that introduces N-linked glycosylation consensus sequences into proteins to increase serum half-life and biological activity, has been examined for EPO^[254]. Darbepoetin alpha is one such example of a hyperglycosylated EPO derivative. Darbepoetin alpha has an increased serum half-life when compared to recombinant EPO^[255] and is considered more potent than recombinant EPO^[256]. EPO mimetic proteins are other avenues being pursued that can be used to activate the EPOR, potentially increase treatment half-life and maintain potency when compared to EPO, and lessen immunogenicity^[257,258]. For example, CNTO 530 has been shown to increase reticulocytes, red blood cells and total hemoglobin in β -thalassemic mice^[259].

A promising investigative course also could target the downstream signaling pathways of EPO that include Akt, mTOR, Wnt signaling, FoxO proteins, SIRT1, and AMPK. EPO employs Akt and mTOR for stem cell maintenance and differentiation, resistance against oxidative stress, and the regulation of autophagy. In experimental models of DM, EPO relies upon Wnt signaling, β -catenin, and the inhibition of GSK-3 β to block apoptotic cell death. EPO also governs FoxO proteins and SIRT1 to protect against DM apoptotic vascular injury, maintain adipose cell energy homeostasis, and modulate autophagic flux to improve cardiac function during metabolic disturbances. Pathways that involve EPO and AMPK also offer interesting targets to maximize clinical efficacy and minimize unwanted side effects. AMPK reduces insulin resistance and lessens oxidative stress through

activation of autophagy, prevents myocardial ischemia in models of DM, and limits adipocyte lipid accumulation and obesity. WISP1 controls AMPK activity for the regulation of cellular metabolism during DM. In addition, SIRT1 and AMPK in conjunction with SIRT1 can increase autophagy activity to provide endothelial cell protection during exposure to oxidized low-density lipoproteins. However, it should be noted that consideration of these pathways may still require use of EPO or an EPO analogue since therapeutic success may be dependent on modulation of more than one of these down-stream pathways of EPO. In addition, one needs to emphasize that each of these pathways also can lead to undesirable biological outcomes under some circumstances such as tumorigenesis, pancreatic islet cell death, and cardiac dysfunction. Carefully targeting future investigations for EPO and its relevant signal transduction pathways for specific clinical disturbances of DM should offer the greatest promise for novel therapeutic strategies.

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Whey protein: The “whey” forward for treatment of type 2 diabetes?

Linda E Mignone, Tongzhi Wu, Michael Horowitz, Christopher K Rayner

Linda E Mignone, Tongzhi Wu, Michael Horowitz, Christopher K Rayner, Discipline of Medicine, the University of Adelaide, Adelaide 5000, Australia

Linda E Mignone, Tongzhi Wu, Michael Horowitz, Christopher K Rayner, Centre of Research Excellence in Translating Nutritional Science to Good Health, the University of Adelaide, Adelaide 5000, Australia

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Correspondence to: Christopher K Rayner, MBBS, PhD, Professor, Discipline of Medicine, the University of Adelaide, Frome Road Adelaide, Adelaide 5000, Australia. chris.rayner@adelaide.edu.au
 Telephone: +61-8-82222916
 Fax: +61-8-82233870

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Abstract

A cost-effective nutritional approach to improve postprandial glycaemia is attractive considering the rising burden of diabetes throughout the world. Whey protein, a by-product of the cheese-making process, can be used to manipulate gut function in order to slow gastric emptying and stimulate incretin hormone secretion, thereby attenuating postprandial glycaemic excursions. The function of the gastrointestinal tract plays a pivotal role in glucose homeostasis, particularly during the postprandial period, and this review will discuss the mechanisms by which whey protein slows gastric emptying and stimulates release of gut peptides, including the incretins. Whey protein is also a rich source of amino acids, and these can directly stimulate beta cells to secrete insulin, which contributes to the reduction in postprandial glycaemia. Appetite is suppressed with consumption of whey, due to its effects on the gut-brain axis and the hypothalamus. These properties of whey protein suggest its potential in the management of type 2 diabetes. However, the optimal dose and timing of whey protein ingestion are yet to be defined, and studies are required to examine the long-term benefits of whey consumption for overall glycaemic control.

Key words: Whey protein; Postprandial glycaemia; Type 2 diabetes; Dietary intervention; Preload; Gastric emptying; Incretins; Gut hormones; Appetite; Amino acids

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Core tip: Whey protein, a by-product of cheese-manufacture, shows promise in the dietary management of diabetes. Whey can slow gastric emptying, stimulate insulin and gut hormones including the incretins, and thereby reduce postprandial blood glucose, especially when consumed some minutes before a meal. Whey may also suppress appetite and reduce food intake. This review will summarise these properties of whey

and examine what further evidence is needed before whey can be recommended in the management of type 2 diabetes.

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INTRODUCTION

It is well established that the risk of microvascular, and to a lesser extent macrovascular complications of both type 1 and type 2 diabetes, is closely related to “average” glycaemic control as assessed by glycated haemoglobin (HbA1c). In people with type 2 diabetes who have relatively good glycaemic control, postprandial hyperglycaemia predominates over preprandial blood glucose in contributing to HbA1c^[1,2]. Accordingly, focusing on postprandial glycaemia in patients with mild or moderate elevation of HbA1c is now appreciated as an important management strategy; indeed, achieving a “target” HbA1c of $\leq 7.0\%$ is difficult without minimising postprandial glycaemic excursions^[3,4]. The potential use of dietary manipulations to reduce postprandial glycaemia is intuitively appealing, particularly given the escalation in health care costs with the rising incidence of type 2 diabetes.

Whey, a by-product of cheese making, is gaining recognition as an important functional food^[5]. Whey protein has been demonstrated to diminish postprandial glycaemia through various interrelated mechanisms including enhancement of insulin and incretin hormone secretion, slowing of gastric emptying, and reductions in appetite and energy consumption (Figure 1). These properties suggest the potential for whey in the management of type 2 diabetes. However, whey protein cannot be endorsed as a potential treatment until further studies show that it improves long-term glycaemic control without significant adverse outcomes.

This review will explore the different forms of whey protein and compare the effects of whey with other sources of protein in reducing postprandial glycaemia. It will address the mechanisms by which whey lowers glycaemia, the factors that need to be considered for optimal use of whey, and the effects of long term consumption of whey protein on glycaemic control, together with its potential adverse effects.

COMPARISON OF WHEY AND CASEIN PROTEINS

Milk proteins are an important amino acid source for young mammals; they facilitate uptake of nutrients and trace elements^[6] and provide a source of bioactive

peptides with a range of physiological functions^[6-8]. Cow's milk contains about 3.5 g of protein per 100 mL, of which whey accounts for about 20% and casein 80%^[9-11].

Whey consists of a heterogeneous group of proteins^[12], including beta-lactoglobulin (35%), alpha-lactalbumin (12%), proteose peptone (12%), immunoglobulins (8%), and bovine serum albumin (5%)^[11,13,14]. When chymosin is used in the cheese-making process, glycomacropeptide - which is high in branched chain amino acids - accounts for about 12% of total protein in whey^[15]. Up to 1% of the total protein content of whey comprises “low abundance” proteins, including lactoferrin, and lactoperoxidase^[14]. All these proteins have been reported to have nutritional and/or physiological functions^[5].

Whey is seen as a more attractive protein for use as a dietary supplement compared to casein, due to differences in the amino acid composition and absorption kinetics between the two proteins^[16]. Whey protein has a higher proportion of branched chain amino acids than casein^[17], and is more soluble in the acidic environment of the stomach, leading to more rapid digestion^[18] - hence it is termed a “fast” protein^[19], while casein is a “slow” protein^[16,20]. Using ¹³C-leucine-labelled whey and casein protein, Boirie *et al.*^[18] demonstrated in healthy subjects that whey protein results in more rapid appearance, and higher peak plasma concentrations of amino acid, when compared with casein, while Stanstrup *et al.*^[21] reported that levels of amino acids after a fat rich meal containing whey were substantially higher when compared to the same meal containing casein. As a result of greater solubility, more rapid digestion, and resultant higher plasma concentrations of amino acids, whey appears to be the more favourable protein to provide nutritional and functional benefits.

FORMS OF WHEY PROTEIN - ISOLATE, CONCENTRATE AND HYDROLYSATE

Whey protein is available in three forms: concentrate, isolate, and hydrolysate. Whey protein concentrate contains 35%-80% protein, with fat, lactose and minerals making up the remainder; whey protein isolate contains 85%-90% protein and very little fat or lactose^[5,15,22]; and whey protein hydrolysate consists of proteins that have undergone hydrolysis by proteolytic enzymes^[14]. Whey hydrolysates and isolates are more costly than whey concentrates, which is an important consideration if whey protein is to be used for a prolonged period of time in the management of type 2 diabetes. It is therefore important to consider the evidence that one form of whey protein is more “functional” than another.

Protein hydrolysates are usually more rapidly absorbed than the intact protein^[23], but since intact whey is already a rapidly digested protein, any difference is likely to be minimal^[24,25]. Some studies have suggested that whey hydrolysates may stimulate insulin and glucose-dependent insulintropic polypeptide (GIP) secretion to

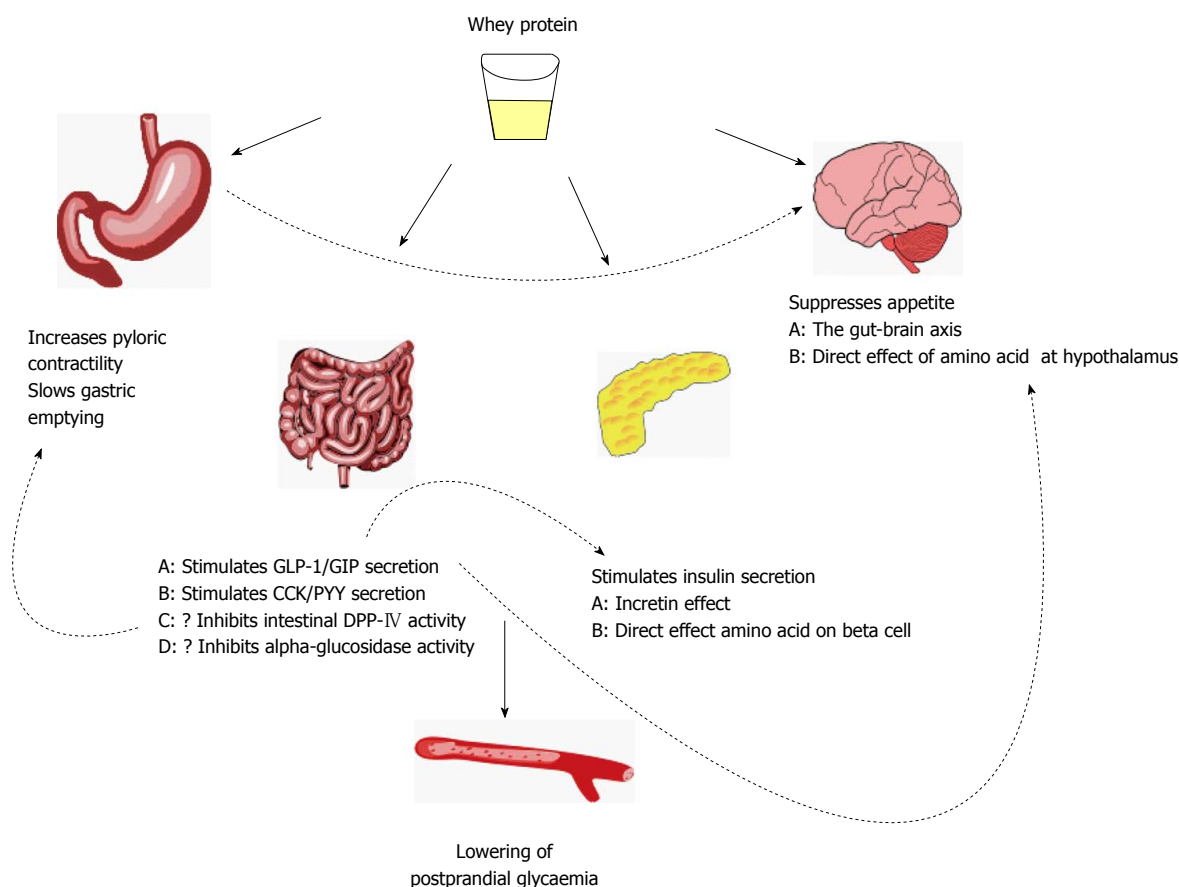


Figure 1 Mechanisms by which whey protein can reduce postprandial glycaemia. GLP-1: Glucagon-like-peptide-1; GIP: Glucose-dependent insulinotropic polypeptide; CCK: Cholecystokinin; PYY: Peptide YY; DPP-IV: Dipeptidyl peptidase-IV.

a greater degree than the intact protein^[26,27]. Mortensen *et al.*^[28] investigated the effects of adding 45 g of four different whey protein formulations (whey hydrolysate, whey isolate, alpha-lactalbumin enhanced whey, and caseinoglycomacropeptide enhanced whey) to a high fat/carbohydrate meal in subjects with type 2 diabetes, and reported that the first phase insulin response (as assessed by the incremental area under the curve (iAUC) up to 30 min) was enhanced after whey hydrolysate compared with the other three supplements, and that whey isolate and whey hydrolysate yielded a greater overall insulin response (iAUC at 480 min) than the other two supplements, without any difference between them. Whey proteins which have been hydrolysed are, however, usually less palatable^[29], which detracts from their potential therapeutic use. There is no compelling evidence that one form of whey protein is significantly more potent than another, particularly in relation to reduction of postprandial glycaemia, so consideration of palatability and cost must also be taken into account.

ROLE OF THE INCRETIN HORMONES, GIP AND GLP-1, IN PROTEIN-INDUCED INSULIN SECRETION

The phenomenon by which insulin secretion is increased

when glucose is given by the enteral route, when compared to an isoglycaemic intravenous glucose infusion, is called the “incretin effect”, and is attributed to the secretion of “incretin” hormones from the gut. The two known incretin hormones, glucagon-like-peptide-1 (GLP-1) and GIP, exert their insulinotropic actions through distinct G-protein-coupled receptors that are highly expressed on beta cells^[30]. After oral glucose, about two thirds of the plasma insulin response can be attributed to the effects of GIP and GLP-1. The insulinotropic effects of both GIP and GLP-1 are glucose-dependent, requiring a substantial elevation of blood glucose (> 8 mmol/L) to be manifest^[31]. Incretin based therapies, such as GLP-1 receptor agonists, are attractive for this reason, as insulin release is only triggered in the presence of elevated glucose concentrations, with consequently minimal risk of hypoglycaemia.

Incretin hormones may play an important role in protein-stimulated insulin release in health and type 2 diabetes^[32]. GIP and GLP-1, when infused intravenously to mimic physiological increments after a meal, have been reported to potentiate the insulin secretory response to IV administration of an amino acid mixture^[33]. In a study of oral administration of protein and amino acids in health, a whey drink resulted in a greater GIP response than a drink containing the essential amino acids found in whey, with an associated augmentation

of the insulin response^[34]. Additionally, the stimulation of insulin secretion from murine islets *in vitro* by whey was inhibited by GIP receptor antagonists^[35]. The effects of the GLP-1 antagonist, exendin 9-39, on whey-induced insulin secretion have not been evaluated. However, it is clear that the insulintropic effects of whey, at least in part, involve the incretin axis.

In humans, fats and carbohydrates are reported to be the most potent stimuli for GLP-1 and GIP secretion^[36], although the effects of protein on incretin secretion are less well studied than the other macronutrients^[37]. Nevertheless, whey protein is reported to stimulate GLP-1 and GIP release^[17,34,35,38-40]. Bowen *et al.*^[41] showed that plasma active GLP-1 concentrations were higher after intake of a whey protein beverage compared to a glucose or fructose drink, but the mechanisms mediating protein-induced incretin secretion remain largely unknown^[37].

Although the capacity for GIP to stimulate insulin is markedly diminished in type 2 diabetes, at least in part due to the effects of chronic hyperglycaemia^[42], GLP-1 retains much of its activity. As whey protein can augment incretin hormone secretion and enhance protein-stimulated insulin release, it seems reasonable to view whey as a potential therapeutic agent in the treatment of type 2 diabetes.

ROLE OF GASTRIC EMPTYING IN MEDIATING THE EFFECTS OF WHEY ON POSTPRANDIAL GLYCAEMIA

It is now well established that gastric emptying plays a major role in determining postprandial blood glucose concentrations, particularly the “early” glycaemic response, and that slowing gastric emptying can diminish postprandial glycaemic excursions in health and diabetes^[43-46]. In healthy humans, the addition of protein to oral glucose lowers postprandial blood glucose concentrations acutely, probably predominantly by slowing gastric emptying^[47]. Similarly, a “preload” of whey has been shown to slow gastric emptying of a subsequent meal in both health^[17], and in type 2 diabetes^[48].

The effects of whey on gastric emptying, postprandial glycaemia, and the secretion of incretin hormones, are interdependent. The incretins not only have major insulintropic effects, but GLP-1 also slows gastric emptying, suppresses energy intake and has glucagonstatic effects to improve postprandial glycaemia^[42]. Reports that GLP-1 secretion is impaired in longstanding type 2 diabetes^[49,50] did not take potential differences in gastric emptying rates into account; furthermore, it has now been shown that in patients with type 2 diabetes managed by diet or metformin only, the GLP-1 response to an intraduodenal glucose challenge is apparently normal^[46]. That GLP-1 secretion is intact in type 2 diabetes adds to the rationale for

using a nutritional approach to enhance the secretion of endogenous GLP-1. Moreover, gastric emptying and appetite are inhibited by gut hormones other than the incretins, including cholecystokinin (CCK) and peptide YY (PYY)^[51-53]. Stimulation of these hormones by nutritional supplements could also be beneficial in reducing postprandial glycaemia.

ANTROPYLORODUODENAL MOTILITY

Interactions between nutrients and the small intestine can induce feedback on gut function to suppress antral motility and stimulate pyloric contractions, with resultant slowing of gastric emptying^[54]. In both healthy young and older humans, intraduodenal delivery of whey suppresses antral and duodenal waves and increases isolated pyloric pressure waves. Such changes in antropyloric motility in response to nutrient ingestion also appear to be independently related to subsequent energy intake in healthy young subjects^[55]. Soenen *et al.*^[56] examined the effects of intraduodenal whey protein infusion on appetite and subsequent *ad libitum* energy intake in relation to antropyloroduodenal motility. They reported that energy intake at a buffet meal was inversely related to the number of isolated pyloric pressure waves, and positively related to the number of antral pressure waves, supporting a relationship between antropyloroduodenal motor activity and feeding behaviour.

POTENTIAL IMPACT OF WHEY ON DIPEPTIDYL PEPTIDASE-IV

The incretin hormones are rapidly degraded to inactive metabolites by dipeptidyl peptidase-IV (DPP-IV). More than 50% of the GLP-1 newly secreted from intestinal L cells is degraded before reaching the systemic circulation^[57], mainly by DPP-IV present in the endothelium of the capillary bed in close proximity to the L cells^[36,57]. Whey hydrolysates, produced using digestive enzymes such as pepsin and trypsin, have been found to inhibit the activity of DPP-IV *in vitro*^[58-61]. For rodents *in vivo*, ingestion of whey protein can reduce DPP-IV activity in the proximal small bowel, thereby increasing intact incretin hormone concentrations^[62]. Further *in vivo* studies, particularly in humans, are required to confirm this phenomenon, and establish its durability with long term ingestion of whey^[63].

EFFECTS OF WHEY ON ALPHA-GLUCOSIDASE

Alpha glucosidase is an enzyme that hydrolyzes starch and disaccharides to enable absorption of glucose at the small intestinal brush border. *In vitro* studies have shown that whey protein hydrolysate has a modest effect to inhibit alpha-glucosidase^[59], which may be

clinically relevant given that alpha-glucosidase inhibitors, such as acarbose, are used widely in the management of type 2 diabetes to improve postprandial glycaemia. Human studies are required to further evaluate this mechanism and the magnitude of the glucose lowering effect attributable to it.

TIMING OF WHEY PROTEIN, “PRELOADS”, AND GASTRIC EMPTYING

The concept of a “preload” refers to administration of a small load of macronutrient at a fixed interval before a meal, so that the presence of nutrients in the small intestine induces the release of GLP-1 and GIP, and other gut peptides such as CCK and PYY, to slow gastric emptying and stimulate insulin secretion in advance of the main nutrient load. In health, whey protein preloads have been shown to slow gastric emptying, as assessed by the plasma concentrations of oral paracetamol given with the meal, and enhance post-prandial GLP-1 levels^[64]. Similarly, whey given immediately before a meal, with or without additional amino acids, reduces the postprandial glycaemic response by over a third (iAUC 0-60 min), associated with an increase in the early postprandial plasma insulin and GLP-1 responses^[65].

The capacity for a whey preload to stimulate incretin hormone secretion and slow gastric emptying has also been established in subjects with type 2 diabetes^[48]. Ma *et al.*^[48] reported in type 2 patients that a 55 g whey protein preload, given 30 min before a meal, slows gastric emptying when compared to either a nutrient-free preload or ingestion of whey with the meal. In this study, gastric emptying was quantified using scintigraphy, which represents the “gold standard”. Whey protein markedly reduced postprandial glucose excursions (iAUC after whey preload about half that of control), and stimulated insulin and CCK, as well as GIP and GLP-1. Both the GLP-1 response and the reduction in postprandial glycaemia were greater when whey was given as a preload, when compared to ingestion with the meal. Accordingly, this study not only established that whey can slow gastric emptying substantially in type 2 diabetes, but that the timing of supplementation is pivotal to the stimulation of incretins and other gut hormones. These acute effects of whey preloads to improve postprandial glycaemia were recently confirmed in another study in type 2 patients^[66]. While whey has been shown to slow gastric emptying acutely, it remains to be seen whether this effect is sustained with long term administration.

AMINO ACIDS AS A STIMULUS FOR INSULIN SECRETION

It has been established for many years that ingested protein stimulates insulin secretion^[47,67], an effect observed in both healthy subjects and in those with type

2 diabetes. This effect is enhanced when protein is co-ingested with carbohydrates when compared with the ingestion of carbohydrate or protein alone, suggesting a synergy between oral protein and glucose^[68-72]. In a recent comparison of four protein sources, the greatest postprandial insulin response was associated with whey compared to casein, gluten or cod, and was attributed to the more rapid appearance of amino acids in plasma when derived from whey^[21].

Whey protein is a rich source of essential amino acids and branched chain amino acids known to have potent insulinotropic properties^[73]. The branched chain amino acids - leucine, valine, and isoleucine - are more insulinogenic than other amino acids^[40,74]. In the 1960s, Floyd *et al.*^[67,75,76] showed that amino acids, given either intravenously or orally, had the capacity to stimulate insulin secretion and reduce blood glucose concentrations. The insulinotropic effect of whey, at least in part, reflects a direct effect of amino acids to stimulate beta cells^[35,77-80]; the underlying mechanisms are complex and involve mitochondrial metabolism^[77].

Amino acids can stimulate insulin secretion in type 2 diabetes as well as in health. van Loon *et al.*^[81] reported that patients with long standing type 2 diabetes who co-ingested an amino acid/protein mixture (wheat protein hydrolysate) with a carbohydrate meal almost trebled their insulin response, when compared to ingestion of carbohydrate alone. This preserved stimulation of insulin by amino acids in type 2 diabetes contrasts with the diminished insulin response to carbohydrates, when compared with healthy controls. Similarly, addition of casein to carbohydrate has also been noted to potentiate insulin secretion in longstanding type 2 diabetes. That amino acids derived from ingested proteins remain a strong stimulus for insulin secretion, even in patients with long standing type 2 diabetes, supports their potential efficacy in the management of this condition^[68].

ROLE OF GLUCAGON

Glucagon, secreted from the alpha cells of the pancreas, primarily acts on the liver to initiate glycogenolysis and gluconeogenesis, which then increases endogenous glucose production. Glucagon secretion is exaggerated in response to a meal in patients with type 2 diabetes^[82], and ingested protein results in an increase in plasma glucagon levels^[83]. It might therefore be expected that protein ingestion would increase blood glucose concentrations, but this is not necessarily the case.

Calbet *et al.*^[84] gave 6 healthy adults four tests meals containing glucose, cow's milk solution, pea and whey peptide hydrolysates, and found that the glucagon response was linearly related to the increase in plasma amino acids. Despite this, plasma glucose levels after whey hydrolysates decreased by about 1.5 mmol/L from baseline to 180 min, most likely due to the effects of insulin, which is stimulated concurrently and is particularly effective at suppressing glycogenolysis.

IS WHEY PROTEIN EFFECTIVE IN REDUCING POSTPRANDIAL GLYCAEMIA IN TYPE 2 DIABETES?

Although it is clear that whey has an insulinotropic effect, it is less clear as to whether the magnitude of insulin stimulation is sufficient to reduce postprandial glycaemia in patients with type 2 diabetes, who tend to be insulin-resistant, and often exhibit hyperinsulinaemia^[40,85-87]. Insulin sensitivity, assessed using a euglycaemic-hyperinsulinaemic clamp, impacts on the capacity for acute administration of protein to reduce blood glucose concentrations in healthy subjects^[88], and this may explain why some studies of patients with type 2 diabetes reported no reduction in blood glucose despite stimulation of insulin after a protein meal^[38,89].

Frid *et al.*^[39] evaluated the effect of adding whey protein to high glycaemic index meals taken at breakfast and lunch in patients with type 2 diabetes. Plasma insulin responses were higher after both breakfast (31%) and lunch (57%) with whey (27.6 g) when compared to lean ham or lactose. There was a reduction in blood glucose excursions after lunch but not breakfast, which might be related to either the differing meal content, or to higher insulin resistance seen in the fasting state^[90] affecting responses after breakfast.

Conversely, other studies in type 2 diabetes have reported up to 3 or 4 fold increases in insulin responses to meals containing protein and carbohydrate, when compared to carbohydrate alone, with concomitant reductions in postprandial glycaemia^[71,91]. Nuttall *et al.*^[70] evaluated nine male subjects with diet controlled type 2 diabetes and showed that the blood glucose response (AUC) to protein and glucose ingestion was one third lower than after glucose alone, and the mean insulin AUC was also considerably greater. While these studies used beef or casein, whey is also effective for both stimulating insulin secretion and reducing postprandial glycaemia in individuals with type 2 diabetes and/or insulin resistance^[48,92].

IS THE DOSE OF WHEY IMPORTANT?

When assessing the magnitude of glycaemic responses after whey protein consumption, one should consider not only the timing of ingestion (*e.g.*, whether giving as a preload), but also the dose, since the effects of whey on glycaemic responses, as well as appetite, appear to be dose-dependent^[19,93]. Preloads of whey concentrate in doses of 5 g, 10 g, 20 g, and 40 g, and control, were given to 22 healthy individuals, followed 30 min later by a standardised pizza meal; the 20 g and 40 g whey preloads suppressed appetite more than control, or 5 g or 10 g whey protein, as assessed by visual analogue questionnaires^[93]. In addition, whey protein reduced postprandial glucose in a dose-dependent manner. Poppit *et al.*^[94] gave 50 overweight women drinks containing 5 g, 10 g or 20 g whey, or control, 120 min after a

standardized breakfast, and found that there was a tendency for hunger and fullness to be dose-related, although this did not reach statistical significance.

In healthy volunteers, whey protein taken with a meal increases insulin and reduces postprandial glycaemia in a dose-dependent manner^[87]. Gunnerud *et al.*^[87] found that a drink containing 25 g glucose and either 4.5 g, 9 g or 18 g whey protein, reduced postprandial glycaemia (iAUC) by 25%, 37% and 46% respectively, compared to a 25 g glucose alone; the reductions with 9 g and 18 g whey were statistically significant. There was also a dose-dependent increase in insulin (iAUC 0 – 120 min), which reached statistical significance with the highest dose of whey.

While whey has convincing dose-dependent effects on glucose, insulin and appetite, the optimal dose for improving long-term glycaemic control in people with type 2 diabetes is yet to be determined.

WHEY AND APPETITE REGULATION

Reduction in energy expenditure and appetite may be achieved through manipulation of dietary macronutrient composition^[95]. Protein has been shown to be more satiating than other macronutrients such as carbohydrate and fat^[16,96], and has also been reported to increase satiety^[97-99]. Whey protein, in particular, has been shown to enhance satiety and reduce food intake at the next meal in acute studies^[93,100], and this effect is thought to be mediated by gut hormones^[17,101], specifically by stimulation of CCK, PYY and GLP-1, and by suppression of the orexigenic hormone, ghrelin^[16].

Bowen *et al.*^[95] reported prolonged postprandial suppression of ghrelin, and elevation of GLP-1 and CCK, after consumption of whey, gluten and soy based preloads compared with glucose, and this was associated with reduction of energy intake at an *ad libitum* meal. CCK is typically associated with satiety; however, in this study there was a trend for an inverse relationship between CCK and subsequent energy intake, which suggests that CCK can also contribute to satiety. Similarly, in a study where hunger scores were reduced after whey ingestion compared to casein, the CCK and GLP-1 responses were higher following whey, which may have contributed to its greater satiating effect^[17]. Other studies have reported that PYY concentrations are higher after whey compared with other proteins, but with comparable CCK and ghrelin responses^[64].

DIRECT EFFECTS OF AMINO ACIDS ON HUNGER

Elevation in plasma concentrations of amino acids after ingestion of whey may affect appetite^[102,103] by hitherto poorly defined mechanisms, including vagal feedback and direct suppression of hunger at the level of the hypothalamus^[104]. The greater suppression of hunger by whey, when compared to soy or casein, is associated

with increased concentrations of the amino acids leucine, lysine, tryptophan, isoleucine, and threonine^[105]. Furthermore, tryptophan is synthesised into serotonin, which itself is known to influence food intake^[103,106].

EFFECT OF WHEY ON ENERGY EXPENDITURE

Energy expenditure from thermogenesis, which increases oxygen consumption and body temperature, is thought to induce feelings of satiety^[107]. Of the macronutrients, dietary protein stimulates thermogenesis and satiety more than carbohydrate or fat^[103]. Acheson *et al.*^[108] reported that whey protein elicits a greater thermic response than protein composed of either casein or soy, where protein accounted for 50% of the energy content of the meal. This may be because whey protein, as a "fast" protein, is rapidly digested to result in greater postprandial protein synthesis^[18]. In particular, leucine, which is present in high concentrations in whey^[109], has been shown to stimulate muscle protein synthesis^[110] and may also increase postprandial energy expenditure^[109].

EFFECTS OF LONG TERM CONSUMPTION OF WHEY PROTEIN ON GLYCAEMIC CONTROL

High protein diets induce weight loss and preserve lean mass^[111]. However, there is a paucity of data relating to whether whey has the capacity to reduce glycated haemoglobin with ongoing treatment in patients with type 2 diabetes.

A 5-wk study in 8 men with type 2 diabetes showed that a diet containing 30% vs 15% of total energy derived from protein, with a corresponding decrease in carbohydrate content, was associated with a greater (by about 0.5%) decrease in glycated haemoglobin^[112]. In another study, 72 non-diabetic obese men were randomised to receive supplements of either whey protein isolate, casein, or glucose (each 54 g/d), 30 min before breakfast and the evening meal for 12 wk. Improvements in fasting insulin and homeostasis model assessment of insulin resistance score of almost 10% were observed with whey compared to control, but there was no difference in the fasting serum glucose^[113].

In considering the use of whey protein in the management of diabetes, it is also important to recognise the potential adverse effects of longer term supplementation. There have been concerns that high protein diets could potentially reduce bone density and impair renal function. However, a recent two year weight loss study in postmenopausal women found no clinically significant effect of a high protein diet on bone density^[114]; nor was there any reduction in renal function in a one year weight loss study in patients with type 2 diabetes with microalbuminuria, assigned to a high protein diet (≥ 90 g protein/d)^[111,115].

The effects of additional energy intake associated with protein supplements should also be considered if using this strategy over the long term. Subjects tend to compensate for the additional energy load by eating less at a subsequent *ad libitum* meal in acute and short term (5 d) studies^[116,117]. This is supported by a 12-wk study in which overweight men received 54 g whey supplements per day, but showed no change in body composition^[113]. Age may be an important determinant of this effect, however; Soenen *et al.*^[56] observed that older men (aged 68 to 81 years), had less capacity to compensate for the additional energy intake associated with whey administration when compared to young men.

Whey's ability to slow gastric emptying is one of the main mechanisms by which postprandial glycaemia is reduced acutely after a meal. However, it is unknown whether the capacity for whey to slow gastric emptying is sustained with prolonged exposure, or whether there is an adaption to this macronutrient of the gut feedback mechanisms that control gastric emptying, as has been demonstrated for carbohydrates and fats^[118]. It would therefore be important to establish whether slowing of gastric emptying induced by whey is sustained with prolonged exposure; this appears to be the case over four weeks in a small pilot study^[119].

CONCLUSION

The acute effects of whey protein on postprandial glycaemic excursions appear promising, but the long term efficacy and optimal application in the management of type 2 diabetes remain to be determined.

Patients most likely to benefit from postprandial glucose lowering by whey protein are those with mild to moderate elevation of HbA1c, who have relatively well controlled fasting glucose, since this is the group of patients in whom postprandial glycaemia makes the greatest relative contribution to HbA1c. However, combining a dietary strategy with pharmacological agents in less well controlled patients should also be evaluated, such as the combination of insulin to control fasting glucose, together with whey protein to reduce postprandial glycaemia; such a concept has proven to be effective with the combination of basal insulin and short-acting GLP-1 receptor agonists^[120]. Moreover, the combination of whey protein with a DPP-IV inhibitor should also be examined, given the potential to augment the stimulation of GLP-1^[121].

The timing of protein ingestion is important when aiming to stimulate incretin secretion and suppress appetite in advance of the main meal^[48], and this, together with the optimal dose of whey protein, requires further refinement.

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Editorial Board Member of *World Journal of Diabetes*, Massimo Collino, PhD, Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, via P. Giuria 9, 10125 Torino, Italy

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Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: editorialoffice@wjgnet.com

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New perspectives on exploitation of incretin peptides for the treatment of diabetes and related disorders

Nigel Irwin, Peter R Flatt

Nigel Irwin, Peter R Flatt, SAAD Centre for Pharmacy and Diabetes, University of Ulster, Coleraine BT52 1SA, Northern Ireland, United Kingdom

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Correspondence to: Dr. Nigel Irwin, SAAD Centre for Pharmacy and Diabetes, University of Ulster, Cromore Road, Coleraine BT52 1SA, Northern Ireland, United Kingdom. n.irwin@ulster.ac.uk
Telephone: +44-28-70324574
Fax: +44-28-70323939

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Abstract

The applicability of stable gut hormones for the treatment of obesity-related diabetes is now undisputable. This is based predominantly on prominent and sustained glucose-lowering actions, plus evidence that these peptides can augment insulin secretion and pancreatic islet function over time. This review highlights the therapeutic potential of glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), oxyntomodulin (OXM) and cholecystokinin (CCK) for obesity-related diabetes.

Stable GLP-1 mimetics have already been successfully adopted into the diabetic clinic, whereas GIP, CCK and OXM molecules offer promise as potential new classes of antidiabetic drugs. Moreover, recent studies have shown improved therapeutic effects following simultaneous modulation of multiple receptor signalling pathways by combination therapy or use of dual/triple agonist peptides. However, timing and composition of injections may be important to permit interludes of beta-cell rest. The review also addresses the possible perils of incretin based drugs for treatment of prediabetes. Finally, the unanticipated utility of stable gut peptides as effective treatments for complications of diabetes, bone disorders, cognitive impairment and cardiovascular dysfunction is considered.

Key words: Diabetes; Obesity; Incretin; Prediabetes; Gut hormones

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Core tip: Stable gut hormones have well defined therapeutic actions for type 2 diabetes mellitus. In addition, simultaneous modulation of gut hormone receptors could increase therapeutic efficacy, but timing and receptor activation profile may be important. Finally, gut-derived peptides could possess benefits for bone disorders, cognitive impairment and cardiovascular dysfunction.

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INTRODUCTION

The human gastrointestinal tract (GIT) comprises the stomach, as well as the small (duodenum, jejunum

and ileum) and large (caecum, colon and rectum) intestines. Aside from nutrient digestion, absorption and assimilation, the GIT also has significant endocrine functions^[1]. To date, the most important endocrine function of the gut relates to evidence that intestinal derived peptides are fundamentally involved in post-prandial insulin release^[2]. This action is termed the “incretin effect”, and relates to the direct beta-cell insulin secretory effect of two hormones, namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) that are secreted from L- and K-cells, respectively (Figure 1)^[3]. A number of other enteric peptide hormones released in response to feeding also have a role in energy regulation and possibly insulin secretion, including cholecystokinin (CCK) and oxyntomodulin (OXM) (Figure 1)^[4,5]. However, only GLP-1 and GIP fulfil the criteria of a true incretin hormone that stimulates glucose-induced insulin secretion at physiological circulating concentrations^[3]. Despite the obvious potential of incretin and incretin-like peptides for the treatment of conditions such as diabetes and obesity, the extremely short biological half-life of these peptides, due to efficient enzymatic degradation and subsequent renal filtration, severely limits therapeutic applicability^[4,5]. However, interest in gut peptides has increased in recent years with knowledge that modified versions of these compounds, with vastly improved pharmacokinetic properties, have sustained beneficial physiological effects^[6].

GLP-1

The biological actions of GLP-1 are largely preserved in type 2 diabetes and pharmacological doses of the peptide evoke robust insulin-releasing and antihyperglycaemic effects^[7]. GLP-1 exerts its beta-cell effects through interaction with specific surface receptors that activate signal transduction pathways including the stimulation of intracellular cAMP mediated events^[8]. GLP-1 also promotes beta-cell proliferation and islet cell neogenesis as well as inhibiting beta-cell apoptosis and alpha-cell glucagon secretion^[8]. Notably, both GLP-1 and GIP expression and secretion has been described in islet alpha cells^[9,10]. Indeed, it is feasible that intra-islet, rather than gut derived, GLP-1 and GIP make a significant contribution to these direct beneficial islet effects^[11-13]. However, it should be noted that positive direct islets effects are still noted in rodents following prolonged exogenous delivery of stable GLP-1 mimetics^[8].

GLP-1 not only targets pancreatic islet cells, but imparts positive actions in terms of inhibition of gastric emptying, suppression of appetite and weight loss^[8]. Given this advantageous biological action profile, there are now several GLP-1 related enzyme-resistant, long-acting analogues available for clinical use in diabetes (Table 1), ranging from regimens that require twice daily injection to those that necessitate only once weekly administration^[14]. Development of

new GLP-1 mimetics, such as those conjugated to an antithrombin III-binding pentasaccharide, are also in the pipeline^[15]. Interestingly, a recent commentary highlights that differences in the structure and pharmacokinetics of currently available GLP-1 mimetics could significantly alter immunogenicity, CNS signalling and overall therapeutic effect^[16]. Thus, physicians may need to re-evaluate the most appropriate GLP-1 treatment strategy for each patient. Encouragingly however, GLP-1-R agonists demonstrate an efficacy approaching that of insulin treatment, but unlike insulin have the added benefits of promoting weight loss with minimal risk of hypoglycaemia^[17].

Despite the widespread use of GLP-1 mimetics (Table 1), there have been recent safety concerns regarding the ability of sustained GLP-1-R activation to cause pancreatitis, pancreatic and thyroid cancer, as well as glucagon-producing neuroendocrine tumours in man^[18,19]. As such, it is well recognised that pancreatitis is a risk factor for pancreatic cancer^[20]. However, a recent meta-analysis did not support increased risk of pancreatitis or cancer associated with GLP-1 therapy^[21]. Indeed, issues with poorly matched patient groups treated with incretin-based vs non-incretin-based medications and problems with specifically identifying glucagon-producing cells also calls into question the validity of these safety concerns^[22]. Thyroid cancer fears appear to stem largely from rodent studies^[23], and reduced expression of the GLP-1 receptors in human, as opposed to rodent, thyroid cells is the likely explanation for this^[24]. The most frequently reported side effect of GLP-1 therapy is dose-dependent and transient mild to moderate nausea, vomiting and diarrhoea^[16]. Thus, taken together the safety profile of GLP-1 based therapeutics is largely reassuring. However, pharmacovigilance with GLP-1 drugs is still required, especially in relation to patients with a history, or increased risk, of pancreatitis or thyroid cancer.

GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE

Although initially thought to play a role in impeding histamine induced gastric acid secretion^[25], the primary physiological role of GIP is now considered to be stimulation of postprandial insulin secretion^[13]. The insulinotropic action of GIP, mediated by specific receptors on the surface of pancreatic beta-cells, is initiated largely by intracellular cAMP generation (Figure 1) and subsequent Ca²⁺ ion influx leading to insulin granule exocytosis^[13]. An additional beneficial action of GIP involves enhanced survival of beta-cells, which is also mediated through cAMP dependent cell signaling pathways^[26,27]. GIP also acts as beta-cell growth factor by stimulating mitogen-activated protein kinase pathways^[28] and modulating K_{ATP} channel expression^[29]. Given this impressive bioactive profile at the level of the beta-cell, there has been significant interest in the potential for GIP-based pharmaceuticals as antidiabetic

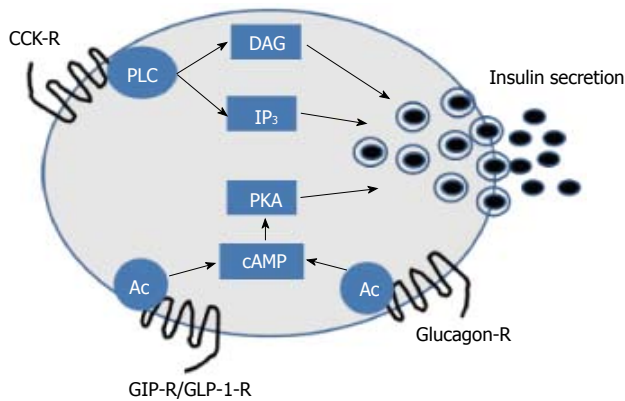


Figure 1 Schematic depicting the major signalling pathways involved in glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1, glucagon and cholecystokinin induced insulin secretion from pancreatic beta-cells. AC: Adenyl cyclase; cAMP: Adenosine 3'-5'-cyclic monophosphate; DAG: Diacyl-glycerol; IP₃: Inositol 1,4,5-trisphosphate; PKA: Protein kinase A; PLC: Phospholipase C; CCK: Cholecystokinin.

drugs. However, like GLP-1 the pharmacokinetic profile GIP is severely hindered due to rapid plasma degradation by the ubiquitous enzyme dipeptidyl peptidase 4 (DPP-4), and clearance cleared from the body by efficient renal filtration^[30]. In addition to this, the biological effects of GIP appear to be markedly reduced in patients with type 2 diabetes when compared to normal individuals^[7].

The first of these barriers has been conquered, as with GLP-1 mimetics, through generation of N-terminally modified enzyme-resistant, long-acting GIP molecules, and these molecules has been reviewed extensively elsewhere^[31,32]. However, the issue of reduced GIP responsiveness in type 2 diabetes still remains, and is thought to be linked to GIP receptor (GIP-R) down-regulation or desensitisation^[7]. However, it is highly likely that that GIP desensitisation is a pathophysiological consequence as opposed to an aetiological factor of type 2 diabetes. In keeping with this, studies correcting hyperglycaemia using insulin or sulphonylureas indicate that GIP sensitivity can be restored^[33,34]. It has also been demonstrated that a K-cell derived peptide co-secreted from the intestine with GIP, xenin-25, can potentiate the insulinotropic action of GIP^[35,36]. As such, a novel long-acting palmitate-derivatised analogue of xenin-25 was shown to significantly augment GIP action *in vitro* and *in vivo*^[37]. Moreover, sustained administration of this acylated xenin peptide exerted a spectrum of beneficial metabolic effects in high-fat-fed mice^[38]. This presumably relates to restoration of GIP action in these diabetic mice^[38]. In harmony with this, a recent study indicates that the impaired insulinotropic response to GIP under diabetic milieu involves mechanisms beyond simple expression of the GIP-R^[39], further highlighting a potential role for xenin. Therefore, there still appears to be significant, as yet untapped, therapeutic potential for GIP-based compounds, especially in combination with molecules that can enhance GIP sensitivity directly or counter hyperglycaemia through other actions.

Table 1 Incretin-based drugs currently approved by the European Medicines Agency

Drug name	Primary mechanism of action	EMA approval date
Exenatide	GLP-1 receptor agonist	Nov-06
Sitagliptin	DPP-4 inhibitor	Mar-07
Vildagliptin	DPP-4 inhibitor	Sep-07
Liraglutide	GLP-1 receptor agonist	Jun-09
Saxagliptin	DPP-4 inhibitor	Oct-09
Exenatide-LAR	GLP-1 receptor agonist	Jun-11
Linagliptin	DPP-4 inhibitor	Aug-11
Lixisenatide	GLP-1 receptor agonist	Feb-13
Alogliptin	DPP-4 inhibitor	Sep-13
Dulaglutide	GLP-1 receptor agonist	Jan-15

DPP-4: Dipeptidyl peptidase 4; GLP-1: Glucagon-like peptide-1; LAR: Long-acting release; EMA: European medicines agency.

OXYNTOMODULIN

Similar to GLP-1, OXM is an L-cell derived proglucagon gene product secreted in response to feeding^[40]. To date a specific OXM receptor has not been described, and the biological actions of OXM are attributed to binding and activation of GLP-1 and glucagon receptors (Figure 1), albeit with reduced potency compared to the native ligands^[41]. *In vitro* and *in vivo* rodent studies suggest that through glucagon receptor agonism, OXM induces catabolic effects that favour weight loss and subsequent improved metabolic control, while glucose homeostasis and insulin resistance are improved through activation of GLP-1 receptors^[5]. Promisingly, data from small clinical studies implies that beneficial effects on energy intake and weight loss also occur in humans^[42,43]. However, as is this case for the incretin hormones, the therapeutic potential of OXM-based molecules is hindered by rapid cleavage of the first two N-terminal amino acids of OXM by DPP-4 in plasma, rendering the peptide inactive^[44]. Nonetheless, structure-function studies show that N-terminal modification can protect against DPP-4 degradation without disproportionately affecting bioactivity of the molecule^[44,45]. Indeed, a recent study of six novel OXM analogues has revealed that Oxm-based peptides with specific N-terminal position 2 modifications are stable and show particular promise for the treatment of diabetes^[46]. These data suggest that further exploration of dual agonism of the GLP-1 and glucagon receptor is required for human diabetes. It is notable that co-administration of GLP-1 and glucagon in humans can replicate the beneficial actions of OXM^[47], although this approach may be more cumbersome in clinical practice.

CHOLECYSTOKININ

CCK is an intestinal I-cell derived gut hormone secreted in response to meal ingestion^[48]. CCK binds to specific CCK₁ receptors present on gastric mucosa and vagal afferent neurons which collectively leads to gallbladder secretions, release of pancreatic digestive juices, satiety and slowing

of gut motility^[1]. CCK₂ receptors are mainly confined to the gastrointestinal tract and brain and may have a role in regulating anxiety and locomotion^[49]. Importantly, CCK has also been shown to stimulate insulin secretion in rodents and man (Figure 1)^[50,51], and act as a growth and anti-apoptotic factor for pancreatic beta-cells^[52]. Thus, CCK agonists could have noteworthy potential for diabetes therapy, since their biological action profile is similar to the incretin hormones. However, native CCK is rapidly degraded by serum aminopeptidases upon secretion into the bloodstream^[53], which hinders therapeutic potential. However, early studies have clearly shown that both N-terminal modification through glycation, or PEGylation, can prevent enzymatic degradation of CCK and extend biological action and therapeutic potential^[53,54]. Following on from this, a more recently developed enzymatically stable, N-terminally modified, CCK analogue, namely (pGlu-Gln)-CCK-8, has been shown to have an improved pharmacodynamic profile, and to both alleviate and protect against obesity-related diabetes in animal models^[51,55], with an encouraging safety profile^[56]. The mechanism of action of (pGlu-Gln)-CCK-8 likely revolves around prominent and sustained reductions of energy intake, possibly related to modulation of central neuropeptide Y and melanocortin related pathways, and enhanced insulin release^[57]. Encouragingly, a PEGylated version of (pGlu-Gln)-CCK-8 has now been fully characterised, that would be resistant to kidney filtration, and suitable for possible once daily dosing in man^[58]. Further investigations relating to translation of beneficial effects to human type 2 diabetes together with safety evaluation are still required, but initial observations with specific and stable CCK₁ receptor agonists are encouraging.

MULTI-TARGET HYBRID PEPTIDE THERAPIES FOR DIABETES

Given the beneficial effects of OXM-based peptides, it follows that design of hybrid peptides capable of modulating more than one receptor pathway could have distinct therapeutic benefits for the treatment of obesity-related diabetes. By utilising the correct ratio of receptor pathway interactions, efficacy should be enhanced with the potential for administration of lower doses, thereby reducing, or removing, adverse side effects. The most logical starting point for design of a synthetic dual acting hybrid peptide would inevitably involve a modified incretin hormones capable of activating both GIP and GLP-1 receptors. As such, GIP/GLP-1 chimeric peptides were characterised almost 20 years ago, and the structural requirements for specific ligand-receptor interactions well defined^[59]. Combined administration of individual long-acting GIP and GLP-1 mimetics has been considered in preclinical studies, with some success^[60]. However, issues of separate drug formulation and dosing still remain, although these may not be insurmountable as indicated by recent

introduction of IDegLira for combined insulin and GLP-1 therapy in type 1 diabetes^[61]. In terms of a single hybrid peptide that can directly activate both GIP and GLP-1 receptors, only MAR701, Marcadia Biotech (now Roche) has progressed to the evaluation of beneficial effects in man. However, since the clinical benefits of DPP-4 inhibitors clearly involves increased circulating levels of both incretin peptides^[62], concomitant activation of GIP and GLP-1 receptors does appear to have promise for the treatment of type 2 diabetes (Table 1).

Further studies have investigated the effects of GLP-1 receptor agonism combined with either glucagon receptor agonism or antagonism^[63,64]. Although somewhat contradictory in nature, these contrasting regimens both utilise the beneficial glucose-lowering effects of GLP-1, combined with either inhibition of glucagon-mediated gluconeogenesis and glycogenolysis^[65], or activation of glucagon pathways involved in energy turnover and weight loss^[64], as is this case for OXM. Other modified hybrid peptides for dual activation of regulatory peptide receptors include, ZP3022, a combined GLP-1-gastrin agonist^[66]. Through activation of GLP-1 and CCK₂ receptors, this peptide improved glycaemic control in *db/db* mice *via* enhancement of beta-cell mass^[66]. However, perhaps more appealing is the potential for combined and sustained activation of GLP-1 and CCK₁ receptors. As such, two independent studies have clearly shown pronounced synergistic metabolic benefits with combined administration of long-acting GLP-1 and CCK₁ receptor agonists in rodent models of type 2 diabetes^[67,68]. These extremely positive effects are believed to occur through activation of complementary pathways that lead to significant weight loss and dramatically improved metabolic control^[67,68]. Furthermore, a novel CCK/GLP-1 hybrid peptide, based on the chemical structures of (pGlu-Gln)-CCK-8 and exenatide, has recently been described and shown to have significant therapeutic potential in high-fat fed mice^[69]. This molecule clearly warrants further study as a potential new treatment option for type 2 diabetes.

Considering the evident therapeutic efficacy offered by dual peptide receptor interactions, single compounds with the ability to concurrently activate three or more regulatory peptide receptors could deliver even greater beneficial effects. Moreover, the celebrated success of bariatric surgery for restoring metabolic control in type 2 diabetic patients, independent of weight loss^[70], results from a culmination of reduced energy intake and modulation of the secretion and biological action of numerous gut-derived peptides^[71]. Thus, there is now significant enthusiasm arising from designer modified peptides with the ability to concurrently modulate GIP, GLP-1 and glucagon receptor signalling^[72,73]. These triple-acting peptides have resulted in dramatic improvements in glucose homeostasis and overall metabolic control in high fat fed mice^[72,73]. Despite their obvious potential, issues regarding the ratio of GIP, GLP-1 and glucagon receptor activation still need to be addressed, As such,

a subsequent study has reported the distinct beneficial effects of a balanced glucagon, GLP-1 and GIP receptor tri-agonist to correct obesity and diabetes in high fat fed mice^[74]. Taken together, there is a clear and attractive rationale for further testing of combinatorial hormone therapies for the treatment of obesity and diabetes in humans.

Although the future trend for peptide-based anti-diabetic drugs seems to be development dual or triple agonists, treatment modalities that incorporate periods of beta-cell rest could be important for glycaemic control^[75]. Thus, antidiabetic drugs that induce direct beta-cell stimulatory effects can erode beta-cell mass over time^[76]. As such, intermittent periods of beta-cell rest may be useful to preserve long-term beta-cell function and lasting glycaemic control^[75]. In contrast to sulphonylureas and meglitinides, incretin based drugs stimulate insulin secretion in a glucose-dependent fashion that should help preserve beta-cell mass and function^[8]. Nonetheless, adequate periods of rest might still allow chronically stimulated pancreatic beta-cells to replenish both cell surface receptors and the immediately secretable insulin granule pool^[77]. Such effects, together with the positive actions of incretins on beta-cell stimulus-secretion coupling, survival and growth, could be highly beneficial. Accordingly, the timing of injections of dual or triple acting therapies, as well as the profile of receptor pathways activated, could be of valuable clinical relevance. In relation to this, inhibition of GIP-R signalling has been shown to improve metabolic control and glycaemic status in animal models of obesity-related diabetes by enhancing insulin action and diminishing insulin secretion^[78,79]. Thus a key aspect underlying the beneficial effects could be related to the induction of pancreatic beta-cell rest. Consistent with this, combination of morning injection of liraglutide, with stable GIP antagonist peptide in the evening, greatly improved glycaemic control in *db/db* mice compared with reciprocal administration or twice daily injection of liraglutide^[80]. Further investigation of this potentially important treatment paradigm, in combination with other agents that stimulate and/or relieve beta cell insulin release, is required to fully explore therapeutic relevance and applicability.

INCRETIN THERAPIES AND PREDIABETES

Prediabetes describes to a situation where blood sugar is high, but not elevated sufficiently to classify as overt type 2 diabetes. However, the condition represents a high risk state for future development of diabetes, most likely linked to progressive beta-cell decline^[81]. Thus, it follows that the positive effects of incretin mimetics on beta-cell function, including possible benefits for beta-cell proliferation and survival, plus additional weight-lowering and extrapancreatic actions^[8], could hold significant promise for prediabetic patients. Moreover, patients with prediabetes have been shown to have

an impaired incretin effect in response to oral nutrient delivery^[82].

To date, there have been several tentative clinical studies conducted on the potential beneficial effects of incretin-based drugs for prediabetes. Studies with DPP-4 inhibitors (Table 1), which prevent incretin peptide degradation and increase active circulating levels of GIP and GLP-1, reported modest positive effects^[83-85]. However, treatment with the stable incretin mimetics, exenatide or liraglutide, generated more positive outcomes^[86,87]. This included significant reductions in the prevalence of prediabetes with reversion to normal glucose tolerance^[86,87]. The inconsistency between DPP-4 inhibitors and GLP-1 mimetics most likely relates to differences in the circulating levels of active hormones achieved. However, issues of oral vs injectable delivery of DPP-4 inhibitors and GLP-1 mimetics, respectively, could significantly affect compliance in this patient subgroup. In addition, the potential adverse side-effect profile of incretin based therapies, as discussed above, would also have to be fully considered. Finally, the cost of therapy with DPP-4 inhibitors and particularly GLP-1 mimetics is greater when compared to other glucose-lowering agents^[88]. Thus, given the limited experience to date regarding the effect of incretin therapies in prediabetes, future clinical trials would be recommended. In terms of GIP, CCK and OXM therapies, clinical effectiveness in type 2 diabetes would need to be fully established before beneficial actions in prediabetic patients could be considered.

UNEXPECTED THERAPEUTIC POTENTIAL OF INCRETIN BASED DRUGS

Bone

Although incretin hormones have been studied extensively for therapeutic effectiveness in diabetes, research has uncovered unexpected benefits in various other tissues. For instance, a role for gastrointestinal derived hormones in bone remodeling is suspected since serum levels of bone biomarkers rapidly alter after a meal^[89]. Indeed, functional GIP receptors have been evidenced on the surface of bone cells^[90]. Notably, GIP has been shown to inhibit bone resorption in humans under both euglycaemic and hyperglycaemic states^[91]. Thus, the beneficial effects of GIP on bone could be independent of feeding state. Indeed, exogenous prolonged administration of an N-terminally modified stable GIP receptor agonist imparted various beneficial effects on tissue-level bone material properties of rats^[92]. In terms of GLP-1 effects on bone, the picture is less clear. This mostly relates to data from animal models being clouded by the fact that GLP-1 receptors are highly expressed on rodent thyroid cells, resulting alterations of circulating calcitonin levels^[93]. Nonetheless, GLP-1 receptors have been found on the surface of human osteoblast-like cells^[94]. Moreover, very recent data suggest that liraglutide has anabolic effects on bone

in diabetic rats^[95]. In keeping with this, a study in double incretin receptor knockout mice^[89], reported a combination of detrimental bone abnormalities that mimicked observations from both GIP^[96,97] and GLP-1^[98] receptor knockout mice. Despite these observations in rodents, a preliminary meta-analysis suggests that GLP-1 mimetics do not modify the increased bone fracture risk in humans with type 2 diabetes^[99], or could even potentially increase fracture risk in this population^[100]. In keeping with this, a retrospective population based cohort study has suggested that DPP-4 inhibition is not associated with reduced fracture risk in humans^[101], whereas bone loss and strength were significantly improved by sitagliptin therapy in diabetic rats^[102]. Care is required therefore when extrapolating data on the effects of incretin-like drugs on bone from rodents to man, particularly in the case of GLP-1. However, actions of GIP are particularly promising and further research is required to determine if incretin hormones can be useful to treat abnormalities of bone encountered in diabetes and osteoporosis.

Brain

In terms of the central nervous system, expression of functional GIP and GLP-1 receptors has been demonstrated in several brain regions^[103]. Much of the therapeutic interest for incretin-like molecules in the CNS revolves around neuroprotective effects for the treatment of Alzheimer's and Parkinson's diseases, as well as cognitive impairments in diabetes^[3,104]. Accordingly, GIP receptor knockout mice exhibit impaired memory learning, synaptic plasticity, and neurogenesis^[105]. In agreement, transgenic mice that over-express GIP exhibit enhanced sensorimotor coordination and memory recognition^[106]. Earlier studies have already shown that stable forms of GIP can beneficially modulate synaptic transmission and enhance the induction of long-term potentiation, an important physiological cellular means of monitoring learning processes^[107]. In addition, prolonged GIP receptor activation improved cognitive function, hippocampal synaptic plasticity and glucose homeostasis in obese-diabetic high-fat fed mice^[108]. In agreement with this, GLP-1 receptor knockout mice display an impairment of synaptic plasticity and memory formation^[109]. Furthermore, sustained treatment with long-acting GLP-1 agonists improves memory and learning in various rodent models of neurodegeneration and diabetes^[108,110,111]. Moreover, liraglutide treatment has recently been shown to restore cerebral and systemic microvascular architecture in a rodent model of genetically-induced cognitive dysfunction^[112]. Based on the positive neuroprotective effects of incretin compounds, there are several ongoing clinical trials with these drugs that should reveal encouraging effects for the potential treatment of Alzheimer's and Parkinson's diseases^[104]. Finally, in harmony with the positive effects of incretin molecules on brain function, sitagliptin treatment was recently shown to improve recognition memory, oxidative

stress and hippocampal neurogenesis in diabetic mice^[113]. Collectively, these observations strengthen the possibility that incretin peptides play a direct role in modulating aspects of brain function and could possess key clinical pharmacological benefits for patients with diabetes and neurodegenerative disorders.

Heart and vasculature

The GLP-1 receptor has been demonstrated in the heart^[114]. Although some controversy still exists as to the exact location of the receptor within the heart, various studies confirm the presence of GLP-1 receptor mRNA transcripts in rodent and human cardiac tissue^[115]. In cardiomyocytes GLP-1 receptor signalling induced elevations in cAMP levels, but surprisingly this was not coupled to an increase in intracellular Ca²⁺ concentrations and cardiomyocyte contractility^[116]. Indeed, there could be a paradoxical reduction in cardiomyocyte contractility despite elevated cAMP levels^[116]. Moreover, GLP-1 receptor knockout mice present with decreased ventricular contractile function^[117]. As such, the exact mechanism of action and physiological relevance of GLP-1 receptor signalling in the heart requires further detailed investigation. Despite this, and similar to the situation in pancreatic beta-cells, GLP-1 appears to have anti-apoptotic effects in cardiomyocytes and improves overall outcomes in mice after myocardial infarction^[118]. Further to this, GLP-1 receptor protein has also been detected in human coronary artery endothelial cells and encouragingly, activation is believed to improve endothelial cell function in diabetic patients^[119]. Thus, prospective clinical trials are ongoing to assess the cardiovascular safety profile of GLP-1 based peptides, and initial observations in humans with diabetes are positive^[120]. Whilst the GIP receptor is believed to be present in the heart and on vasculature^[103], there is a paucity of knowledge in relation to GIP effects on these tissues. Stimulation of GIP receptors may induce conflicting effects in different vascular beds^[121], and this could explain for its unaccounted physiological effects in these tissues. In keeping with this, the overall effect of DPP-4 inhibition on cardiovascular function is still not clear^[122].

FUTURE DIRECTIONS

Stable gut hormones have considerable potential for the treatment of obesity-related diabetes, and possibly other related pathologies. Whilst disorders of bone, cognitive function and the cardiovascular system can be considered as complications of diabetes, they are also standalone distinct illnesses in their own right. Thus, the therapeutic outlook of incretin mimetics may stretch well beyond diabetes. However, to date only GLP-1 based drugs are clinically available, exclusively for the treatment of type 2 diabetes and associated obesity. Concerns regarding the safety of GLP-1 analogues in man appear to have been allayed, but pharmacovigilance is still required. The potential promise of incretin based drugs

such as GLP-1 mimetics for the treatment of prediabetes still requires detailed investigation. Stable forms of GIP, OXM and CCK also appear to offer distinct therapeutic possibilities for the treatment of type 2 diabetes based on data from animal models and preliminary human studies. Given this, and the multifactorial pathological nature of diabetes, it is not unexpected that concurrent activation of more than one regulatory peptide receptor signalling pathway appears to have promise for the future treatment of diabetes. This may be achieved through the development of double or triple acting agonists or use of a cocktail of existing peptidergic drugs. However, note should be taken of emerging evidence suggesting the utility of sequential peptide exposures to facilitate essential periods of beta-cell rest. Taken together, future advances in our understanding of gut peptide biology, coupled with therapeutic application, should lead to an expansion of clinically available gut peptide-based drugs with far-reaching benefits to the patient.

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Role of adiponectin and some other factors linking type 2 diabetes mellitus and obesity

Chandra Kanti Chakraborti

Chandra Kanti Chakraborti, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela 769015, Odisha, India

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Correspondence to: Dr. Chandra Kanti Chakraborti, Professor, Kanak Manjari Institute of Pharmaceutical Sciences, Chhend, Rourkela 769015, Odisha, India. chandrakanti_12@rediffmail.com
 Telephone: +91-0977-6145092
 Fax: +91-0661-2480752

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Abstract

Because of the intimate association of obesity with type 2 diabetes mellitus (T2DM), during the last two decades, extensive research work is being conducted to find out whether the coexistence of the two is a simple association or there is a positive correlating link between the two. In this article, an attempt has been made to collect and analyse the recent developments in this

field and to arrive at a conclusion on the subject. The possible role of several important factors (obtained from adipocytes/not of adipocyte origin) in linking the two has been discussed in detail. Some of the agents, specifically adiponectin, are beneficial (*i.e.*, reduce the incidence of both), while others are harmful (*i.e.*, increase their incidence). From the analysis, it appears that obesity and T2DM are intimately linked.

Key words: Obesity; Insulin; Insulin resistance; Type 2 diabetes mellitus; Adipocyte

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Core tip: The objective of this article is to establish the connection of obesity with that of insulin resistance (IR) and type 2 diabetes mellitus (T2DM) by analyzing the recent developments in this field. The factors linking the three have been found to be some adipocytokines as well as certain other factors not of adipocyte origin. Of these, adiponectin appears to play the most beneficial role (so also leptin, peroxisome proliferator-activated receptors, apelin, *etc.*), while others (tumour necrosis factor- α , interleukin-6, resistin, retinol binding protein-4, dipeptidyl peptidase-4, plasminogen activator inhibitor-1, visfatin, free fatty acid, angiotensin II and toll-like receptors) are harmful. Agonists and antagonists of these factors may be designed to fight against obesity, thereby achieving protection for IR and T2DM.

Chakraborti CK. Role of adiponectin and some other factors linking type 2 diabetes mellitus and obesity. *World J Diabetes* 2015; 6(15): 1296-1308 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i15/1296.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i15.1296>

INTRODUCTION

It is practically established that type 1 diabetes

mellitus is an autoimmune disorder where the tissue-specific antibodies target and cause complete or near complete destruction of islet- β -cells, leading to absolute insulin deficiency. In contrast, type 2 diabetes mellitus (T2DM) is usually a hereditary disorder, commonly (80%) associated with obesity, where deficient insulin action may be due to a real deficiency of insulin or a relative one associated with normal or even elevated plasma concentrations of insulin, *i.e.*, insulin resistance (IR). Such simultaneous occurrence of the two (T2DM and obesity) suggests the possibility of a strong link between them, and during the past two decades several positive correlations between them have been established by many workers^[1-4]. Besides obesity which is directly linked to T2DM *via* adipocytokines, some nonadipocytokines have been found to be related with T2DM indirectly by interfering with the growth, development and functions of adipocytes (mentioned later). In this article, an attempt has been made to collect and analyse some such authentic work-results together that will help the reader to comprehend and assess the developments in this field.

The intimate association of T2DM and obesity is a world-wide phenomenon. Though much knowledge about the pathophysiology, course and consequences of T2DM has been gathered, it is not so with obesity, which was almost practically considered as a cosmetic problem. But recently, because of its frequent association with T2DM as well as with hypertension, extensive work is being continued on the adipocyte anatomy, distribution pattern, physiological function, pathological role and its possible link with T2DM and hypertension.

PHYSIOLOGICAL ROLE OF ADIPOCYTES AND ADIPOSE TISSUE

Primary physiological role of adipose tissue is to insulate and cushion the body, to store fat when it is in excess and to supply it when needed^[5]. The exogenous and endogenous pathways of lipid metabolism, during which free fatty acids (FFAs) are released from the lipoprotein (chylomicron, very low density lipoprotein, *etc.*) - triglyceride (TG) content upon hydrolysis by the enzyme lipoprotein lipase (LPL), their (FFAs) subsequent storage in fat depots as TG again, and their remobilisation into the periphery by hydrolysis of these stored TGs by the hormone sensitive lipase (HSL), is well established^[5,6]. Insulin plays a major role for maintenance of adipocyte-fat content as it is a potent activator and inhibitor of LPL and HSL, respectively^[5].

SECRETIONS OF ADIPOCYTES (ADIPOCYTOKINES)

Recently, adipocytes are considered as endocrine structures because of their wide variety of chemical secretions (adipocytokines), which affect many diverse physiological functions and related pathological processes

of the body, like metabolism of carbohydrates and lipids, coagulation of blood, maintenance of blood pressure, feeding behaviour and inflammation, affecting almost all the organs of the body. Increased adipocyte number and adipose-tissue mass have been found to result in increased plasma adipocytokine level except adiponectin, whose plasma concentration is actually low in obesity^[5]. Diseases like obesity, T2DM and metabolic syndrome are associated with altered plasma adipokine levels.

A brief discussion of the adipocytokines known till-date along with their possible roles in genesis or amelioration of IR and T2DM is made below. Besides the adipokines, possible involvement of certain other factors (not of adipocyte origin) has also been taken into account (Figure 1).

Leptin

Several physiological functions of leptin along with its source and metabolism have been extensively discussed. This adipokine, which is a product of "*ob*" gene but mediates its function through the receptor coded by "*db*" gene, is involved in energy homeostasis of the body by interfering with the food-behaviour of the animal centrally (hypothalamus) *via* several hormones^[7].

Many studies on mice and human beings have shown a beneficial and balancing complementary relationship between leptin and insulin where leptin has been found to reduce appetite, obesity and IR along with improvement of metabolic disturbances associated with T2DM. Moreover, mice with *db/db* gene (deficient leptin action) have been found to be obese and diabetic^[7].

Though the receptors for insulin and leptin are different, both of them mediate their action through some common second messengers. Therefore, it is possible that leptin may trigger some of the same downstream events triggered by insulin. Increase in tissue sensitivity of insulin by leptin may be due to later's action on oxidation of FFAs which is increased in skeletal muscles leading to its (FFAs) decreased blood concentrations^[7].

Because of such functional cooperation, it may be assumed that obesity due to inadequate leptin action may predispose or get associated with IR and T2DM.

Tumour necrosis factor- α

The role of tumour necrosis factor- α (TNF- α) as a pro-inflammatory cytokine is well established^[8]. It is produced by macrophages (mainly) as well as by some other cell types including visceral adipocytes^[8-10]. Recently, it has been shown that besides its pro-inflammatory property, increased TNF- α inhibits insulin transduction mechanism, resulting in inadequate glucose metabolism, IR and obesity. Because visceral fat is a source of TNF- α , increase in such fat (obesity) leads to increased production of this cytokine, which aggravates obesity and a vicious cycle is established leading to predisposition, onset and progression of T2DM along with IR. Hence, reduction of obesity, which in

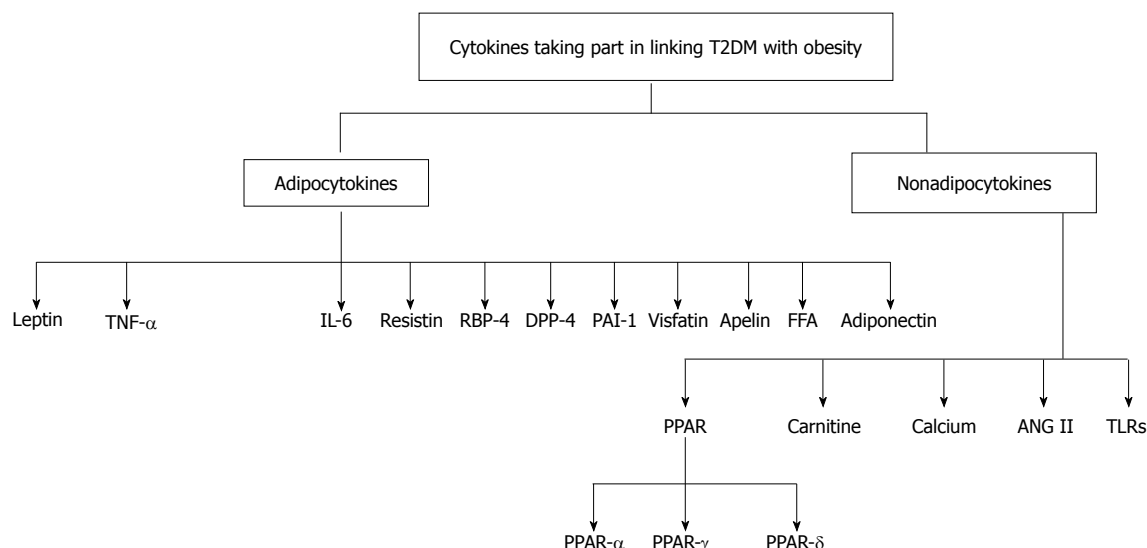


Figure 1 Cytokines linking type 2 diabetes mellitus with obesity. TNF- α : Tumour necrosis factor- α ; IL-6: Interleukin-6; RBP-4: Retinol binding protein-4; DPP-4: Dipeptidyl peptidase-4; FFA: Free fatty acid; PPAR: Peroxisome proliferator-activated receptor; Ang II: Angiotensin II; TLRs: Toll-like receptors; T2DM: Type 2 diabetes mellitus; PAI-1: Plasminogen activator inhibitor-1.

turn may lead to decreased formation of TNF- α , may help to prevent genesis, progression and complications of T2DM^[8]. Besides inhibiting insulin signalling mechanism, TNF- α also has been found to inhibit glucose-induced insulin secretion from β -cells, cause damage to insulin strand and enhance β -cell apoptosis. However, such functions of TNF- α have been demonstrated *in vitro* with concentrations of the cytokine, which was much higher than *in vivo* plasma concentrations^[5]. Moreover, besides visceral adipocytes, macrophages and other cells also produce TNF- α , which may contribute towards the elevated level of this cytokine in obesity^[10]. Therefore, obesity and increased TNF- α levels cannot be directly and definitely implicated with T2DM, although they seem to have a role which needs further investigations^[5,8,9].

Interleukin-6

It is another pro-inflammatory cytokine produced by many cell types (fibroblast, endothelial cells, monocytes) in the body including adipocytes, the production (by adipocytes) being increased in obesity. *In vitro* studies as well as investigations on mice have shown interleukin (IL)-6 to upregulate the production of vascular endothelial growth factor, which is thought to support angiogenesis during adipose tissue growth, leading to increase in the production of IL-6 further (similar to TNF- α)^[5,10].

IL-6 action is mediated through a cytokine class one receptor subtype involving Janus kinase/signal transducers and activators of transcription (JAK/STAT) signal transduction pathway, whereas insulin action is mediated through a receptor family having intrinsic tyrosine kinase activity, signal transduction being carried out through insulin receptor substrate (IRS) proteins. It has been clearly demonstrated that inspite of entirely different receptor involvement, a strong interaction

occurs between the receptor signalling pathway of IL-6 and insulin, leading to impaired biological effect of the later. Though not fully clear, the interaction may involve activation of tyrosine phosphatase, leading to dephosphorylation and inactivation of tyrosine kinase activity or an interaction between suppressor of cytokine signalling proteins and insulin receptors, resulting in deficient insulin action^[10]. Therefore, it appears that elevated plasma levels of IL-6 due to any cause (not necessarily of body fat) may get associated with IR and hence, increased risk of diabetes^[5].

Resistin

This pro-inflammatory cytokine, besides monocytes and macrophages, is also produced by adipocytes. It is so named, because of its capacity to resist insulin action^[1,10,11]. It has a molecular weight of 12.5 kDa and possesses 108 amino acid residues in humans. Unlike adiponectin, this polypeptide has a low circulatory level, which is increased in subjects with IR, T2DM and metabolic syndrome^[3].

Several workers have demonstrated a definite role of resistin in linking obesity to T2DM, during which the cytokine has been found to modulate the insulin signalling pathway, leading to development of IR^[2]. Increased production of resistin has been found to be a result of adipocyte differentiation as well as increase in their number. Locally (from adipocytes) released resistin may play a paracrine role, resulting in inhibition of insulin-induced glucose uptake by adipocytes, which prevents their (adipocytes) further differentiation, thereby reducing its own synthesis and release. This observation may suggest a reciprocal relationship between the two hormones which may further be supported by the fact that rosiglitazone (an oral antidiabetic drug) decreases the circulating concentration of resistin, whereas diet-

induced and genetic forms of obesity increases it^[11]. Moreover, neutralization of resistin has been found to increase the insulin-induced uptake of glucose by adipocytes, whereas resistin itself decreased it.

Recently, it has been observed that resistin-knockout mice show lower fasting blood sugar with increased glucose tolerance and insulin sensitivity associated with reduced hepatic output of glucose. The possible mechanism of this observation may be an overactivity of AMP-activated protein kinase (AMPK) resulting from lack of resistin, leading to reduced expression of genes responsible for hepatic neoglucogenesis. This possible mechanism suggests an opposite role of resistin to that of adiponectin. Again, it was observed that when these resistin-knockout mice were fed with high fat diet, they became obese and IR like their wild counterparts^[10]. All these observations suggest a potential positive link between obesity and T2DM^[11].

Retinol-binding protein-4

This adipocytokine, which is primarily a vitamin A -transport protein, has been recently shown to be linked with IR. Down-regulation of adipocyte GLUT-4 (glucose transporter) has been found to increase the secretion of retinol-binding protein-4 (RBP-4) from adipocytes. In mice, increased serum levels of RBP-4 has been found to be associated with decreased uptake of glucose by skeletal muscles and increased hepatic neoglucogenesis. On the other hand, insulin sensitivity was found to be increased when serum RBP-4 levels were low^[12]. Similar positive correlations between raised plasma RBP-4 level and IR, plasma glucose, BMI and homeostatic model assessment-IR have also been shown in nondiabetics with a high genetic predisposition for T2DM. Interestingly, in this experiment, it was observed that serum RBP-4 levels were raised before significant appearance of diabetic markers^[13]. Such an observation indicates the "elevated plasma RBP-4 level" to be a signal for development of insulin resistance and subsequent T2DM in future^[12,13]. In another experiment, it has been shown that excess of RBP-4 relative to retinol (RBP to retinol ratio) is more accurate in predicting the development of T2DM than raised RBP-4 levels alone^[14].

Dipeptidyl peptidase-4

The incretins (glucagon-like peptide-1 and glucose-dependent insulinotropic hormone) are known to possess favourable effect on carbohydrate and lipid metabolism as they increase postprandial insulin release along with a decrease in release of glucagon. The two incretins, like several other glycoprotein and peptide substrates, are metabolically degraded by the enzyme dipeptidyl peptidase-4 (DPP-4), which reduces their favourable metabolic effects in relation to diabetes and therefore may be considered as diabetogenic. Hence, DPP-4 inhibitors (sitagliptin, vildagliptin, etc.) are now used extensively for management of T2DM along with other antidiabetic agents^[15].

Recently, it has been shown that like other cells, adipocytes also express DPP-4 and substantial over-expression is found in visceral fat of obese persons. Experiments have demonstrated that DPP-4 expression and circulating DPP-4 concentration are well-correlated with adipocyte size and adipose tissue inflammation. This may suggest a stimulatory role of pro-inflammatory adipokines on expression of DPP-4 from adipocytes and other tissues. Thus, increased release of DPP-4 from visceral adipocytes of obese persons may enhance the metabolic degradation of incretins in an autocrine or paracrine manner, thereby reducing their favourable effect on carbohydrate and lipid metabolism which in turn may predispose the concerned obese person for development of T2DM and metabolic syndrome. In another study, it has been shown that explants from subjects release more DPP-4 and the release is reduced after weight reduction^[15]. Moreover, in insulin-sensitive obese patients, plasma concentration of DPP-4 has been found to be lower than those of insulin-resistant obese diabetics^[16]. All these physiological and experimental observations suggest a strong link between T2DM and obesity, where the linking factor appears to be DPP-4.

Plasminogen activator inhibitor-1

This prothrombotic cytokine, besides being produced by vascular endothelial cells, is also produced by adipocytes, production being more from omental adipose tissue than that of subcutaneous adipocytes^[17]. Some recent studies have found a direct contribution of this cytokine towards the complications of obesity like T2DM and coronary thrombosis, as well as increased accumulation of visceral fat^[18]. Nowadays, plasminogen activator inhibitor-1 (PAI-1) is being considered as a strong predictor of T2DM, and has been found to stimulate adipocyte differentiation, which may be mediated through reducing *peroxisome proliferator-activated receptor* (PPAR)- γ activity, resulting in more production of resistin. It has been demonstrated that adipocyte-PAI-1 increases the production of TNF- α (an autocrine action) in adipocytes that reduces insulin action and predisposes to T2DM. Moreover, PPAR- γ receptor has been found to be downregulated both by PAI-1 and TNF- α . Hence, inhibition of PAI-1 action on adipocytes may prevent obesity and IR, and retard adipocyte differentiation and fat accumulation by removing not only its (of PAI-1) own antiinsulin action but also that of resistin and TNF- α ^[7,17].

Visfatin

This adipocytokine, a pro-inflammatory marker of adipose tissue, is mainly produced by visceral adipocytes of humans and mice, whose plasma concentration increases along with the progression of obesity^[19-21]. Its production is upregulated by hypoxia, inflammation and hyperglycemia, and downregulated by insulin, somatostatin and cholesterol reducing statins. Besides visceral fat, intracellular presence of visfatin has also

been demonstrated in many other tissues and organs, the location being both cytoplasmic and nuclear^[21].

Functions of visfatin are difficult to explain as they appear to be contradictory. The cytokine has been found to possess insulinomimetic effect in cultured cells^[19,20] and lowers plasma glucose concentration in mice^[19]. It has also been shown to cause hypoglycaemia by reducing hepatic output of glucose and increasing utilisation of glucose in adipocytes and monocytes^[21]. In spite of such favourable insulinomimetic action^[19,20], this cytokine has been found to be associated with IR and possesses a direct relationship between its plasma concentration and T2DM^[21,22]. This anomaly may be explained by the fact that it also produces hyperlipidemia, which may be responsible for IR and hence T2DM (As T2DM may either be due to deficiency of insulin or IR)^[22]. The resultant effect seems to be favouring the development of T2DM, which in turn suggests the pernicious role of visceral adipose tissue (VAT) in human obesity-related T2DM and accompanying metabolic disorders^[20].

Besides these T2DM-related pathological functions, visfatin, by its endocrine, autocrine as well as paracrine function, has been found to cause increase in cell proliferation and biosynthesis of nicotinamide mono- and dinucleotides^[16], significance of which is yet to be ascertained.

Apelin

Apelin, a small peptide adipokine, has also been found to be present in a number of tissues. It is the ligand of the G-protein-coupled receptor (GPCR) APJ, and has several active forms, which include apelin 13, apelin 17 and apelin 36. It is considered as a beneficial adipokine as it has been found to possess antiobesity and anti-diabetic properties, because of its potent positive role^[23,24] in energy metabolism and insulin sensitivity improvement^[24]. Such actions appear to be due to promotion of complete lipid combustion^[23] in muscle of IR mice through mitochondrial biogenesis and tighter matching between fatty acid oxidation and TCA cycle. Such apelin-stimulated improvement of FA oxidation led to decreased levels of acyl-carnitines and enhanced insulin-stimulated glucose uptake in soleus muscle^[25]. For such beneficial actions, apelin may be considered as a promising useful therapeutic agent for T2DM and other metabolic disorders^[23].

FFA

FFAs, which are produced during the metabolism of exogenous and endogenous lipids, play an important role in the development of IR and hence, genesis of T2DM, when their plasma concentration is abnormally raised^[26].

Mechanisms of FFA-induced IR include inhibition of insulin-induced release of NO from endothelial cells, resulting in decreased blood flow, inhibition of insulin-stimulated glucose transport across the cell membrane and/or inhibition of intracellular phosphorylation of

glucose by interfering with insulin signal transduction pathway. Acute elevation of FFA in plasma has been found to be associated with IR, which may account for 50% of IR in obese individuals with T2DM^[27].

Intracellular mechanism of FFA-induced IR has been demonstrated both *in vivo* and *in vitro*, where there was an activation of pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway. It has been shown *in vivo* that acute increase in FFA level resulted in activation of NF- κ B pathway in human skeletal muscle and rat liver, leading to increased production of pro-inflammatory cytokines, *i.e.*, TNF- α , IL-1 β and IL-6, in both the test organs along with an increase in the concentration of macrophage chemoattractant protein-1 (MCP-1) in circulation. In health as well as in T2DM, insulin tends to reduce FFA-induced-IR by lowering the plasma concentration of FFAs through its lipogenic as well as antilipolytic action along with increased intracellular oxidation of FFA. However, in obesity, which is considered as an inflammatory state, there is not only an increase in FFA, but also an increase in the plasma concentration of pro-inflammatory cytokines, which together are liable to cause IR and T2DM^[27].

Thus, obesity alone or along with increased FFA, can create and maintain a low grade inflammatory state by production of pro-inflammatory cytokines (TNF- α , IL-6, etc.), which may induce IR and T2DM. The condition may be further aggravated by antiinsulin action of FFA on glucose metabolism^[27].

Adiponectin

This adipocytokine is being extensively studied worldwide since the past decade because of its remarkable insulin sensitizing property (IR is the major problem in T2DM) as well as antiatherogenic action (dyslipidemia, commonly associated with T2DM, is responsible for atherosclerotic complications of T2DM), thereby playing an important role in delaying and suppressing the metabolic derangements, which result in IR, T2DM, metabolic syndrome and complications of diabetes including vascular and cardiac. These two important functions of adiponectin involves myriads of interrelated molecular mechanisms, which interconnect it with other diabetogenic/antidiabetic adipokines as well as with many physiological and biochemical processes associated with maintenance of energy balance from metabolism of carbohydrates and lipids^[3]. Because of such widespread metabolic involvement, an attempt has been made to discuss the pathophysiological role of this key adipocytokine in detail, which in concert with its siblings appears to play an important role in linking T2DM with obesity.

Source and location: Adiponectin, secreted by both white and brown adipose tissue, has several other names like gelatin binding protein-28, AdipoQ, Adipocyte complement-related protein-30 and OP-MI.

Adiponectin mRNA presence is lower in VAT than that of subcutaneous adipose tissue^[4,10]. Normal plasma concentration of this cytokine varies from 5–30 µg/mL and is inversely proportional to abdominal obesity, IR and T2DM. In some animal models, a decrease in plasma adiponectin concentration was found to precede the onset of T2DM and was parallel with decreased insulin sensitivity. The cytokine circulates in blood in multimeric forms, like trimeric, hexameric and high molecular mass species, each of which plays a specific role in maintenance of energy homeostasis^[4,7].

Control of secretion: Control of adiponectin secretion is effected by: (1) some hormones; (2) many adipokines; (3) certain receptor families including its own; (4) endoplasmic reticulum (ER) and oxidative stresses; and (5) several other factors.

Hormonal control: Sex hormone: Adiponectin plasma concentration has been found to be higher in women than men, which may be due to the difference in concentration of oestrogen and androgen, suggesting a presumably stimulating role of oestrogen on synthesis and secretion of this adipokine^[5,7]; Insulin: The relationship between plasma insulin concentration and adiponectin secretion appears to be peculiar, confusing and contradictory, as the experimental observations do not correlate as expected.

Though insulin favours adiponectin biosynthesis through PPAR-γ *via* inhibition of FoxO1 (an inhibitor of PPAR-γ), type I diabetic patients who practically have no circulating insulin, contrary to the expectations, show elevated levels of plasma adiponectin. Moreover, patients, with defective insulin receptors due to abnormal genes coding for them, also show raised circulating adiponectin levels. Furthermore, adiponectin concentration, unlike other insulin-resistance-inducing adipokines, has been found to be decreased in obesity and insulin-resistant models. From such observations it seems that IR decreases plasma adiponectin concentration. This may be explained by taking into account the role of oxidative stress which is known to increase IR and to decrease adiponectin production. In obesity, adipocytes may develop oxidative stress, leading to decreased expression of adiponectin by them. That IR decreases adiponectin expression may further be supported by the observation that hyperinsulinemia associated with euglycemia (an IR state) significantly decreases the plasma adiponectin concentration and selectively downregulates its high molecular weight (HMW) form. The disparity between the above mentioned experimental observations in relation to role of insulin on adiponectin formation is not known and appears to be complicated^[4].

Control by adipokines: TNF-α and IL-6 are considered to be established inhibitors of adiponectin synthesis^[28]. As their synthesis and secretion increase in obesity, adiponectin plasma concentration decreases

accordingly^[4,29].

Control by certain receptors: PPAR-γ: This PPAR subfamily transcription factor, which is mainly found in adipocytes, has been shown to possess a positive regulatory role on adiponectin gene expression leading to increased production of proteins like Erol-La and DsbA-L, which take part in synthesis and secretion of adiponectin^[4]; Own receptors: Circulating adiponectin concentration has been found to be inversely related to muscle AdipoR_{1/2} (receptor subtypes), but directly related to subcutaneous AdipoR₂^[4,30].

ER and oxidative stresses: ER is known to be an intracellular fine network of microtubules. It is continuous with the nuclear membrane, and is called sarcoplasmic reticulum in muscles. It controls intracellular calcium ion uptake and release besides its other functions, thereby effecting muscular contraction and relaxation. ER stress, which is produced in obesity, has been shown to be negatively related to adiponectin production by adipocytes. The molecular mechanism involved has been studied in 3T3LI-cells, where oxidative stress in ER lead to increased production of H₂O₂, which, *via* protein kinase B (Akt) and JAK/STAT pathway, appreciably suppressed the expression of adiponectin mRNA and consequent reduction in synthesis of proteins required for adiponectin formation. Moreover, in this model H₂O₂ has been found to increase the production of PAI-1 and IL-6, which are known to inhibit adiponectin synthesis^[31].

Other factors: Obesity: Unlike other adipokines, adiponectin secretion has been found to be decreased in obesity. Though the exact cause of such reduction is not known, the suggested causes include increased production of TNF-α and IL-6^[28], generation of a hypoxicmicroenvironment in the adipocytes of increased fat mass, and obesity-induced increased production of insulin like growth factor binding protein-3, which inhibits adiponectin transcription *via* hypoxia inducible factor-1α dependent pathway^[4,32]; Drugs: PPAR-γ agonists (thiazolidinediones-TZDs), which increase insulin sensitivity, have been found to increase the plasma concentration of adiponectin, whereas anti-HIV drugs like protease inhibitors decrease it^[29].

Physiological functions of adiponectin: Adiponectin, along with other adipokines, interferes in several metabolic functions, like lipid synthesis and storage, neoglucogenesis and peripheral utilisation of glucose, which have been demonstrated in skeletal and cardiac muscles, adipocytes and hepatocytes^[31]. But, it differs from other adipokines in several aspects. Unlike others, its circulating concentration has been found to be decreased in obesity (particularly abdominal obesity) and T2DM, and instead of increasing insulin resistance, it decreases it in addition to possessing antiatherosclerotic effect. In animal models and in

patients with obesity and T2DM, the cytokine has been shown to stimulate fatty acid (FA) oxidation, reduce lipid accumulation in muscles, decrease plasma FA concentration and increase insulin sensitivity. Because of such beneficial involvement in metabolic functions (lipids and carbohydrates), IR and atherosclerosis, this adipokine is expected to impart protection against coronary heart diseases, steatohepatitis, non-alcoholic fatty liver diseases and a wide variety of cancers^[33].

Cellular basis of mechanism of action: Functions of adiponectin have been found to be mediated by three receptor subtypes namely, AdipoR₁, AdipoR₂ and T-cadherin. AdipoR₁ and AdipoR₂ are 7 transmembrane proteins but dissimilar to GPCRs. Its receptor distribution pattern varies from cell type to cell type - AdipoR₁ being found abundantly in muscles, while AdipoR₂ is mainly expressed in hepatocytes. Both the receptors are present in almost every tissue, but in a particular tissue, usually one type predominates. Moreover, degree of affinity of these receptors for different forms of adiponectin also varies^[4,7]. AdipoR₁ has high affinity for globular adiponectin (a cleaved part of full-length adiponectin) but low affinity for full-length adiponectin, whereas AdipoR₂ has intermediate affinity for both forms. Hypoadiponectinemia, associated with IR, upregulates both the receptor types. Such upregulation also occurs in physical activity, suggesting an association between adiponectin hormone system and exercise-induced improvement in IR^[7]. Adiponectin, binding to its cell surface receptors, activates several intracellular signalling molecules like p38MAPK, PPAR, the RAS-associated protein Rab5, PI3K, Akt and AMP-activated protein kinase (AMPK), of which AMPK system and PPARs play an important and dominant role leading to modification of lipid and carbohydrate metabolism^[4,7,29].

As has already been mentioned, in this article emphasis would be given on two important protective physiological functions of adiponectin, *i.e.*, protection against IR and atherosclerosis. Obesity, T2DM, dyslipidemia and IR are intimately related, where one leads to the other and once developed, aggravate each other thereby establishing a vicious cycle, leading to development of practically all the dangerous complications of T2DM^[34]. Increased fatmass, as found in obesity, not only increases the production of bad adipokines who enhance this cycle further but also decreases the production of the good one-adiponectin, deficiency of which contributes significantly towards the development, continuation and aggravation of this cycle. Adiponectin has been shown to prevent the development as well as to break this dangerous cycle, thereby posing itself as a potential therapeutic agent in such condition.

Mechanisms of antiatherosclerotic and IR preventing actions of adiponectin: As these two actions are interrelated, it is convenient to discuss them together. It has already been mentioned that

adiponectin increases FA oxidation in mitochondria that leads to a decrease in plasma concentration of FA. Reduced level of FA in circulation prevents the development and progression of atherosclerosis and IR. Multiple biochemical actions at cellular level are modified for this action of adiponectin that needs an extensive discussion and correlation between them to arrive at a conclusion.

Adiponectin-induced FA oxidation is primarily mediated by phosphorylation (activation) of AMPK - a multi-subunit protein kinase, which appears to be a sensor of intracellular energy status through activation of PPAR- α receptor. It has been demonstrated that when muscles were treated with adiponectin or when its receptors were expressed ectopically, there occurred an increase in AMPK phosphorylation and FA oxidation in the muscles that was abolished by dominant-negative AMPK use. Stressful conditions, like heat shock, hypoxia, starvation and exercise, *etc.*, which need expenditure of more energy (denoted by high AMP - to - ATP ratio) have been found to cause AMPK activation. This important signalling molecule (AMPK) is also directly activated by other upstream kinases, where they cause phosphorylation of its threonine residue in the kinase domain. In skeletal muscle, activated AMPK increases FA oxidation by stimulating the phosphorylation (leading to inactivation) of the key enzyme acetyl-CoA carboxylase (ACC). Reduced ACC activity, in turn, decreases intracellular malonyl-CoA concentration along with stimulation of carnitine palmitoyl transferase 1 (CPT1) activity, leading to increased entry of long-chain FAs into mitochondria and hence, more of their peripheral oxidation. The fact, that adiponectin increases insulin sensitivity by decreasing plasma FA concentration, has been demonstrated in obese and T2DM patients, where serum adiponectin concentration is low. In such patients, administration of adiponectin has been found to increase insulin sensitivity by decreasing their plasma FA and TG^[33].

Metabolic stressful conditions like muscle contraction, hypoxia, ischemia and hyperosmolality, *etc.*, not only increase AMPK activation (as mentioned before), but also stimulate the activity of p38MAPK (a signalling molecule activated by inflammatory cytokines). This indicates an association between the two signalling molecules during signal transduction, though the agonists (adiponectin, inflammatory cytokines) inducing the signals are different. In fact, adiponectin has been found to stimulate the activity of not only AMPK but also that of p38MAPK and PPAR- α in target tissue though the subsequent signal transduction pathway following these three activations is not fully known. Other evidences in muscles suggest a sequential activity of these three, leading to increased FA oxidation and increased glucose uptake by muscles. But it has been shown that when primary hepatocytes are treated with adiponectin, their FA oxidation is not increased, which suggests a differential effect of the cytokine on FA oxidation of muscles and liver^[33].

ER stress decreases adiponectin secretion:

Several workers have shown that ER stress in adipocytes decreases adiponectin secretion. It has been demonstrated that properly integrated mitochondrial function in adipocytes is necessary for adequate secretion of adiponectin. Like other cells, growth and development of adipocytes occur through differentiation and hypertrophy, which need increased mitochondrial function, because of greater energy requirement. Newly differentiated adipocytes are small in size, because of less accumulated TG due to increased FA oxidation in them, as the mitochondrial content and activity are more^[29].

It has been shown that these small adipocytes synthesise and secrete more adiponectin because of their high mitochondrial functional level, whereas large hypertrophied fat cells, as found in obesity, produced the cytokine to lesser extent because of impaired mitochondrial function. Though till now, adiponectin synthesis has not been properly correlated with increased mitochondrial function, it may be due to much greater consumption of energy for the synthesis of this cytokine protein in comparison with other proteins. Therefore, it appears that synthesis of adiponectin in adipocytes needs high consumption of energy, which is produced by elevated (adequate) mitochondrial function. In support of this, it has been shown that rosiglitazone and others agents like Ad-NFR-1, which increase mitochondrial biogenesis, also cause an increase in adiponectin synthesis. This observation points the finger towards mitochondrial dysfunction as the cause of low adiponectin level in obesity^[29].

Moreover, several evidences have been put forward where obesity-induced mitochondrial dysfunction has resulted in ER stress, which decreases adiponectin secretion and development of IR. Both ER stress and mitochondrial dysfunction have been demonstrated to activate a series of reactions involving sequential activation of JNK and activating transcription factor 3 (ATF3), which in turn decrease the transcription of adiponectin. When JNK and ATF3 are inhibited, adiponectin transcription is restored. It has also been suggested that ER stress and impaired mitochondrial function are separately responsible for genesis of IR in various tissues of obese persons^[29].

Adiponectin-induced increase in FA oxidation *via* activation of AMPK and phosphorylation of ACC is of short duration, as ACC phosphorylation is short-lived. Hence, this pathway cannot be considered to be fully responsible for the long term effect of adiponectin in causing weight loss and FA oxidation, for which action through PPAR- α is thought to be involved, because PPAR- α action has been found to persist even after initial signalling is over. This is so, because adiponectin has been found to increase transcriptional activity of PPAR- α and subsequent expression of its target genes *via* activation of AMPK. Involvement of AMPK is supported by the fact that when PPAR- α agonists were administered to obese animals, there occurred an

equivalent and sufficient lowering of lipids, as was found with adiponectin. This fact was further supported by *in vivo* administration of 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) to lean and obese Zucker rats where the compound was found to decrease plasma FA and TG levels significantly, because AICAR is known to increase the transcriptional activity of PPAR- α *via* activation of AMPK^[33].

Anti-inflammatory action of adiponectin: Mention has been made about the decreased secretion of inflammatory cytokine TNF- α by adiponectin from macrophages that contribute towards its antiatherogenic effect. This anti-inflammatory property is also likely to be involved in its IR reducing action, because TNF- α and IL-6 are known to decrease adiponectin formation and to induce IR^[10,28].

Recently, it has been shown that NF- κ B activation in endothelial and monocytic cells, which is involved in causation of inflammation and metabolic alteration in obesity, is suppressed in these cells by adiponectin. Moreover, both forms of adiponectin-globular as well as full-length, have been found to decrease the production of pro-inflammatory cytokines IL-6 and MCP1 from inflamed adipocytes that may be due to inhibition of NF- κ B activity as well as PPAR- α expression^[35].

Insulin sensitizing actions of adiponectin: Adiponectin aids to insulin sensitivity by several novel mechanisms, which include - increased FA oxidation, decreased ER stress, improvement in insulin signalling pathway, increased (improved) mitochondrial number and function, increased insulin secretion, decreased hepatic output of glucose, increased uptake of glucose by liver and muscle, and increased glucose metabolism.

Adiponectin-induced increase in FA oxidation has been demonstrated by several workers^[5,10,33,36]. This action of adiponectin contributes significantly towards its insulin sensitizing action and prevention of development of IR, as increased plasma FA concentration is the most important cause of IR. In some animal models, adiponectin has been shown to decrease FFA concentration in plasma by increasing its uptake and oxidation in skeletal muscles. On the other hand, acute reduction of plasma FFA has been found to be associated with low adiponectin concentration, though the exact role of FFA in such action is not known^[36]. It is well documented that the key enzyme responsible for FA oxidation is AMPK, which is activated by adiponectin^[4,10,31,33]. It has already been mentioned that once activated, AMPK inhibits the activity of ACC, which not only leads to reduced contents of intracellular malonyl-CoA but also increases activity of CPTI. Such an action increases the entry of long-chain FAs into mitochondria and hence, an increase in their oxidation. Works on FA oxidation in skeletal muscles have shown a sequential activation of AMPK, p38MAPK and PPAR- α to be responsible for increased FA oxidation. But the signalling pathways and components involved in such sequential activation is

not known. PPAR- α , a ligand-activated nuclear receptor, plays an important role in FA oxidation. This receptor is abundantly expressed in tissues like liver, heart, kidney and skeletal muscles, who meet their metabolic energy consumption from oxidation of FAs. It has been shown that HMW adiponectin fraction increases the PPAR- α target gene expression. Moreover, in IR rodent models, PPAR- α ligands have been found to reduce lipid levels and to improve insulin sensitivity. Several studies on humans and rodents have shown that both forms of adiponectin, HMW as well as low molecular weight (LMW), not only increase target gene expression of PPAR- α but also increase the phosphorylation of AMPK and p38MAPK. But such activity is more pronounced and better correlated with HMW fraction than that of LMW, suggesting a differential efficacy between the two fractions or involvement of multiple pathways in increasing FA oxidation in muscles^[33].

It has already been mentioned that mitochondrial dysfunction in adipocytes induces ER stress, which in turn reduces adiponectin transcription, leading to decreased production of this adipokine along with development of IR^[29,31]. Moreover, as discussed earlier, adiponectin, *via* activated AMPK, also improves mitochondrial number and function in skeletal muscles^[29]. From these two observations it may be inferred that adiponectin, by counteracting mitochondrial dysfunction (through improvement of mitochondrial function), decreases ER stress and improves its own secretion, which in turn may contribute towards reduction of IR. Mention has already been made about the IR-inducing and diabetogenic adipocytokine resistin^[1,10,11], whose plasma concentration is high in IR, T2DM, metabolic syndrome and cardiovascular diseases^[3]. In contrast, its sibling adiponectin plasma concentration is low in such conditions^[4], and it has favourable effects on them. Such contrasting effects of the two adipokines may be due to their comparable domain architecture, assembled in a multimeric form, which suggests a common regulatory mechanism (opposite to each other) on insulin-signalling pathway, as well as on mechanisms involved in glucose and lipid homeostasis. In IR and T2DM, hypoadiponectinemia along with hyperresistinemia have been found to antagonise insulin signalling by causing dephosphorylation and deactivation of the key enzyme AMPK in skeletal muscles and liver along with increased expression of genes coding for the synthesis of neoglucogenic enzymes as well as reduced expression of IRS-2 and glucose transporter, GLUT-2. The resultant effects of such action were decreased FFA oxidation in muscles, decreased hepatic uptake of glucose, increased neoglucogenesis and glycogenolysis leading to hyperglycemia and increased plasma FFA. Impaired FFA oxidation may be further aggravated by downregulated PPAR- α action^[3].

It is well established that insulin resistance is very often associated with inadequate functioning of post receptor signalling molecules including IRS. It has been demonstrated that adiponectin upregulates

IRS-2 by activation of STAT-3 in liver. Such activation was also associated with increased production of IL-6 from macrophages - an adiponectin action mediated through activation of NF- κ B, which does not require activation of classical AdipoR₁ and AdipoR₂ receptors. Upregulation of IRS-2 definitely improves insulin sensitivity, but exact mechanisms of such upregulation are not known. Probably, it is effected by an IL-6 dependent pathway, which is initiated by adiponectin, through its combination with yet another unidentified adiponectin receptor. Moreover, though adiponectin activates AMPK and PPAR- α through activation of its classical AdipoR₁ and AdipoR₂ receptors leading to increased FA oxidation and insulin sensitisation, it has not been possible to link AMPK and PPAR- α activation with the proper functioning of post-receptor insulin signalling molecules^[37]. Experiments on skeletal muscles have demonstrated that AMPK activation by adiponectin occurs by two pathways, out of which one is a major one while the other plays a minor role. In the major pathway (the APPL1/LKB1-dependent pathway), AMPK activation needs the binding of adapter protein APPL1, which promotes the translocation of APPL1-dependent LKB1 into the cytosol where it is anchored. The same pathway has been found to be followed by the insulin sensitising drug metformin. Through the minor pathway (the phospholipase C/Ca²⁺/Ca²⁺/calmodulin-dependent protein kinase kinase-dependent pathway), *via* activation of phospholipase C, Ca²⁺ is released from the intracellular calcium ion stores that plays a minor role in activation of AMPK^[38].

Works on skeletal muscles have shown that adiponectin, through AMPK activation, not only increases mitochondrial function, but also increases their number. As activated AMPK in skeletal muscles has been found to stimulate mitochondrial biogenesis under conditions of chronic energy deprivation or endurance training, it appears that adiponectin-induced-increase in mitochondrial number is due to stimulation of mitochondrial biogenesis. This action of the adipokine points towards its insulin sensitising action, because mitochondrial function in skeletal muscles is taken as an indicator of whole-body insulin sensitivity. Thus, it may be presumed that adipocyte-mitochondrial action, which regulates adiponectin synthesis in adipocytes (already discussed), also regulates skeletal muscle-mitochondrial (or metabolic) activity and insulin action in skeletal muscles through adiponectin^[29].

Though adiponectin does not have any effect on normal insulin secretion, the adipokine has been found to increase it in insulin resistant mice fed with high fat diet. But in such mice, augmentation of secretion occurs only in response to high plasma glucose, but actually inhibited when plasma glucose was low. Adiponectin appears to possess a protective effect on islet- β -cells, as it has been found to reduce the pro-apoptotic effect of FFAs and other cytokines on β -cells^[5].

Several workers have demonstrated the capacity of adiponectin to decrease hepatic output of glucose,

thereby contributing towards reduction of plasma glucose concentration and hence, increased insulin sensitivity^[5,10]. One of the important causes of increased hepatic output of glucose in diabetes mellitus is increased neoglucogenesis due to inadequate insulin action. As mentioned earlier, adiponectin inhibits hepatic neoglucogenesis by decreasing the formation of two important enzymes concerned, through interference with the mRNA expression that is necessary for the synthesis of these enzymes^[10]. Moreover, adiponectin, by increasing the oxidation of FAs, decreases their availability for utilization in the process of neoglucogenesis.

Adiponectin has been found to increase the uptake of glucose by liver and muscles which appears to result from improvement in insulin signalling pathway, leading to better insulin action and hence, decreased blood sugar and increased insulin sensitivity^[5].

It has been observed that in obese individuals with IR and in patients having metabolic syndrome (who are IR), adiponectin receptors are downregulated, which suggests inadequate adiponectin action as the cause of IR. *In vitro* and *in vivo* experiments on skeletal muscles have shown adiponectin to increase glucose metabolism and insulin sensitivity *via* activation of AMPK^[31].

AR (adiponectin: Resistin) and IR_{AR} indices: Upregulated resistin, which is followed by PPAR- α downregulation, has been found to impair adipocyte differentiation, leading to dramatic decrease in adiponectin formation. Because of such inverse relationship with respect to both secretion and function, it seems to be more predictive to use their ratio (AR index-adiponectin: Resistin) in linking obesity with T2DM than using either of them alone^[3].

Besides AR index another novel IR_{AR} index has been coined that seems to be a strong indicator of degree of IR in T2DM. The index appears to relate IR with AR. As expected, AR index value gets smaller and smaller according to the degree of obesity (which determines the magnitude of hypoadiponectinemia with hyperresistinemia), resulting in a parallel rise of IR. Hence, greater the IR_{AR} index value, more is the degree of IR in T2DM.

As IR in T2DM is the major determinant of progression into metabolic syndrome, which in turn, lays the foundation for other complications of diabetes, this index may also be used to predict the arrival of T2DM complications^[3].

FACTORS NOT OF ADIPOCYTE ORIGIN

In addition to these adipokines, there are some other factors (not of adipocyte origin), whose role in linking obesity with T2DM cannot be ignored. These factors include PPARs, carnitine, calcium, angiotensin II and toll-like receptors (TLRs).

PPARs

This nuclear receptor family, consisting of PPAR- α , PPAR- γ and PPAR- δ , are primarily related with lipid metabolism

having fatty acids and their derivatives as their endogenous ligands.

PPAR- α : Besides interference with several steps of lipid metabolism, the main results of this receptor activation is increased oxidation of FA that leads to decreased plasma level of TG by decreasing its synthesis and storage in adipocytes. Moreover, PPAR- α activation, along with activation of PPAR- γ , has been found not only to increase the formation and secretion of adiponectin but also to upregulate AdipoR₁/AdipoR₂^[7].

PPAR- γ : These receptors, mainly expressed in liver and adipose tissue, on stimulation, cause gene expression necessary for differentiation of fibroblasts into adipocytes, and for lipid synthesis and storage in adipocytes. Because of their lipogenicity, they seem to decrease insulin sensitivity rather than increase it. But, their exogenous agonists-TZDs, have been found to decrease IR and increase insulin sensitivity. Such paradoxical actions of TZDs, have been shown to be due to reduced lipotoxicity in liver and skeletal muscles because of lipid storage in adipocytes, and increase in number of small adipocytes, which are not only more sensitive to insulin action, but also secrete large quantity of adiponectin (insulin-sensitising), while decreasing the release of resistin and TNF- α (both are IR-inducing)^[7].

PPAR- δ : Main result of this receptor activation is increased FA oxidation, which contributes towards decreasing IR and increasing insulin sensitivity^[7].

It may be noted that the results of activation of these three receptors, particularly activation of those of PPAR- α and PPAR- γ , are beneficial in IR and insulin sensitivity through their interference with adipocyte number (increased number of small adipocytes) and function (increased production of adiponectin and decreased production of resistin and TNF- α), FA oxidation (which decreases TG formation in adipocytes resulting in decreased obesity) and upregulation of AdipoR₁ and AdipoR₂ (decreased IR and increased insulin sensitivity). As all these functions finally lead to reduced obesity, this receptor family can be considered to play a role in linking obesity and T2DM.

Carnitine

This vitamin and amino acid, which is derived from yeast, milk, liver and muscles (in large quantities), increases FFA oxidation through carnitine shuttle reactions. In this reaction, carnitine has been found not only to favour entry of long-chain FFAs across the mitochondrial membrane, but also facilitate the transport of fatty acyl-CoA into mitochondrial matrix for β -oxidation. Therefore, carnitine deficiency, which is commonly found in several IR cases, leads to increased concentration of plasma FFA and hence, their increased conversion into TG in adipocytes, resulting in obesity and further aggravation of IR. Moreover, relative carnitine deficiency may occur in prolonged metabolic stress, which may add to mito-

chondrial dysfunction, leading to reduced glucose tolerance. These two factors may contribute towards obesity-associated IR in T2DM. Therefore, like PPAR-receptor family action, carnitine function in the body may contribute towards linking obesity with diabetes as its deficiency is reflected upon genes of obesity and IR^[7].

Calcium

Role of calcium in various cellular secretory processes^[39], including secretion of insulin from islet β -cells, is well established. Improper regulation of intracellular calcium has been found to affect insulin secretion and its tissue sensitivity adversely^[40]. High calcium intake alone or with vitamin D has been shown to reduce not only body weight and fat mass, but also to decrease weight gain and adipocyte fat accumulation. The mechanisms suggested for such beneficial actions include adipocyte apoptosis and reduced adipogenesis along with deranged lipid metabolism^[40,41]. Moreover, epidemiological studies have shown that low calcium intake and poor vitamin D status are associated with increased risk of obesity^[36]. From such observations, it may be inferred that obesity, thus developed, may lead to increased production of IR-inducing and diabetogenic adipokines, thereby linking it (obesity) with IR and T2DM.

Angiotensin II

Renin-angiotensin-aldosterone system, whose primary function is to maintain water and electrolyte balance of the body and to regulate blood pressure, is known to mediate its function by formation of angiotensin II (Ang II). Ang II formation occurs through several steps where renin of renal origin converts angiotensinogen of hepatic origin to Ang I, which is then converted to Ang II by the enzyme angiotensin-converting enzyme (ACE) of endothelial cell origin^[42]. But recently, a local RAAS has been demonstrated in several tissues of the body including adipose tissue, which is involved in several functions of the adipocytes including adipose tissue growth and cell differentiation. It has been shown that when AT₂ receptors (one of the subtypes of angiotensin receptor) are deleted from adipocytes, the cell size is reduced, and there is protection from diet-induced obesity and IR^[43]. Such observations suggest an additional beneficial role of ACE inhibitors and AT₂ receptor blockers, when used as antihypertensives in patients having hypertension with obesity and T2DM^[44]. Moreover, like low Ca²⁺ and poor vitamin D status, locally generated Ang II, via its action on adipocytes, may link obesity with T2DM.

TLRs

TLRs are transmembrane glycoprotein receptors whose known function is antigen recognition^[6,45]. Recently, substantial evidences have been put forward which suggest their pathological role in genesis of obesity. In this respect, both TLR-2 and TLR-4 have been found

to be overexpressed on adipocytes in obese persons having T2DM. Such overexpressed TLR receptors along with similarly overexpressed adipokines in adipose tissue of obese individuals may play an important role in obesity-associated meta inflammation resulting in IR and T2DM. It has been demonstrated that inhibition of TLR-2 in skeletal muscles and white adipose tissue of mice fed with high fat diet, improves insulin sensitivity and signalling^[43].

Moreover, overexpression of TLRs on adipocytes may also suggest an important role of adipose tissue in the regulation of inflammation and innate immunity in human beings by modulating TLR/NF- κ B regulatory pathway. Such observations suggest a modulatory role of TLRs in the interaction between the pathways of inflammation and metabolism^[43]. The above-discussed roles of TLRs in genesis of obesity, reduction of insulin signalling and sensitivity, and modulation of the interacting pathways of inflammation and metabolism appear to support the correlation between obesity and T2DM.

CONCLUSION

From the discussions made so far, it may be observed that results obtained from extensive research work on the factors supposed to link obesity with T2DM, very clearly show an intimate relationship between the two, for which both adipocytokines as well as some factors not derived from adipocytes have been implicated. Of them, few (Adiponectin, Leptin, PPAR, Carnitine, Apelin and Calcium) are beneficial, while others (TNF- α , IL-6, Resistin, RBP-4, DPP-4, PAI-1, Visfatin, FFA, Ang II and TLR) are harmful, but all of them play a definite role in linking obesity with T2DM (mentioned earlier). Among these, adiponectin has been found to play a crucial and seemingly complicated but definite role. Such studies may be extended to all concerned factors giving emphasis on mitochondrial and ER stresses. Finally, using these agents, drugs may be designed which will be helpful to prevent the development of obesity, thereby producing a beneficial response in prevention, progression and treatment of T2DM.

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Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

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E-mail: editorialoffice@wjgnet.com

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From non-obese diabetic to Network for the Pancreatic Organ Donor with Diabetes: New heights in type 1 diabetes research

Lourdes Ramirez, Abdel Rahim A Hamad

Lourdes Ramirez, Abdel Rahim A Hamad, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States

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Correspondence to: Abdel Rahim A Hamad, BVSC, PhD, Associate Professor of Pathology and Medicine, Department of Pathology, Johns Hopkins University School of Medicine, 720 Rutland Ave, Ross 664G, Baltimore, MD 21205, United States. ahamad@jhmi.edu
 Telephone: +1-410-6143021
 Fax: +1-410-6143548

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Abstract

Since the discovery of therapeutic insulin in 1922 and

the development of the non-obese diabetic spontaneous mouse model in 1980, the establishment of Network for Pancreatic Organ Donor with Diabetes (nPOD) in 2007 is arguably the most important milestone step in advancing type 1 diabetes (T1D) research. In this perspective, we briefly describe how nPOD is transforming T1D research *via* procuring and coordinating analysis of disease pathogenesis directly in human organs donated by deceased diabetic and control subjects. The successful precedent set up by nPOD is likely to spread far beyond the confines of research in T1D to revolutionize biomedical research of other disease using high quality procured human cells and tissues.

Key words: Type 1 diabetes; Network for the Pancreatic Organ Donor with Diabetes; Non-obese diabetic mouse; Transitional type 1 diabetes research

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Core tip: Type 1 diabetes (T1D) strikes early in life with monumental impact on life style and long term health of affected children. There is currently no cure for T1D or mechanisms to protect at risk individuals. A major obstacle is the difficulty in translating the interventions that succeeded in preventing or reversing the disease in the non-obese diabetic mouse model into human immunotherapies. Network for Pancreatic Organ Donor with Diabetes has been established in 2007 to study the disease directly in humans by procuring and offering well preserved tissues to investigators. These efforts, as indicated by published results, are paying off by providing critical new insights that are expected to facilitate development of efficacious immunotherapies.

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MAIN TEXT

Type 1 diabetes (T1D) is a form of diabetes mellitus that results from the autoimmune destruction of the insulin-producing beta cells in the pancreas, manifested clinically as hyperglycemia. T1D accounts for 5% to 10% of diabetes cases in the world and about 80000 children develop the disease each year. During the last century, research of T1D passed through several critical milestones (Table 1). Prior to the discovery and use of insulin as a replacement therapy in 1922, T1D was invariably fatal^[1,2]. Advances in insulin delivery and formulation are allowing many patients to live out their respective life expectancies. Nonetheless, insulin replacement is not a cure and it has to be taken daily. In addition, the dose needs to be adjusted frequently for successful management of blood glucose levels and their maintenance within an acceptable range. Achieving these goals is challenging and patients develop bouts of hypo- and hyper-glycemia as well as serious long term cardiovascular complications^[3]. To alleviate these problems, there have been intensive and sustained efforts to develop a cure that protects high risk individuals and perhaps reverses hyperglycemia in new-onsets. For this purpose, scientists have been using small animals to understand disease pathogenesis better, which is critical for finding a cure. Indeed, the discovery of the pancreas as the sole source of insulin almost a century ago was based on the development of severe diabetes in depancreatized experimental animals^[2]. In the 1980s, these efforts were seriously boosted by the development of the non-obese diabetic (NOD) mouse as a spontaneous model of the diseases^[4]. Extensive studies of NOD mice over the years led to significant understanding of the disease pathogenesis and identification of large numbers of molecules and cell types that stood out as potential therapeutic targets^[5]. Clinical relevance of the findings derived from NOD mice are substantiated by identification of their counterparts in humans. Strategies to block or reverse disease in NOD mice were mostly successful^[6], raising the hope of translating them into effective and safe immunotherapies. Results of clinical trials, however, were rather disappointing as they largely failed to achieve the expected efficacy to preserve C-peptide or protect high risk individuals, dashing hopes. There were several comprehensive reviews of the major trials and their outcomes including an excellent concise review by Atkinson *et al*^[7].

Assessment of the reasons behind the failure of the selected agents in the clinic is leading to more appreciation of biological differences between the highly heterogeneous human population and the NOD inbred mouse and to the role of the environment as barriers that challenge the assumption that “what works in NOD mice will work in humans”. Changes in environmental

factors (including viral infections, changes in diet) rather than changes in allele frequency, which would not occur so rapidly, are likely responsible for the rapid increase of the incidence of T1D in western countries. Together these factors pointed to the importance of studying the disease directly in humans. However, regular access to well characterized human organs has been very difficult and not available at central facilities. Consequently, most of the clinical research has been limited to the analysis of peripheral blood mononuclear cells. Facing the reality that most if not all of what worked in NOD mice failed in the clinic, a foresighted group of researchers conceived and implemented the idea of studying the disease directly in humans using donated organs. This led to the established of the Network for Pancreatic Organ Donors with Diabetes (nPOD). For complete information about nPOD, supporting agencies and how to get involve, please visit: <http://www.jdrfnpod.org/>. As indicated in their website, nPOD biobank receives organs from donors, worldwide, and distributes tissues and cells to nPOD researchers. These efforts are allowing scientific investigation of T1D directly in well-preserved high quality human tissues and organs by researchers with diverse scientific specialties and interests. The ultimate goal of this diverse group of research converges on studying and understanding different aspects of the disease and eventually developing therapeutic modalities to protect high risk individuals and perhaps reverse disease at onset. Fruitfulness of these efforts are indicated by a stream of new discoveries some of which confirmed similarities to what have been known in the NOD mouse, whereas others identified significant departures (for complete list of these publication, please visit nPOD website). Most of these notable differences, particularly in the pancreas and potential roles of viral infections in driving disease pathogenesis have been elegantly described in recent reviews by Pugliese *et al*^[8] and Kaddis *et al*^[9].

CONCLUSION

Access to donated human organs procured by nPOD is providing the opportunity to study the disease directly in humans. Studies of these organs is already leading to new critical insights that are expected to help better understanding of T1D and development of new modalities that can prevent T1D or preserve C-peptide in new-onset patients. However, NOD mouse still remain valid model for identification of new targets such as FasL^[10] and for functional understanding of observations made in humans. Particularly useful will be development of robust humanized mice that can be reconstituted by lymphocytes isolated from different human organs, particularly the pancreas. In addition, new innovative studies are directed towards combining reconstitution of humanized mice with peripheral mononuclear cells (PMNCs) with transplanted organs. At least but not least, success of the nPOD model is likely to inspire establishment of similar networks to study various

Table 1 Milestones in type 1 diabetes research

Use of depancreatized mice showed that T1D is due to the absence of internal pancreas secretion (1889)
Identification and synthesis of insulin for therapy (1922)
Development of spontaneous NOD mouse model (1980)
Establishment of nPOD to study disease using organs and tissues procured from patients, high risk individuals and non-diabetic controls (2007)

T1D: Type 1 diabetes; NOD: Non-obese diabetic; nPOD: Network for Pancreatic Organ Donors with Diabetes.

human diseases.

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Basic Study

Simple calculator to estimate the medical cost of diabetes in sub-Saharan Africa

Koffi Alouki, Hélène Delisle, Stéphane Besançon, Naby Baldé, Assa Sidibé-Traoré, Joseph Drabo, François Djrolo, Jean-Claude Mbanya, Serge Halimi

Koffi Alouki, Hélène Delisle, Department of Nutrition, Faculty of Medicine, University of Montreal, Downtown Station, Montreal H3C 3J7, QC, Canada

Stéphane Besançon, Santé-Diabète (NGO), Mali Chapter, Bamako, Mali

Naby Baldé, Endocrinology Department, Donka Teaching Hospital, Conakry, Guinea

Assa Sidibé-Traoré, Internal Medicine Department, Bamako University Hospital, Bamako, Mali

Joseph Drabo, Internal Medicine Department, Ouagadougou University Hospital, Ouagadougou, Burkina Faso

François Djrolo, Endocrinology Department, National University Health Centre, Cotonou, Benin

Jean-Claude Mbanya, Department of Internal Medicine and Specialties, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon

Serge Halimi, University Joseph Fourier and University Hospital Centre, 38043 Grenoble Cedex, France

Author contributions: Delisle H, Besançon S, Baldé N, Sidibé-Traoré A, Drabo J and Djrolo F designed the study; Alouki K, Delisle H, Besançon S, Baldé N, Sidibé-Traoré A, Drabo J and Djrolo F developed the calculator and collected the data; Mbanya JC and Halimi S advised on the methods; Alouki K and Delisle H analyzed the data; Alouki K and Delisle H drafted and finalized the manuscript; Besançon S, Baldé N, Sidibé-Traoré A, Drabo J, Djrolo F, Mbanya JC and Halimi S revised and corrected the manuscript.

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of hospital department heads in all four countries (members of the working group) and these professors of medicine have the required authority to collect and use the data. Furthermore, the identity of the hospitals and pharmacies where price information was collected was not divulged. Additionally, the prices are known to the public and displayed in hospitals and pharmacies. Therefore, no further authorization or special permission to use the data was necessary.

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Correspondence to: Hélène Delisle, PhD, Professor Emeritus, Department of Nutrition, Faculty of Medicine, University of Montreal, PO Box 6128, Downtown Station, Montreal H3C 3J7, QC, Canada. helene.delisle@umontreal.ca
Telephone: +1-514-3436111-25219
Fax: +1-514-3437395

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Abstract

AIM: To design a medical cost calculator and show that diabetes care is beyond reach of the majority particularly

patients with complications.

METHODS: Out-of-pocket expenditures of patients for medical treatment of type-2 diabetes were estimated based on price data collected in Benin, Burkina Faso, Guinea and Mali. A detailed protocol for realistic medical care of diabetes and its complications in the African context was defined. Care components were based on existing guidelines, published data and clinical experience. Prices were obtained in public and private health facilities. The cost calculator used Excel. The cost for basic management of uncomplicated diabetes was calculated per person and per year. Incremental costs were also computed per annum for chronic complications and per episode for acute complications.

RESULTS: Wide variations of estimated care costs were observed among countries and between the public and private healthcare system. The minimum estimated cost for the treatment of uncomplicated diabetes (in the public sector) would amount to 21%-34% of the country's gross national income per capita, 26%-47% in the presence of retinopathy, and above 70% for nephropathy, the most expensive complication.

CONCLUSION: The study provided objective evidence for the exorbitant medical cost of diabetes considering that no medical insurance is available in the study countries. Although the calculator only estimates the cost of inaction, it is innovative and of interest for several stakeholders.

Key words: Diabetes; Non-communicable diseases; Africa; Advocacy; Cost-of-illness

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Core tip: The costs of medical treatment of diabetes are poorly documented in sub-Saharan Africa, while such data are of interest for several stakeholders and useful for advocacy. There is a lack of tools to make these estimations. We describe a standardized, innovative and user-friendly medical cost calculator and provide the results of its use in four countries. It was developed in West-Africa but it is also relevant for other African countries and perhaps even in Asia provided the standard treatment protocol is deemed appropriate.

Alouki K, Delisle H, Besançon S, Baldé N, Sidibé-Traoré A, Drabo J, Djrolo F, Mbanya JC, Halimi S. Simple calculator to estimate the medical cost of diabetes in sub-Saharan Africa. *World J Diabetes* 2015; 6(16): 1312-1322 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i16/1312.htm> DOI: <http://dx.doi.org/10.4239/wjcd.v6.i16.1312>

INTRODUCTION

Non-communicable diseases (NCDs) including diabetes

pose a serious challenge to health systems already overstretched with acute and infectious diseases in Africa^[1]. Following the High Level Meeting of the United Nations on NCDs in New-York in 2011^[2], heads of governments made commitments towards prevention and control. However, the misconception that NCDs are diseases of the affluent is still widespread in low-income countries in spite of accumulating evidence against this myth. Strong advocacy is therefore required and the International Diabetes Federation (IDF), for instance, has developed a series of tools and guidelines to influence decision makers^[3]. The following definition of advocacy is relevant for all health professionals^[4]: "Blending science, ethics and politics, advocacy is self-initiated, evidence-based, strategic action that health professionals can take to help transform systems and improve the environments and policies which shape their patients' behaviours and choices, and ultimately their health". Simple and culture-sensitive advocacy tools are direly needed to foster the prevention and control of chronic diseases such as diabetes.

Several methods for identifying the economic consequences of diseases have been defined. Standard costs of diabetes in Africa, including the direct and indirect costs of the illness, have been estimated based on secondary data^[5]. Such cost-of-illness (COI) studies, in addition to direct and indirect costs, may also include the intangible costs although these are seldom measured owing to the complexity of such measurements^[6]. COI methods are simpler than, and very different from, the macro- and micro-economic models that have been developed to estimate the economic burden of the disease (cost of inaction) and the cost-effectiveness of action, such as CORE^[7] and other Markov-based models, and WHO CHOICE^[8]. Markov models are computer simulations of probabilistic progression of a disease in a hypothetical cohort which have been adapted to forecast mortality and complications of diabetes, as well as medical costs^[9]. WHO CHOICE has been used to estimate the cost-effectiveness of interventions to combat chronic diseases, including diabetes, at the regional level, thereby assisting decision makers^[10]. Comprehensive computer models of diabetes economic burden are many and they are in constant evolution^[11,12]. However, such models are highly complex and ill-suited to the field.

As part of a university partnership project on the double burden of malnutrition in French-speaking West-Africa^[1], diabetes advocacy instruments that are more specific to sub-Saharan African countries were developed. The principal tool was a simple medical cost calculator which is described and the results discussed in the present paper.

MATERIALS AND METHODS

The process and the study sites

A Diabetes Advocacy Working Group was set up among the partnership project institutions, with members from Benin, Burkina Faso, Mali, Guinea and Canada (University of Montreal). The focus on diabetes, rather than obesity, was a strategic choice because obesity is not as yet

commonly perceived as a health problem while diabetes certainly is.

The working group held regular sessions between 2009 and 2014. A medical cost calculator for diabetes treatment, in the absence or presence of complications, was designed. The team was inspired by the positive experience with PROFILES in the area of nutrition for advocating investments to eradicate malnutrition and micronutrient deficiencies^[13]. The costing tool was applied after pretesting in the four West-African countries represented on the working group. A training workshop on diabetes advocacy with the introduction of this tool was held in Benin for members of the project's institutional partners and for graduate students in health and nutrition.

The definition of a realistic diabetes care protocol

The approach was based on COI, which is quite different from a cost-effectiveness approach^[6,14]. In our study, the COI included only medical care from the patient perspective, that is, estimated individual, out-of-pocket expenditures. Medical cost estimates would also be relevant for third-party payers if and when health insurance becomes available. Other direct costs, for instance for transportation, are highly variable across individual patients and cannot be estimated in a standardized fashion. Although the instrument was based on the PROFILES conceptual model^[13], only the first component was considered at the present stage, that is, the cost of the disease (cost of inaction), as there are as yet insufficient relevant data for Africa on the cost-effectiveness of primary prevention, screening and secondary prevention to also include the cost of action.

Prior to data collection on medical costs, a detailed protocol for the basic treatment of type-2 diabetes and for the treatment of main complications was developed by the working group. Components of care were listed under physicians and allied health professionals' services, hospital care, lab tests and controls, and drugs and medical supplies, first for uncomplicated diabetes and then for each of the main acute and chronic complications. Acute complications included ketoacidosis, acute diabetic foot, kidney failure and stroke. Chronic complications were proliferative retinopathy, hypertension, nephropathy, cardiac ischemia, foot ulcer and the chronic phase of stroke. These complications were considered as the most common according to published data^[15-19]. The treatment protocol was based on realistic medical care for uncomplicated and complicated diabetes in the African context, rather than on optimal care as may be available in high-income countries. The working group selected the components of care based on the latest IDF clinical guidelines^[20], on IDF guidelines for Africa in 2006^[21] taking account of needed update, as well as on a thorough literature review and on the clinical experience of endocrinologists of the group. Standards of care of the American Diabetes Association^[22] were also examined for their relevance. The level of medical care was usually between "standard" and "minimal" as defined by IDF^[20]. The care components as detailed for

the basic treatment of uncomplicated type-2 diabetes and its complications are shown in Table 1. The frequency of medical check-ups, controls and tests is indicated, as well as drug posology. It will be noted that there are some alternatives, for instance, for hypoglycemic agents, as well as for drugs for complications. This is so because a cheaper alternative and a more expensive one are provided in order to estimate a range of medical costs instead of a single value such as the mean. Regarding tests, medical supplies, specialized treatments and drugs, two alternatives are also sometimes listed, a cheaper and a more expensive one.

Price data collection

Diabetes care costs were computed from price data (in local currency) collected in hospitals, clinics and pharmacies of the targeted countries, and not on the basis of actual patients' expenditures. Unit prices or rates for services and supplies were collected and entered in the tally forms designed for the purpose and including each care component previously identified. Prices were retrieved in public hospitals, private clinics, hospital pharmacies and private pharmacies of the capital city. The two hospitals included a university hospital (there is usually only one, serving as reference hospital) and a secondary hospital. Price data were also collected in two private clinics, including one offering specialized diabetes care if available. Prices for drugs and medical supplies were obtained in the pharmacies of the selected public hospitals and in two private pharmacies. Prices or rates were as charged to patients, irrespective of government subsidies that may exist in a given country. Forms for entering price data were designed (available from the corresponding author upon request). Unit costs were entered in the unshaded parts of the forms. If an element of care was only offered in one public hospital or only in one private clinic, the same price was entered for the other public or private structure. In public pharmacies, prices of available generic drugs were collected. If a given drug was only available in private pharmacies, the same price would be entered for the public pharmacies as well. The cost of drugs was to be entered for the number of units as generally packaged, but if the number was different, it had to be specified on the form. General and specific guidelines were developed to assist the users of the cost calculator in filling the forms.

Estimating medical costs for individuals living with diabetes

A user-friendly software was designed on Excel 2010 for Windows with the assistance of a computer specialist in order to compute the medical costs of diabetes, in the absence or presence of complications. The estimated costs per individual are given as a range in the public and private health sector of a given country. It was hypothesized that incurred costs would be higher in the private healthcare sector and this was the rationale for collecting data in the public and private sectors. The cost data could be entered directly in the Excel software,

Table 1 Care parameters for basic treatment of uncomplicated diabetes and for the treatment of chronic and acute complications

Medical conditions	Care component	Guidelines
Uncomplicated diabetes	Consultation of diabetes specialist or a general practitioner	4/yr
	Fasting glucose test	4/yr
	Urine glucose test	6/yr
	Glycated hemoglobin	2/yr
	Proteinuria test	1/yr
	Blood lipid test TG, HDL-cholesterol, LDL-cholesterol	1/yr
	Electrocardiogram	1/yr
	Chest X-ray	1/yr
	Ophthalmology consult	1/yr
	Oral hypoglycemic agents	
	Glibenclamide 5 mg OR in combination with metformin	3 tablets/d
	Metformin® 500 mg	3 tablets/d
	Glucophage® 850 mg OR in combination with Amarel®	3 tablets/d
	Amarel® 4 mg	1 tablet/d
	For insulin users	
	Syringes	1/wk (min)-1/d (max)
	Insulin	30 UI (min) et 60 UI (max)/d
	Strips for blood glucose control	1 strip/d (min) 3 strips/d (max)
	Glucometer	1 (Duration: 2 yr)
Chronic complications		
Proliferative retinopathy	Consultation in ophthalmology	3/yr
	Retinography	1/yr
	Laser photocoagulation	1/yr
Overt nephropathy	Consultation in nephrology	2/yr
	Blood creatinine test	2/yr
	Serum protein test	2/yr
	Serum electrolytes test (sodium, potassium)	2/yr
	Urinary electrolytes test (sodium, potassium)	2/yr
	Urine creatinine test	2/yr
	Proteinuria	2/yr
	Hemogram	2/yr
	Urine bacteriology	4/yr
	Urine culture (ECBU)	1/yr
	Antiplatelet drugs	
	Aspirin® 100 mg OR	1 tablet/d
	Plavix® 75 mg	1 tablet/d
	Antihypertensive (ARA2)	
	Valsartan® 80 mg	1 tablet/d
	Diuretics:	
	Laxilix® 40 mg OR	3 tablets/d
	Laxilix® special 500 mg	Half tablet/d
	Calcium carbonate (added to antiplatelet therapy, maximum cost)	2 tablets/d
	Statin (added to antiplatelet therapy, maximum cost)	1 tablet/d
Renal failure	Dialysis	2 session/wk
	Potex® 4000 UI (EPO)	50 UI/kg weight (max) 2 sessions/wk
	Calcium carbonate 500 mg	1.5 g or 3 tablets/d (max)
Ischemic heart disease	Consultation in cardiology	2/an
	Antiplatelet drugs	See under nephropathy
	Statins	
	Simvastatin (Zocor®) OR	1 tablet/d
	Atorvastatin (Tahor®)	1 tablet/d
	Exercise electrocardiogram testing	1/yr
	Echo doppler	1/yr
	Cardiac ultrasound	1/yr
	Coronarography	1/yr
Hypertension	Consultation in cardiology	1/yr
	Antihypertensive drugs (ACEI)	
	Captopril® 25 mg OU	3 tablets/d
	Ramipril® 5 mg	1 tablet/d
	Diuretics	See under nephropathy

Diabetic foot	Aggregation inhibitors	See under nephropathy
	Semi quantitative urine protein test	2/yr
	Blood creatinine test	2/yr
	Proteinemia	2/yr
	Blood electrolytes test (Na, K, Ca)	2/an
	Stroke (chronic phase)	
	Consultation in cardiology	2/yr
	Antiplatelet drugs	See under nephropathy
	Consultation in podiatry	1/yr
	Arteriography of the lower limbs	1/yr
Acute complications	Physiotherapy sessions	10-20 sessions/yr
	Echo doppler	1/yr
	Statins	See under ischemic heart disease
	Antiplatelet drugs	1 tablet/d
	Orthopedic shoes	2 pairs/yr
	Ketoacidosis	
	Hospitalization	7 d
	Blood glucose test	Done once during hospitalization
	Glycated hemoglobin	Done once during hospitalization
	Hemogram	Done once during hospitalization
Diabetic foot (acute)	Blood lipids test	Done once during hospitalization
	Blood electrolytes test (Na, K)	Done once during hospitalization
	Blood creatinine test	Done once during hospitalization
	Blood urea test	Done once during hospitalization
	Electrocardiogram	Done once during hospitalization
	Chest X- ray	Done once during hospitalization
	Echo doppler	Done once during hospitalization
	Keto-Diastix® box of 50 strips (blood biology)	3 times/d for 3 d
	Perfusion	3 d
	Hospitalization	90 d
Foot surgery	Antibiotics	
	Oxaciline® 500 mg	4 tablets/d
		3 wk of treatment without bone involvement (min); 10 wk when bone involved (max)
	Vasodilator: Vastarel® 35 mg	2 tablets/d, 22 wk
	Biopsy	Done once during hospitalization
	Antibiogram	Done once during hospitalization
	Bone radiography	Done once during hospitalization
	Vascular ultrasound	Done once during hospitalization
	Dressings	1/wk, 22 wk
	Minor surgery (56%)	
End stage renal disease	Major surgery, amputation (44%)	
	Prosthesis	
	Hospitalization	30 d
	Ultrasound	Done once during hospitalization
	Electrolytes blood test (Na, K)	Done once during hospitalization
	Creatinine blood test	Done once during hospitalization
	Proteinemia	Done once during hospitalization
Stroke	Hospitalization	18 d
	Vasodilator: Vastarel® 35 mg	2 tablets/d, 18 d
	Anticoagulants	1 tablet/d, 18 d
	Rehabilitation/physical therapy	18 d
	Scanner	1 examination during hospitalization

TG: Triglycerides; HDL-cholesterol: High density lipoprotein cholesterol; LDL-cholesterol: Low density lipoprotein cholesterol; ARA2: Angiotensin receptor blocker; ACEI: Angiotensin converting enzyme inhibitors; EPO: Erythropoietin.

but the algorithms could not be changed. They are quite complex as several assumptions and empirical solutions had to be made. For instance, in computing the annual cost of basic care in the absence of complications, an

assumption had to be made regarding the proportion of subjects taking insulin since glucose monitoring regimen is different from those on oral hypoglycemic agents. It was estimated that roughly 20% of all patients were on

Table 2 Medical costs per individual per year for uncomplicated diabetes and for complications (in United States dollars) in the four countries

Medical condition	Bénin				Burkina Faso				Guinea				Mali			
	Public sector		Private sector		Public sector		Private sector		Public sector		Private sector		Public sector		Private sector	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Uncomplicated diabetes	212	542	310	828	224	451	363	859	126	422	437	1093	137	335	390	869
Chronic complications - Basic medical cost for uncomplicated diabetes plus additional annual cost for single complication																
Retinopathy	320	686	421	987	306	532	465	984	187	488	511	1243	177	374	524	1002
Stroke	233	576	672	1360	241	469	1644	2155	135	432	1267	1937	147	345	687	1850
Nephropathy	850	1920	1614	3198	783	1299	2578	3412	808	1578	2366	4256	491	1808	1516	4069
Hypertension	345	751	1256	2065	377	605	2074	2590	367	667	1784	2655	293	556	1212	2408
Ischemic heart disease	535	1297	1050	2188	496	785	1943	2577	313	612	1484	2553	441	1324	1002	2881
Diabetic foot	694	1533	1103	2386	923	1337	2447	4294	527	877	1717	3393	484	1424	1043	3172
Acute complications - Basic annual medical cost for uncomplicated diabetes plus additional cost per episode of acute complication																
Keto acidosis	406	802	576	1297	387	665	1036	1936	222	531	726	1758	243	463	606	1391
Infected diabetic foot requiring hospitalization	728	1539	1020	3860	729	1702	6886	12043	667	1129	2897	6679	698	1340	1709	5802
Stroke (acute phase)	637	1117	834	1935	576	1064	2316	4147	510	933	1427	3315	455	705	1003	2266

insulin, based on clinical practice and published data^[15]. Another example refers to dialysis. Some hospitals have a package rate, whereas others have a rate per session, with a separate charge for the catheter. For any care component, four prices were obtained; the software computed the range of cost per care item and for the total in the private and public sectors.

Based on the recommended frequency of "treatment" units, the total cost was computed per year and per individual for the medical cost of uncomplicated diabetes. Similarly, the additional annual cost for the treatment of chronic complications (one by one to prevent dual counting) was computed. For acute complications, the additional cost was calculated per episode. Costs were computed in local currency and they can then be converted automatically into Euros or United States dollars. The software provides the results in table and figure format. It is also possible to estimate with the software the total theoretical medical costs at country level, based on prevalence of diabetes and its main complications, but this is beyond the scope of the present paper (The software, which includes the spreadsheet for price data entry, is available from the corresponding author).

RESULTS

Individual medical costs for uncomplicated diabetes and additional costs associated with complications are shown in Table 2 for each study country (in United States dollars). Uncomplicated diabetes costs and chronic complication costs are given on an annual basis. Costs for acute complications include uncomplicated diabetes annual costs plus additional costs per episode of a given complication. Medical costs of uncomplicated diabetes ranged from 126 United States dollars to 1093 United States dollars in Guinea, 137 United States dollars to 869 United States dollars in Mali, 212 United States dollars to 828 United States dollars in Benin, and 224 United States dollars to 859 United States dollars in Burkina-Faso. Wide ranges were also observed within countries,

with at least a twofold increase from the minimum to the maximum cost in the public healthcare system as well as in the private sector. The minimum cost in the public sector was lower by a factor of 4 to 8 than the maximum cost in the private sector in all countries. In the treatment of uncomplicated diabetes, drugs and medical supplies represented the highest cost share, ranging from 52% to 75%. Figure 1 illustrates the range of medical costs per person per year for uncomplicated diabetes, in the public and private healthcare systems and for each study country. The medical costs increased steadily from the public to the private sector, except in Benin and Burkina-Faso where the maximum medical cost in the public sector was higher than the minimum cost in the private sector. Figure 2 provides illustrated examples of the software outputs for incremental medical costs per person per year in the presence of one chronic complication, in this instance for Mali. It shows that except for retinopathy, the additional cost for treating complications represents at least twice the medical cost of basic diabetes care. Of all chronic complications considered, the most costly was nephropathy in all countries.

Table 3 shows for each study country the estimated cost range in current United States dollars and in percentage of gross national income (GNI) per capita (<http://wdi.worldbank.org/table/1.1>) and of an economic poverty threshold (2\$ per day or 730\$ a year according to the World Bank^[23]) for uncomplicated diabetes, for diabetes with retinopathy, the complication with the lowest incremental cost, and for diabetes with nephropathy which entails the highest additional cost. GNI per capita in current United States dollars 2013 ranged from 460\$ in Guinea to 790\$ in Benin. Uncomplicated diabetes cost amounted to a minimum of 21%-34% of the GNI, with a maximum reaching 238% in Guinea. With chronic complications, the share of the GNI soared. The minimum cost of diabetes with nephropathy represented 73%-176% of the GNI. The medical costs of diabetes without complications ranged from 17.2% to 150% of the annual income corresponding to the poverty line.

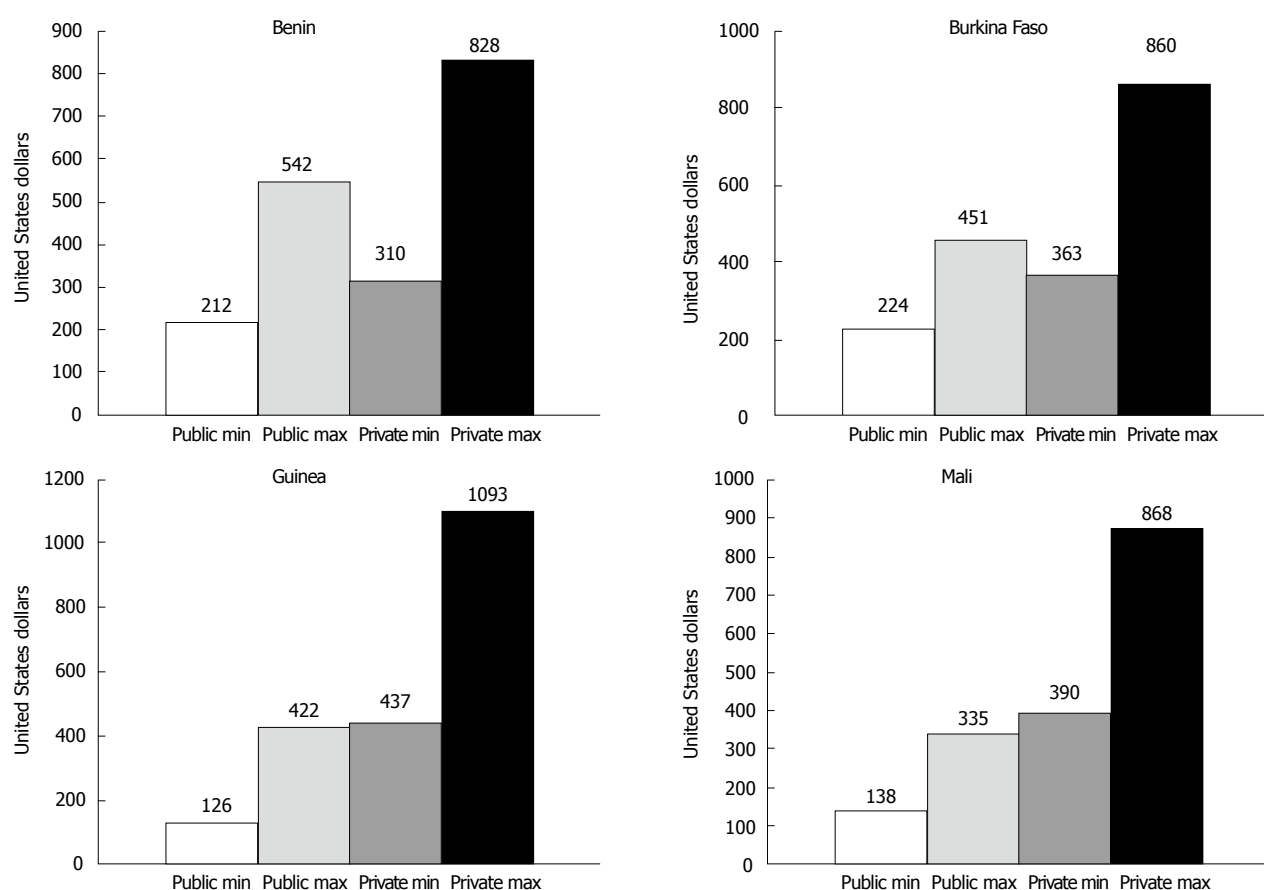


Figure 1 Computed medical costs per individual per year, basic care, no complications.

Table 3 Diabetes medical costs and national income in the study countries (United States dollars)

Countries		Mali	Benin	Burkina Faso	Guinea
GNI per capita (2013)		670	790	670	460
% of population with < \$2/d (= \$730/yr)		78.7	74.3	72.6	72.7
Uncomplicated diabetes	Minimum cost	137.21	211.63	224.42	125.58
	Maximum cost	868.60	827.91	859.30	1093.02
% of GNI (% of poverty threshold ¹)	Minimum	20.5 (18.7)	26.8 (28.9)	33.5 (30.7)	27.3 (17.2)
	Maximum	129.6 (118.9)	104.8 (113.4)	128.3 (117.6)	237.6 (149.7)
Diabetes + retinopathy	Minimum cost	176.74	320.93	305.81	187.21
	Maximum cost	1002.33	987.21	983.84	1243.02
% of GNI (% of poverty threshold)	Minimum	26.4 (24.2)	40.6 (43.9)	45.6 (41.9)	46.8 (25.6)
	Maximum	149.6 (137.3)	125.0 (135.2)	141.6 (134.7)	270.2 (170.3)
Diabetes + nephropathy	Minimum cost	490.70	850.00	782.56	808.14
	Maximum cost	4068.60	3197.67	3411.63	4256.98
% of GNI (% of poverty threshold)	Minimum	73.2 (67.2)	107.6 (116.4)	116.8 (107.2)	175.7 (110.7)
	Maximum	607.3 (557.3)	404.8 (438.0)	509.2 (467.3)	925.4 (583.1)

¹Threshold of 2 dollars/d (\$ 730/year) as set by the World Bank. GNI: Gross national income.

With retinopathy, the least costly complication, medical costs varied from 24.2% in Mali to 170% in Guinea. For nephropathy, the most costly complication, medical costs amounted to at least 67% of the annual poverty line.

DISCUSSION

The aim of this study was to develop and test a simple medical cost calculator for diabetes to be used primarily for

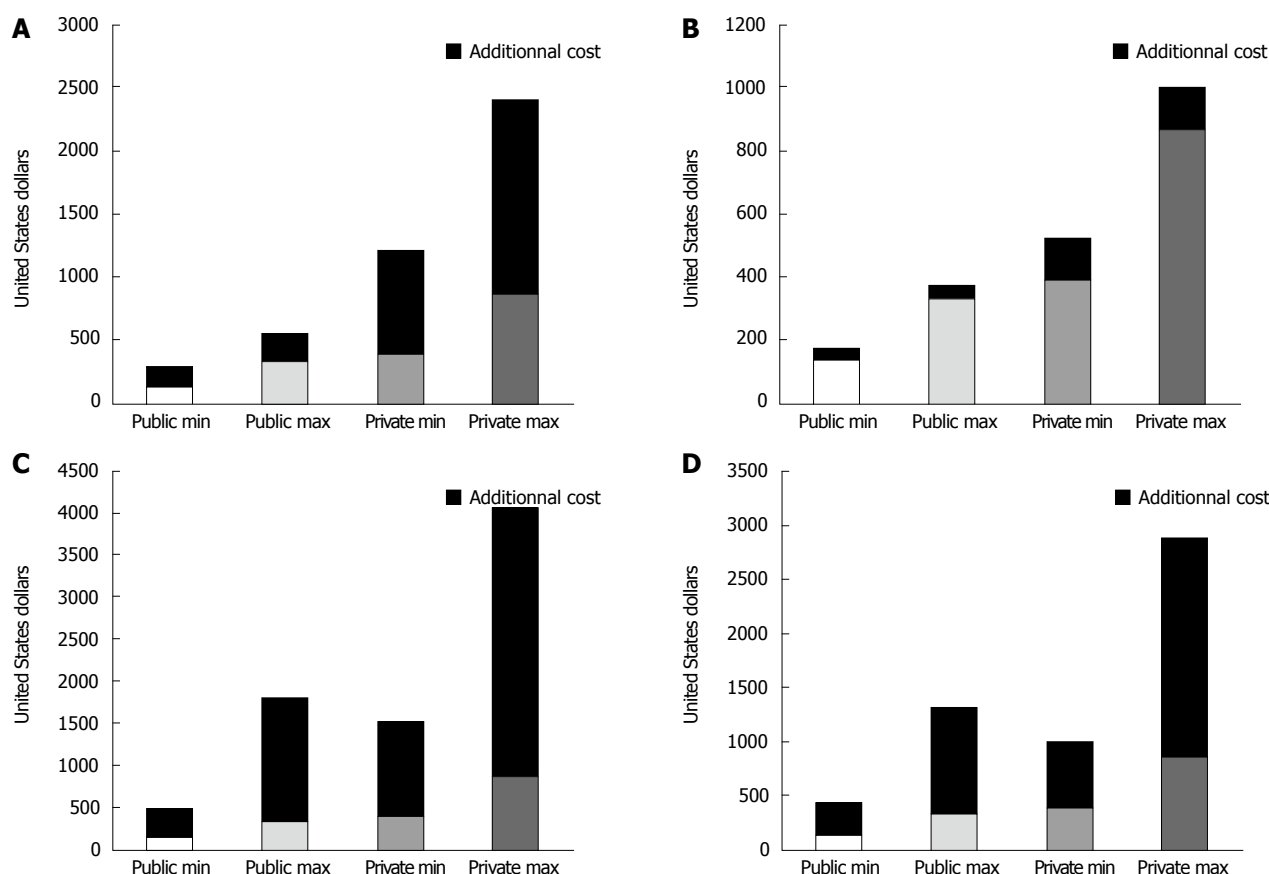


Figure 2 Computed annual medical costs per individual, basic care plus complications (Mali). A: Annual medical costs per individual, basic diabetes care plus hypertension; B: Annual medical costs per individual, basic diabetes care plus retinopathy; C: Annual medical costs per individual, basic diabetes care plus nephropathy; D: Annual medical costs per individual, basic diabetes care plus cardiopathy.

advocacy purposes. Research evidence is not consistently used for policy formulation^[24], even by international organizations^[25]. Demonstration of unbearable economic and human costs of a chronic disease such as diabetes may be regarded as concrete and therefore more promising as a means to influencing decision makers. While considerations other than financial, whether political or other, are at play, economic arguments are important even if not sufficient by themselves to induce changes in public policy and programmes^[26]. Furthermore, figures are compelling for decision-makers who have to address competing priorities. Cost indeed plays a critical role in decision making regarding health, in any country.

What the study showed is that the medical cost calculator was quite user-friendly and that collecting country-specific price data was highly relevant, considering the wide differences observed across countries in estimated medical costs of uncomplicated and complicated diabetes. The country-specific cost data collected using this instrument could actually be used to update regional estimates that are needed for macroeconomic models. The diabetes cost calculator is certainly not intended as a substitute for highly technical and validated models such as CORE^[7] and WHO-CHOICE^[8]. However, their use is constrained by their complexity, the global scale of the figures, and the numerous assumptions underlying

the calculations. Expertise is required to manipulate and adjust the models. Furthermore, since data on local effectiveness of interventions are scanty, particularly in Africa, assumptions have to be made. The costing tool was designed so as to remain simple in order to encourage its use at country level. The participants of the advocacy training workshop during which the calculator was presented indeed felt that the instrument was transparent and easy to use, but at the present time, we have no feedback on the effective use of the calculator for advocacy purposes.

The results confirmed that medical costs vary considerably between the private and public healthcare systems and even within each sector. Similarly, in India, direct costs were nearly four times higher in Chennai than in Delhi, illustrating wide variations within a country^[6]. Providing ranges of medical costs was considered more representative than means. Medical costs in other hospitals, clinics and pharmacies of the country would most likely fall within the computed range. Additionally, in order to compute means that would be representative of a whole country's medical costs, the process would be much heavier and expensive, with the need for a large number of randomly selected health facilities to include in the study. The cost calculator also allows to better size up the medical cost discrepancies between the private and public

sector. Public subsidies on drugs, hospitalization and other treatment components were not taken into account and likely contribute to these discrepancies among countries and between the private and public sector, for example in Benin, where the government gives subsidies for dialysis and for hospitalization. Additionally, costs for drugs will depend on the type of molecules and on whether or not generic formulations are available. The specific guidelines provided with the cost calculator regarding the type of drugs to consider (available on demand) contribute to standardize as much as possible cost data compilation.

The costing tool that we developed is the first of its kind and there is no equivalent in the published literature, where only out-of-pocket expenditures based on patients' surveys are to be found. The cost calculator refers to a standardized medical care protocol so that costs can be compared across countries. The tool is flexible in that prices can be updated as required in the Excel file, with automatic adjustment of the outputs (tables and graphs). This allows for the tool to be updated whenever new data become available. However the treatment components cannot be modified in the software in order to allow comparisons.

The estimated total medical cost for the basic treatment of diabetes at the individual level in a given country using this standard procedure may be of value to health professionals, governments and other potential payers such as insurance companies in many ways. Firstly and of foremost importance, the burden of the expenses that households or individuals would have to incur for basic care of uncomplicated diabetes can be appraised based on income levels and income distribution in the country, where such data are available. In the absence of recent household budget surveys, a basis for comparison could be the country's minimum wage, the GNI per capita, or else an economic poverty threshold, as was done in the present study. This may allow to clearly show that several patients are not minimally treated because they simply cannot afford the medical follow-ups and even the drugs, in the absence of government subsidies or insurance (or even when these are available). These cost data may therefore represent in themselves powerful advocacy arguments. In Cameroon, a study on out-of-pocket expenditures of more than 350 diabetes patients revealed that monthly medical costs reached 148\$ United States in 2009-2011 (89.40\$ for medicines, 10.40\$ for consultations, 35.0\$ for tests, and 13.20\$ for glycemia monitoring), which amounted to more than twice the minimum wage^[27]. According to the present study, basic treatment cost range of uncomplicated diabetes would represent 27%-105% of GNI per capita in Benin, 21%-130% of GNI in Mali, 27%-238% in Guinea, and in Burkina, where the minimum estimated cost was the highest of the four countries, from 34% to 138%. Actual expenditures of patients probably lie somewhere in-between the minimum and the maximum. Analysis of data from a survey of several hundred individuals living with diabetes in Mali showed that annual medical expenditures of

subjects free from complications were within the range of estimated costs using the calculator, at least in the public sector (unpublished data). A study on the economic burden of diabetes in Africa^[5] showed that average medical costs (including the same components as the present study) represented 36% of GNI for countries with a GNI lower than 2000\$ United States, which includes all four countries of the present study. Furthermore, this is the basic cost only. Treatment costs soared when diabetes complications are present, which is the case for a majority of persons living with diabetes -70% according to the survey in Mali^[28]. In India, the cost-ratio for those having complications vs those without was around 2.0^[6]. In our study, for instance, the treatment of nephropathy, considering only the minimum cost in the public sector, would more than treble the yearly basic cost, ranging from a factor of 3.5 in Burkina Faso to 6 in Guinea. A few studies have shown that the cost of diabetes is indeed beyond reach of a sizeable proportion of the population. In Côte d'Ivoire, for instance, 35% to 55% of the household income would have to be spent for diabetes care^[29]. In Mali, it was estimated that for insulin only, households with a diabetic member spent 38% of their total income^[30]. This confirms that acceptable diabetes care is likely unaffordable to most. In our study, we used the GNI per capita as a proxy of income, as well as the World Bank poverty threshold of 2\$/d (or 730\$/year). It is noteworthy that the GNI per capita itself is below this poverty threshold in three of the four study countries. Roughly 75% of the population of these countries lives with less than 2\$/d, which betrays rampant poverty. It is therefore likely that a good majority of the people would not afford even the most basic medical treatment of type-2 diabetes. The diabetes cost calculator provides for an estimation of minimum incurred medical expenses as a percentage of income proxy without having to conduct lengthy and expensive surveys for that purpose. Furthermore, demonstrating the cost increment associated with complications related to a late diagnosis may help convince decision-makers to make the screening more efficient at primary health care level. Comparing the medical costs of diabetes in neighbouring countries may also be of interest for policy purposes, even if only the cost of the disease is computed at this stage.

There are obviously several limitations to this costing tool. Only direct medical costs are estimated, on the basis of diabetes treatment component price data collected locally in public and private care institutions. Other direct costs incurred by families, such as transportation, traditional medicine, time of the care-provider and extra-expenditures for the diet are not taken into account and besides, these can hardly be standardized. However, according to other studies on actual expenditures, direct costs tend to be higher than indirect costs^[6].

Although the treatment protocol was considered realistic for Africa, all included elements of care may not be absolutely necessarily in spite of their relevance. For instance, some could argue that care may be acceptable

even if some costly tests are not performed. Moreover, although consensus was required in the working group to define the treatment components, some arbitrariness was unavoidable, whether in the frequency of medical visits or in medicine posology.

The cost calculator does not include either the indirect costs to the health system (salaries and training of health personnel; health facilities; subsidies, etc.), the families and the society as a whole (loss of productivity, of income, of healthy life years...). The medical costs estimated with the calculator represent only a fraction of the total economic burden of the disease.

A useful addition to the costing tool would be to estimate cost-effectiveness of interventions for primary prevention among high-risk individuals, combined with secondary prevention among diagnosed individuals, in order to demonstrate the savings that may accrue from earlier detection and treatment of diabetes, using the costing tool. However, there are no cost-effectiveness data that are relevant for Africa and the only controlled interventions in low- and middle-income countries were conducted in India^[31] and China^[32]. Additionally, cost-effectiveness analyses do not take into account non-health benefits, such as income gains, which may be important^[33].

In conclusion, the study confirms in an objective and standardized fashion that the basic medical cost of diabetes is likely beyond reach of a majority of people in West-African countries considering that no medical insurance is available in most of them. In spite of its limitations, the medical cost calculator, which can be used in different countries, is deemed important in Africa, considering the paucity of data on diabetes cost in the whole region. It is also flexible enough since cost data can easily be changed. No study had so far designed a simple costing tool which would take into account the various components of medical care of diabetes and its complications in sub-Saharan Africa.

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COMMENTS

Background

Non-communicable chronic diseases such as diabetes are taking a heavy social and economic toll in low income countries already overburdened by acute diseases. However, diabetes remains far from the top of health priorities in most of sub-Saharan Africa. In addition to global calls for action, specific advocacy strategies and instruments are therefore timely. The study describes a standardized calculator that was developed to estimate the medical costs of diabetes in sub-Saharan Africa. Showing the exorbitant cost of inaction is regarded as potentially convincing for decision makers.

Research frontiers

Economic models of diabetes and other chronic diseases exist but their complexity is a serious barrier to their wider use notably for advocacy purposes. The development and testing of simple yet standardized estimators of medical costs of diabetes (and other chronic diseases) is a relevant research area for low- and middle-income countries who now face a staggering escalation of chronic diseases.

Innovations and breakthroughs

Previous studies on this topic in Africa focused on medical costs of acute complications in the hospital or of specific care components such as insulin treatment, whereas this study included all direct medical costs for basic treatment and for the treatment of chronic and acute complications. To develop the medical cost calculator, a detailed protocol for the basic treatment of type-2 diabetes and for the treatment of main complications was first developed by a working group consisting of medical and public health specialists from the four West African study countries. Care components for minimally adequate treatment included physicians and allied health professionals' services, hospital care, lab tests and controls, and drugs and medical supplies. Price data for the care components were then collected in the public and private health sectors of the study countries. The user-friendly software designed on Excel 2010 for Windows allows to compute ranges of total medical costs for patients. Total yearly medical costs for a person with diabetes convincingly show that the treatment is unaffordable for many, particularly when taking into account local incomes and if complications are present, which is the case for a majority of patients.

Applications

The medical costs calculator developed in the study can be used in other African countries since the treatment protocol is standardized; only local prices vary. This tool can be used by health professionals or other stakeholders for advocacy so that action is taken for type 2 diabetes prevention and control. The calculator allows to show the prohibitive cost of inaction vis-à-vis type 2 diabetes, whether at the individual or country level. The tool would have to be complemented with data on alternative interventions in order to show the cost of action.

Terminology

Direct medical costs of diabetes: The calculator provides an estimate of out-of-pocket expenditures of patients with diabetes for their treatment in the study countries of West Africa. Other direct costs (transportation, diet...) are not included. Gross national income (GNI) per capita: This figure is often used as a proxy for income, as done in the study countries. The GNI is converted to international dollars using purchasing power parity rates. An international dollar has the same purchasing power as a United States dollar in the United States.

Peer-review

The authors describe a tool that is a simple medical cost calculator; they report and discuss the results of this tool. The manuscript is well written and well organized.

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World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
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PUBLISHER
Baishideng Publishing Group Inc
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Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
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Skeletal muscle as a therapeutic target for delaying type 1 diabetic complications

Samantha K Coleman, Irena A Rebalka, Donna M D'Souza, Thomas J Hawke

Samantha K Coleman, Irena A Rebalka, Donna M D'Souza, Thomas J Hawke, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON L8S 4L8, Canada

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Correspondence to: Thomas J Hawke, PhD, Department of Pathology and Molecular Medicine, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4L8, Canada. hawke@mcmaster.ca
 Telephone: +1-905-5259140

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Abstract

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease targeting the pancreatic beta-cells and rendering the person hypoinsulinemic and hyperglycemic. Despite exogenous insulin therapy, individuals with T1DM will invariably develop long-term complications such as blindness, kidney failure and cardiovascular disease. Though often overlooked, skeletal muscle is

also adversely affected in T1DM, with both physical and metabolic derangements reported. As the largest metabolic organ in the body, impairments to skeletal muscle health in T1DM would impact insulin sensitivity, glucose/lipid disposal and basal metabolic rate and thus affect the ability of persons with T1DM to manage their disease. In this review, we discuss the impact of T1DM on skeletal muscle health with a particular focus on the proposed mechanisms involved. We then identify and discuss established and potential adjuvant therapies which, in association with insulin therapy, would improve the health of skeletal muscle in those with T1DM and thereby improve disease management—ultimately delaying the onset and severity of other long-term diabetic complications.

Key words: Type 1 diabetes mellitus; Skeletal muscle; Exercise; Myostatin; Leptin; Adiponectin; Metabolism

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Core tip: Skeletal muscle is adversely affected in type 1 diabetes mellitus and strategies to maintain/improve muscle health will positively impact disease management and delay diabetic complications.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic disease characterized by the autoimmune destruction of the pancreatic beta cells. Without the insulin produced by these cells, the body is no longer able to manage blood

glucose, leading to hyperglycemia. Even in the case of tightly regulated insulin therapy, it is extremely difficult to maintain blood glucose levels within an acceptable range^[1]. Complications, such as blindness (retinopathy), kidney failure (nephropathy), peripheral nerve damage (neuropathy), cardiovascular disease and impairments to muscle health (myopathy), invariably arise as a direct/indirect result of the inability to manage blood glucose.

In healthy individuals, insulin is typically released postprandially and is responsible for promoting an influx of glucose into adipose, hepatic and skeletal muscle cells for storage or metabolism. Of these insulin-sensitive cells, skeletal muscle is the largest of these organs by mass in the body^[2,3] and thus plays a prominent role in glucose homeostasis. Skeletal muscle is also capable of uptaking large amounts of glucose in a non-insulin mediated manner^[4] such as is seen during muscle contraction. Not surprisingly then, if the health of skeletal muscle is sub-optimal, management of blood glucose will also be sub-optimal. Despite the vital role played by skeletal muscle in whole body metabolic control and blood glucose management, our understanding of changes to the health of this organ system in both acute and long-term T1DM is still in its infancy. Much of our current knowledge is derived from rodent models with uncontrolled hyperglycemia for a period of weeks or months. The resultant impairments to skeletal muscle health, referred to as “diabetic myopathy” manifests as impaired muscle growth and strength^[5-8], altered metabolic capacity^[5-7] and reduced regenerative and stem cell capacities^[9-15]. Though human studies investigating diabetic myopathy are sparse, the results to date suggest consistency in the observations with rodent models^[6]. Specifically, reductions in muscle mass, fiber size, work capacity and maximal force production^[6] are seen in persons with T1DM.

In this review, we will introduce some of the key factors impacting skeletal muscle health in those with T1DM and then discuss established and possible therapeutic strategies focused on improving skeletal muscle health as a means of improving skeletal muscle health with the ultimate goal of attenuating the development of other diabetic complications.

METABOLIC STRESS

In a state such as T1DM, excessive accumulation of glucose in the blood incites excessive stress on the entire body. Specifically within the muscle, damaging metabolites, such as reactive oxygen species (ROS), wreak havoc within the tissue causing damage to cellular structures with resultant functional impairments. The oxidative capacity of T1DM skeletal muscle is altered when compared to healthy, non-diabetic muscle. In the *Ins2*^{Akita+/-} model of T1DM, glycolytic fibers exhibit atrophy, as demonstrated through a decreased proportion of type II B/X fibers, as well as a decrease in type II A and II B/X fiber area^[5]. Studies in human

T1DM populations also displayed alterations in fiber type variability through an increased proportion of fast glycolytic fibers, and an increased amount of glycolytic enzyme activity^[16,17]. Correspondingly, changes in the normal fiber type distribution are accompanied by changes in fuel oxidation and metabolic capacity of the muscle. Due to the reduced ability of skeletal muscle to access carbohydrates in times of inadequate/low insulin, diabetic skeletal muscle must promote the use of other fuel sources. Skeletal muscle of individuals with T1DM is associated with the excessive deposition of intramyocellular lipids (IMCL)^[5,18]. This high level of IMCLs is noted in the muscle following food consumption, and very low levels in the fasted state, as this fuel source is heavily relied upon. Muscle from the streptozotocin (STZ) T1DM mouse model also demonstrates increased acetyl CoA/CoA ratio, hypothesized to be due to increased fatty acid oxidation^[19], as well as increased fat utilization and mobilization^[20], as the muscle tries to deal with the increased fat content. Along with these changes in the skeletal muscle of both the *Ins2*^{Akita+/-} and STZ models, there is an upregulation of CD36, a fatty acid transporter^[5,21-23]. The alloxan-induced T1DM model similarly demonstrates an increase in free fatty acid levels in cardiac and skeletal muscle tissues^[24]. It is believed that as the levels of IMCL deposition increase, lipotoxicity ensues^[25], enhancing stress to the tissue. Despite a heavier reliance on triglycerides, diabetic myopathy is accompanied with decreased activity of lipid metabolism enzymes citrate synthase^[5,26,27], β -hydroxybutyrate^[5], and 3-hydroxybutyrate dehydrogenase^[26]. The trend of increased IMCL persists in human populations of T1DM, and is correlated with the degree of insulin resistance observed in these subjects^[28]. Contrarily, the *Ins2*^{Akita+/-} mouse model does not show the same increase in intramuscular triglyceride content^[5,29] seen in the (disease duration-matched) STZ model, and does not demonstrate a decrease in citrate synthase or β -hydroxybutyrate activity^[5]. It is worth noting, however, in the case of the STZ-induced diabetic model, that STZ itself has been implicated in the generation of oxidative stress within muscle cells, even in the absence of hyperglycemia^[30]. Thus the STZ model could be held to represent a much more severe model of T1DM due to the elevated levels of oxidative stress than may be seen in diabetes alone.

Studies have shown that hyperglycemia and T1DM specifically display elevated markers of oxidative stress in the skeletal muscle^[31,32], resulting in insulin resistance^[33]. Accumulation of damaging ROS in skeletal muscle has been linked with a loss of protein mass^[34] and disrupted protein turnover^[35]. This oxidative stress has an effect on transcription of glucose transporters which contributes to the development of insulin resistance^[32]. Specifically in STZ rats, oxidative stress was seen to upregulate atrogin-1 and MuRF-1, markers of muscle atrophy, and downregulate MyoD, Myogenin and JunD, genes required for normal muscle growth and repair^[15]. Though there is clear evidence that accu-

mulation of IMCL deposits causes dysfunctional fatty acid oxidation, generation of ROS, and stress on the muscle, future studies are needed in other diabetic models to more fully elucidate the contribution(s) of these stressors to diabetic myopathy development and progression.

VASCULAR DYSFUNCTION

An intricate network of vasculature supplying the skeletal muscle with adequate blood supply is required for optimal muscle performance. In T1DM, however, there is dysfunction of the capillary network and endothelial cells. Hyperglycemia has been found to alter the capillary bed, reducing capillary diffusing capacity and disrupting hemodynamic regulation to skeletal muscle^[36,37]. T1DM mice demonstrate both a decrease in capillary-to-fiber ratio^[5,38] and dysregulated angiogenesis^[38]. Moreover, thickening of the basement membrane of skeletal muscle blood vessels in T1DM rats has been found to be positively related to their level of dysglycemia^[39-41]. Thickening of the basement membrane in skeletal muscle capillaries is also greater in patients experiencing worsening retinopathy, a serious complication of T1DM^[42]. Furthermore, studies show that peripheral microvascular dysfunction could also be seen as an indicator of atherosclerotic damage in individuals with T1DM^[43]. In the case of ApoE^{-/-} STZ mice, a T1DM rodent model which mimics macrovascular complications, mice which were returned to normoglycemia exhibited expansion of the vasa vasorum microvascular network^[44]. This expansion was directly correlated with attenuation of atherogenesis^[44]. Overall, early attenuation of vascular dysfunction within the skeletal muscle would help prevent further long-term complications.

INSULIN RESISTANCE

Brownlee^[31], in his unifying theory of diabetic complications, has suggested that a large part of cardiovascular disease risk in those with diabetes is due to insulin resistance. Though insulin resistance is more commonly associated with the development of type 2 diabetes, individuals with T1DM also demonstrate insulin resistance^[29,45,46]. In fact, insulin resistance has been observed in T1DM youth^[45] and long-duration type 1 diabetics^[47,48], and occurs independent of glycemic control^[49]. Impairment of glucose transporters^[50] and glucose transport following exercise^[51] have been observed in insulin resistant T1DM, further enhancing the diabetic phenotype. Insulin resistance in T1DM has been linked directly with skeletal muscle pathology^[52] through increased IMCL deposition and dysregulation of fatty acid oxidation^[53].

Interestingly, exposure to a long-acting human insulin analogue, insulin detemir, has been shown to result in more significant insulin resistance, oxidative stress, skeletal muscle ectopic fat accumulation and mitochondrial impairments compared to hyperglycemia alone^[54]. These results indicate that insulin resistance

may in fact be a response to insulin treatment as opposed to hyperglycemia. Therapeutic strategies targeting an improvement in peripheral insulin sensitivity would reduce exogenous insulin needs, preventing insulin resistance and thus delaying the onset of diabetic complications^[55].

In response to T1DM, skeletal muscle is negatively impacted, as is evident by increased metabolic stress, vascular impairments and insulin resistance (Figure 1). With all of these decrements, muscle is not able to respond optimally to stressors or combat the elevated glycemic and lipid loads frequently experienced in T1DM. It is believed that maintaining or improving skeletal muscle health in T1DM can contribute significantly to delaying diabetic complications. For example, improving muscle metabolic health would reduce oxidative stress, and increasing insulin sensitivity would have the combined effect of improving glycemic control and reducing exogenous insulin needs. In the following section we propose a variety of skeletal muscle-centric therapeutic strategies as a means to both improve the overall health of those with diabetes mellitus and reduce the complications associated with this disease state.

EXERCISE TRAINING

Exercise therapy is now being regarded as an important component in the management of T1DM due to its resultant improvements towards attenuation of microvascular complications and improvements of insulin sensitivity^[56]. In a variety of metabolic disorders (independent of T1DM) exercise is associated with improvements in glucose and lipid metabolism^[57-59], enhanced glucose transport^[60], increased insulin sensitivity^[61,62], reductions in daily insulin requirement, and a decreased risk of related co-morbidities^[63,64]. Accordingly, it is predicted that improvements in skeletal muscle health, by way of exercise, would promote a greater state of well-being in individuals with T1DM.

Due to the onset of myopathy with T1DM disease advancement^[6], as well as the presence of disease onset during the critical growth period, it is not surprising that the physical fitness of T1DM children is often observed to be reduced when compared to their healthy age-matched counterparts^[6,65]. This disparity has been attributed, in part, to the inverse association between glycemic control and skeletal muscle function, resulting in reduced aerobic fitness. As mentioned, T1DM individuals commonly experience both functional and growth impairments^[5-8]. A decrease in cardiorespiratory fitness has similarly been observed in T1DM adolescents and adults with poor glycemic control^[66,67]. Based on these data, the implementation of an exercise training program would be considered an effective therapeutic strategy to improve muscle health and delay the onset and progression of diabetic complications.

A primary clinical measure to define the risk for complications development in those with T1DM is glycosylated haemoglobin (HbA1c). Changes to long term

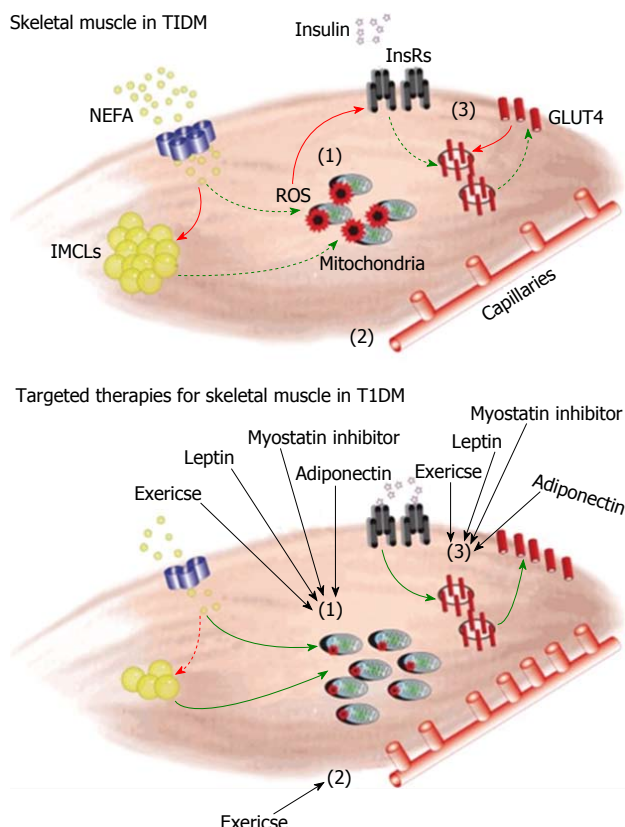


Figure 1 Schematic figure representing skeletal muscle dysfunction in type 1 diabetes mellitus and possible therapeutic approaches targeting skeletal muscle. (1) In T1DM, due to dyslipidemia and/or the reduced ability for muscle to uptake carbohydrates, an increased amount of non-esterified fatty acids (NEFA) are shuttled into the skeletal muscle. The majority of this excess fat is deposited in the form of intramyocellular lipid droplets (IMCLs) as there is a reduced ability to efficiently oxidize lipids due to impairments to oxidative capacity. An increased amount of metabolic stress and reactive oxygen species (ROS) production within the mitochondria is observed in T1DM and appears to be a causative factor; (2) T1DM also induces dysfunction with regard to the vasculature network. There is a thickening of the basement membrane and downregulation of angiogenesis resulting in a decreased capillary-to-fiber ratio. Impairments to microvasculature have also been linked with generation of macrovascular complications (e.g., atherosclerosis), a serious long-term diabetic complication; and (3) Insulin resistance results in disruptions to the insulin signalling pathway. Improper insulin signalling prevents excess glucose in the blood from being taken up by the muscle via decreased translocation of the GLUT4 glucose transporter. Our proposed treatments of exercise, myostatin inhibition, leptin and adiponectin target the specific pathways mentioned above in skeletal muscle. We hypothesize that if diabetic myopathy is attenuated it will allow muscle to contribute a greater amount towards reducing hyperglycemia. Since muscle is an important large metabolic organ, if skeletal muscle health was improved there would be resultant decreases in oxidative stress, improvements to glycemic control and a reduction in the need for exogenous insulin. T1DM: Type 1 diabetes mellitus; GLUT4: Glucose transporter type 4.

glycemic control (measured by HbA1c) are a contributing factor to disease progression, and it has been shown that hyperglycemia is prone to induce an assortment of co-morbidities that further perpetuate the disease state^[68] including muscle morphology and function^[69-71]. Many studies investigating the therapeutic benefit of exercise on the overall health of those with T1DM have relied on HbA1c as a primary outcome measure. Indeed, while exercise has been shown to increase glucose uptake and improve insulin sensitivity, information on

changes to HbA1c remains largely controversial. Studies assessing the impact of either aerobic and/or strength training protocols in T1DM rodents and humans fail to establish a consensus on whether or not increasing physical activity improves glycemic control. For instance, a number of studies have reported a decrease in HbA1c levels following a period of aerobic training^[72,73], while others report no difference in HbA1c following a period of comparable training volume^[74-76]. Similarly, investigations incorporating strength training protocols have reported no effect on HbA1c levels^[77,78], while others indicate beneficial effects incorporating both strength and aerobic exercise^[79]. Nevertheless, longitudinal data suggests that improvements in glycemic control are still observed despite minimal improvements in HbA1c levels following aerobic training^[80,81]. Discrepancies in HbA1c improvements amongst the studies reported are thought to be a result of variations in insulin dosage (reducing dosages as a means to prevent exercise-induced hypoglycaemia) and carbohydrate uptake, which override any quantifiable changes in glucose disposal. Although increased fitness may not dramatically improve glycemic control, physical activity is still encouraged for all T1DM individuals due to the additional skeletal muscle health benefits incurred, including the attenuation in microvascular complications, improved insulin sensitivity, reductions in inflammation, and enhanced muscle growth and repair. For a thorough review on exercise and T1DM, see^[56].

As noted previously, the progression of T1DM promotes the onset of various microvascular complications. These complications not only promote a worsened disease state, but may also interfere with the individual's physical capacity^[82]. It is critical to address the role of vascular complications in the skeletal muscle in T1DM, as maladaptive changes to the diabetic muscle often precede the advancement of other complications^[6,69,83]. The effect of exercise therapy on skeletal muscle vasculature is largely positive, with many studies reporting increases in angiogenesis-related genes^[38], and enhanced vascular function^[84,85]. In humans, an inverse correlation exists between physical activity and the development of macro- and micro-vascular complications in long-standing T1DM^[86], however specific adaptations in skeletal muscle vasculature following exercise training remain largely unknown.

Elevations in markers of inflammatory and oxidative stress have also been identified in T1DM patients^[87-89]. Inflammation is known to negatively impact skeletal muscle health, as observed by the positive correlation between inflammatory factors and muscle wasting^[90,91]. Skeletal muscle from T1DM mice show an increased expression of inflammatory-related factors^[92,93]. Exercise does elicit anti-inflammatory effects^[94,95], which are dependent on exercise type, duration, intensity, endurance capacity and muscle morphology^[96-98]. Recently, diabetic rats demonstrated reductions in inflammatory cytokine levels [*i.e.*, interleukin 1B (IL-1B), IL-4, *etc.*] following exercise intervention^[99]. Furthermore, T1DM children

subjected to an acute bout of exercise demonstrated dysregulation in the expression of inflammatory and oxidative stress variables^[100], thereby providing evidence for the importance of exercise training in the reduction of inflammation associated with T1DM disease progression. While exercise reduces pro-inflammatory cytokines, it has also been found to promote the expression of anti-inflammatory cytokines that enhance muscle health. For instance, STZ rats subjected to a 5-wk resistance exercise training regimen displayed an increase in IL-15, an anabolic cytokine that is known to induce hypertrophy in skeletal muscle^[101,102], while hindering apoptosis^[103]. The cytokine IL-6, while primarily believed to be pro-inflammatory in nature, is also known to exert beneficial effects on skeletal muscle following training. Specifically, increased IL-6 production promoted greater glucose uptake during exercise^[104] and an up-regulation of additional anti-inflammatory cytokines^[105]. These data, while not explicitly investigated within the context of T1DM, suggests a protective role of IL-6 release from skeletal muscle following exercise. While these studies implicate exercise in the support of muscle health *via* attenuation of the inflammatory state associated with T1DM development, future work using human data is needed to further delineate the role of exercise training in the regulation of chronic inflammation in T1DM.

Overall physical capacity is negatively affected by the presence of T1DM, particularly in those with long-standing disease, and thus it is predicted that any form of activity (endurance, resistance, etc.) will benefit the individual by maintaining and/or enhancing skeletal muscle health and the benefits therein. The literature to date makes a clear case that exercise training can positively affect the skeletal muscle of those with T1DM through its influence on skeletal muscle endothelial cell function, inflammation and insulin sensitivity. What remains to be clearly elucidated is the impact of exercise training on the modulation of long-term glycemic control; a measure hampered by subject variability in insulin dosage, intensity of exercise training, and degree of disease advancement between studies.

MYOSTATIN

Myostatin (GDF-8), primarily synthesized by skeletal muscle and a negative regulator of muscle growth, was originally discovered in 1997 when a mutation in the myostatin gene was shown to be responsible for phenotypically hypermuscular cattle^[106]. In the case of myostatin deficiency, muscle growth was observed to reach 2-3 times that of typical muscle size^[106]. Instances of loss-of-function myostatin mutation have been observed in human populations to the same effect^[107].

Myostatin levels have been measured in the STZ-diabetic mouse, and consistently show elevated protein^[108] and gene expression^[109,110]. Human populations of T2DM also demonstrate increased levels of myostatin^[111-113]. This increase of myostatin in T1DM is consistent with the decreased muscle mass and myopathic phenotype

observed. In a study of food deprivation, a state similar to that as found in uncontrolled T1DM, increased expression of myostatin was found to contribute to the observed muscle atrophy^[114].

Methods of inhibiting or knocking down elements of the myostatin pathway have been, and are currently being investigated in a variety of disease states. Naturally, myostatin inhibition therapy *via* MYO-029^[115], PF-06252616^[116] and ACE-031^[117], amongst others, was originally investigated in patient populations with genetic muscular diseases and muscle wasting disorders (e.g., cancer cachexia). More recently, blockade of the myostatin pathway has been linked to improvements of metabolic pathologies in animal studies. For instance, high-fat diet fed mice with myostatin reduction therapy did not gain weight as wildtype counterparts did^[118-120] and myostatin inhibition is seen to prevent diabetes development in a model of lipodystrophy^[121]. Furthermore, in the case of T1DM specifically, STZ animals treated with follistatin, a known inhibitor of myostatin, demonstrate improvements in the regenerative capacity of skeletal muscle^[14].

In the case of other metabolic diseases, increased myostatin expression has been implicated in the development of insulin resistance^[122] and reduction or inhibition of myostatin has been seen to improve insulin sensitivity^[119,123-126]. It is clear that myostatin plays a role in glycemic control of skeletal muscle. Models examining mutated myostatin or myostatin inhibition coincide with significantly elevated levels of GLUT4^[127,128] and GLUT1^[128], resulting in increased glucose uptake^[127]. This evidence demonstrates how myostatin plays an important role in increasing glucose disposal both dependent and independent of insulin. Reductions in circulating myostatin in T1DM may therefore aid in both reducing exogenous insulin needs and preventing the insulin resistance which may develop as a result.

Increased levels of myostatin may contribute to the elevated oxidative stress noted in diabetic myopathy. Myostatin is thought to operate both through^[129] and independent^[130] of the nuclear factor κ B pathway to produce ROS, leading to muscle atrophy. In STZ-induced T1DM, myostatin was shown to contribute to oxidative stress leading to DNA damage^[131]. Since myostatin contributes to oxidative stress, it is possible that in the case of myostatin inhibition, decreased oxidative stress (ROS production) could lead to functional problems as have been reported in rodents without myostatin^[132]. It is important to remember however that in T1DM the fulcrum is already shifted towards increased ROS levels. Thus, reductions in myostatin could serve to restore balance resulting in healthier muscle and the associated benefits therein.

Myostatin inhibition has more recently been linked to the "browning" of white adipose tissue^[133-136]. One study has postulated this effect is mediated through the 5' AMP-activated protein kinase (AMPK)-PGC1 α -Fndc5 pathway originating in skeletal muscle^[137]. While this is an indirect positive effect of myostatin inhibition

(i.e., not specifically related to skeletal muscle), it would also provide benefits in reducing the diabetic condition. Gunawardana *et al.*^[138] have shown that a transplant of brown adipose tissue into STZ-diabetic mice resulted in normalization of glucose and attenuation of the diabetic state. This effect is thought to occur through recovery of subcutaneous white adipose tissue, resulting in the normalization of adipokines leptin, adiponectin and insulin-like growth factor-1 (IGF-1).

Although downregulation of myostatin shows promise in the treatment of T1DM *via* decreasing oxidative stress, upregulating glucose transporters, preventing insulin resistance and browning white adipose tissue, there are still many areas left to be explored. Production of ROS is a delicate balance, and a drastic decrease in ROS levels can cause harm to an organism as well. Further, Wang *et al.*^[139] explored a soluble myostatin receptor to downregulate the effects of myostatin in conjunction with STZ diabetes, and saw worsened hyperglycemia. Authors of this study observed severely low insulin levels and significantly elevated glucocorticoid levels, common to the STZ rodent model^[139]. The lack of effect of myostatin reduction therapy may be the result of the rise in glucocorticoids (resulting in elevated blood glucose) or the absence of circulating insulin. Since the inhibition of myostatin may have its greatest metabolic effects *via* increasing insulin sensitivity, the lack of insulin seen in the STZ model may have been detrimental to any potential blood glucose lowering capacity of myostatin inhibition^[139]. Overall, there is certainly enough compelling evidence to further investigate myostatin inhibition strategies as an adjuvant therapeutic strategy for T1DM.

LEPTIN

Leptin, a hormone predominantly produced by adipose tissue, has been heavily implicated in metabolism. First unwittingly examined in the 1950s, the leptin knockout mouse (*ob/ob* mouse) demonstrated excessive hyperphagia and in turn, excessive weight gain^[140]. The discovery of leptin itself in 1994 led to the understanding of leptin as an important hormone with regard to appetite control^[141], and has further been implicated in reproductive health^[142], bone metabolism^[143], the immune response^[144], and importantly in regulating fat metabolism, insulin resistance and overall metabolism. The identification of leptin brought about an understanding that adipose tissue was an endocrine organ. Currently, more than 19 different adipocyte-derived cell-signaling proteins, termed adipokines, have been identified^[145]. Adipokines include inflammatory mediators, angiogenic proteins, and metabolic regulators. With the global rise in obesity, the relationship between adipose tissue and its systemic effects has attracted much interest. Adipokines are thought to influence multiple processes, including glucose and fatty acid metabolism, and insulin sensitivity.

It has been noted that children and adults with poorly controlled T1DM demonstrate low levels of

leptin regardless of gender^[23,146]. Leptin levels can be normalized *via* insulin treatment in T1DM children^[146], but not in adults^[23]. Furthermore, poorly managed diabetes has been associated with an increase in the soluble leptin receptor, leading to leptin resistance^[147]. This same trend is seen in STZ diabetic rodents, in which the induction of T1DM caused a decrease in circulating leptin, which was reversed by insulin therapy^[148,149].

Leptin therapy has been found to attenuate many of the effects of T1DM, most notably restoring euglycemia^[150-153]. Considering the restoration of euglycemia coupled with leptin's ties to appetite control, leptin treated STZ diabetic rodents demonstrate diminished hyperphagia^[154]. While Fujikawa *et al.*^[155] have hypothesized that the improvements observed in T1DM *via* leptin treatment occur *via* CNS-dependent mechanisms, and Unger's group has targeted leptins ability to decrease plasma glucagon levels^[152,156-158], there is growing evidence that leptin therapy provides benefits through skeletal muscle as well. Leptin treatment has been found to increase insulin sensitivity and glucose uptake in skeletal muscle specifically^[159-161]. Yu *et al.*^[162] demonstrate that hyperleptinemia leads to euglycemia independent of insulin. This causes an upregulation of IGF-1 and pIGF-1 receptor, which further leads to increases in skeletal muscle IRS-1, P13K and ERK phosphorylation^[162]. Specifically in the soleus muscle, leptin was implicated to act in an insulin-like fashion, leading to increases in a variety of muscle metabolic factors including glucose uptake, glycogen synthesis, lactate formation and glucose oxidation^[163].

Leptin has also been demonstrated to play a role in both regulating fatty acid oxidation and preventing insulin resistance in skeletal muscle. Skeletal muscle of STZ diabetic animals treated with leptin exhibit evidence of restored glucose uptake, but also enhanced skeletal muscle markers of fatty acid utilization and oxidation, notably independent of differences in food consumption^[164]. Leptin has also been seen to direct lipids towards the muscle to be burned rather than stored^[165], as well as increase fatty acid oxidation in the skeletal muscle^[166]. These metabolic benefits are thought to occur through the activation of AMPK and the inhibition of acetyl-CoA carboxylase^[167]. Insulin resistance in T1DM has also been found to be reversed through leptin therapy^[168]. Interestingly, however, this was thought to occur in a method independent of skeletal muscle^[168]. Kusakabe *et al.*^[169] found that leptin treated STZ mice fed high fat diet to induce insulin resistance demonstrated enhanced insulin sensitivity. This was again seen by Lin *et al.*^[170], although was attributed to neurological changes. Although leptin's role in diminishing insulin resistance is clear, further work is necessary to elucidate the mechanism of its action in this role.

As leptin appears to mimic many of the effects of insulin, leptin may indeed be used as an adjuvant therapy to insulin^[152,171]. When leptin and insulin were given in conjunction to STZ rodents, much smaller doses

of insulin were required to achieve normoglycemia than would be required with each treatment alone^[172]. Metreleptin, a leptin analogue, is currently under clinical trials (NCT01268644) in conjunction with insulin therapy in order to investigate the effectiveness of this combination seen in the literature. Considering both the prevalent development of insulin resistance and the difficulty in maintaining normoglycemia in T1DM patients, even in the presence of insulin therapy, this adjuvant therapy warrants further investigation in the human T1DM population.

ADIPONECTIN

Adiponectin, first characterized in 1995^[173], is an insulin-sensitizing adipokine; capable of increasing both insulin-mediated uptake of glucose and β -oxidation of lipids^[174-177]. Individuals with T2DM exhibit significantly lower levels of circulating adiponectin than healthy, non-diabetic individuals^[178]. With adiponectin behaving as an insulin sensitizing factor, it is not surprising that this deficiency in adiponectin closely correlates with an individuals' degree of insulin resistance^[179]. Systemic injection of adiponectin has been shown to decrease resting blood glucose levels and attenuate insulin resistance^[174,175,180]. Furthermore, stimulation of adiponectin production in an animal model of T2DM improves skeletal muscle insulin sensitivity^[181]. Paradoxically, when compared to healthy non-diabetic subjects, adiponectin is present in elevated levels in individuals with T1DM, regardless of their level of glycemic control^[28,182,183] and these elevations are positively correlated with duration of T1DM^[184,185].

The presence of metabolic syndrome in patients with T1DM has previously been associated with insulin resistance^[186]. Interestingly, T1DM patients with metabolic syndrome present with significantly lower levels of serum adiponectin than T1DM patients that do not present with metabolic syndrome^[186]. Similar to the relationship between insulin sensitivity and adiponectin in non-diabetic individuals, levels of adiponectin are positively correlated with insulin sensitivity in T1DM^[184]. Insulin sensitivity in T1DM individuals, however, is lower than in non-diabetic subjects at any given level of circulating adiponectin^[184]. The preservation of the positive relationship between adiponectin and insulin sensitivity in T1DM coupled with the overall decrease in insulin sensitivity in T1DM individuals suggests a modification in the homeostatic regulation of adiponectin in the T1DM state^[184].

Upon binding to adiponectin receptors in the pancreatic beta cells, adiponectin increases insulin gene expression and secretion^[187]. The presence of insulin, on the other hand, has been shown to downregulate adiponectin gene expression^[188]. In this light, it is possible that the overabundance of adiponectin in the T1DM state is a compensatory mechanism; an attempt at upregulating insulin production. As previously mentioned, however, despite higher levels of adiponectin

being associated with insulin sensitivity, individuals with T1DM still have a lower insulin sensitivity than non-diabetic individuals^[184].

Adult T1DM human and rodent muscle has been observed to have higher levels of intramyocellular lipids (IMCL) than muscle of healthy, non-diabetic subjects^[5,28,189]. This accretion of IMCLs has been associated with insulin resistance in T1DM^[189]. Interestingly, previous reports indicate no differences in IMCL content between T1DM and non-diabetic children^[190], potentially indicating that, similar to circulating levels of adiponectin, IMCL content is affected by, and positively associated with T1DM disease duration. Furthermore, Krause *et al.*^[191] found a positive correlation between intramyocellular adiponectin expression and IMCL density in non-diabetic mice; elevated levels of adiponectin were detected in muscle fibers displaying a greater IMCL density, while adiponectin was virtually undetectable in muscle fibers with a low IMCL content. In the T1DM disease state, however, it is possible that this positive relationship may be a compensatory mechanism to remove lipid from circulation, and further investigation into this relationship in the diabetic state must be conducted. In 2007, Behre^[192] proposed that adiponectin may in fact be a defense mechanism of the body in response to starvation (as can be compared to overt T1DM), resulting in increased fatty acid oxidation and glucose uptake *via* activation of AMPK and PPAR- α .

Overall, a great deal of research must still be conducted to elucidate the role of adiponectin in both overall health and skeletal muscle health in T1DM. While adiponectin levels are elevated in the T1DM state, adiponectin appears to act in a compensatory mechanism to improve insulin sensitivity in the absence of insulin. As insulin resistance develops in T1DM individuals that develop metabolic syndrome, adiponectin levels demonstrate a decline. Evidence suggests that it may be beneficial to supplement adiponectin in the T1DM disease state in order to boost insulin production and increase insulin sensitivity in order to prevent this insulin resistance.

CONCLUDING THOUGHTS

The presence of insulin resistance, altered lipid metabolism, impaired vascularization and oxidative stresses are clear indicators of the presence of pathology in T1DM skeletal muscle. Exercise training, myostatin, leptin and adiponectin have been identified as potential therapeutic avenues to investigate with regard to improving skeletal muscle health (Figure 1). It is our hypothesis that, by improving skeletal muscle health in T1DM, the muscle will be better able to contribute to the reduction of diabetic symptoms. This would, in turn, lead to systemic benefits and delayed diabetic complications, increasing the quality and quantity of life of individuals with T1DM.

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Observational Study

Evaluation of the Finnish Diabetes Risk Score to predict type 2 diabetes mellitus in a Colombian population: A longitudinal observational study

Diego Gomez-Arbelaiz, Laura Alvarado-Jurado, Miguel Ayala-Castillo, Leonardo Forero-Naranjo, Paul Anthony Camacho, Patricio Lopez-Jaramillo

Diego Gomez-Arbelaiz, Laura Alvarado-Jurado, Leonardo Forero-Naranjo, Paul Anthony Camacho, Patricio Lopez-Jaramillo, Dirección de Investigaciones, Clínica de Síndrome Metabólico, Prediabetes y Diabetes, Fundación Oftalmológica de Santander - FOSCAL, Floridablanca 681004, Colombia

Diego Gomez-Arbelaiz, Patricio Lopez-Jaramillo, Instituto de Investigaciones MASIRA, Facultad de Medicina, Universidad de Santander - UDES, Bucaramanga 680003, Colombia

Diego Gomez-Arbelaiz, División de Endocrinología, Escuela de Medicina, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

Miguel Ayala-Castillo, Escuela de Medicina, Universidad Autónoma de Bucaramanga, Bucaramanga 680003, Colombia

Author contributions: Gomez-Arbelaiz D, Camacho PA and Lopez-Jaramillo P conceived and designed the study; Alvarado-Jurado L, Ayala-Castillo M and Forero-Naranjo L contributed to data acquisition; Gomez-Arbelaiz D and Camacho PA analyzed the data; Gomez-Arbelaiz D and Lopez-Jaramillo P wrote the paper; Gomez-Arbelaiz D, Alvarado-Jurado L, Ayala-Castillo M, Forero-Naranjo L, Camacho PA and Lopez-Jaramillo P contributed to editing, reviewing and final approval of article.

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Correspondence to: Patricio Lopez-Jaramillo, MD, PhD, FACP, Director de Investigaciones, Dirección de Investigaciones, Clínica de Síndrome Metabólico, Prediabetes y Diabetes, Fundación Oftalmológica de Santander - FOSCAL, Calle 155A N. 23-09, Torre Milton Salazar, Primer piso, El Bosque, Floridablanca 681004, Santander, Colombia. jplopezj@gmail.com
 Telephone: +57-7-6386000
 Fax: +57-7-6388108

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Abstract

AIM: To assess the performance of the Finnish Diabetes Risk Score (FINDRISC) questionnaire for detecting and predicting type 2 diabetes mellitus (DM2) in a Colombian population.

METHODS: This is a longitudinal observational study conducted in Floridablanca, Colombia. Adult subjects (age ≥ 35 years) without known diabetes, were included. A modified version of FINDRISC was completed, and the glycemia values from all the subjects were collected from the hospital's database. Firstly, a cross-sectional analysis was performed and then, the subsample of prediabetic participants was followed for diabetes incidence.

RESULTS: A total of 772 subjects were suitable for the study. The overall prevalence of undiagnosed DM2 was 2.59%, and the incidence of DM2 among the prediabetic participants was 7.5 per 100 person-years after a total of 265257 person-years follow-up. The FINDRISC at baseline was significantly associated with undiagnosed and incident DM2. The area under receiver operating characteristics curve of the FINDRISC score for detecting undiagnosed DM2 in both men and women was 0.7477 and 0.7175, respectively; and for predicting the incidence of DM2 among prediabetics was 71.99% in men and 67.74% in women.

CONCLUSION: The FINDRISC questionnaire is a useful screening tool to identify cross-sectionally unknown DM2 and to predict the incidence of DM2 among prediabetics in the Colombian population.

Key words: Finnish diabetes risk score; Type 2 diabetes mellitus; Prediabetes; Screening; Colombia

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Core tip: The prevalence of type 2 diabetes mellitus (DM2) is rapidly increasing worldwide, and unfortunately its diagnosis is being made when vascular complications are already exhibited. Since laboratory-based diagnostic methods are costly, the International Diabetes Federation suggests to do an early detection of undiagnosed DM2 patients, and to identify individuals at risk for developing DM2 by simple risk-scoring questionnaires. The present study assesses the performance of the Finnish Diabetes Risk Score questionnaire in a Colombian population, and aims to establish the specific cutoff values for detecting subjects at increased risk of undiagnosed DM2 and for predicting the incidence of DM2 in prediabetic individuals.

Gomez-Arbelaez D, Alvarado-Jurado L, Ayala-Castillo M, Forero-Naranjo L, Camacho PA, Lopez-Jaramillo P. Evaluation of the Finnish Diabetes Risk Score to predict type 2 diabetes mellitus in a Colombian population: A longitudinal observational study. *World J Diabetes* 2015; 6(17): 1337-1344 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v6/i17/1337.htm> DOI: <http://dx.doi.org/10.4239/wjd.v6.i17.1337>

INTRODUCTION

The prevalence of type 2 diabetes mellitus (DM2) is

rapidly increasing worldwide^[1]. Recently, it has been estimated that the number of adults with DM2 will increase between 2010 and 2030 by 69% and 20% in developing and industrialized countries, respectively^[1]. In Colombia the overall prevalence of DM2 is 5.51%, however significant differences are observed and in some regions the prevalence rises to 8.2%^[2]. The increase in the prevalence of DM2 across the world has become an important public health concern given that it is a major risk factor for death and numerous nonfatal complications. Hence, this situation will form a large burden to the patients, their families, and the health care system^[3].

Several studies have demonstrated that DM2 could be prevented, and its complications can be limited when a timely and appropriate intervention is started^[4,5]. However, in the majority of cases detection is delayed and at the time of diagnosis many patients already exhibit signs of microvascular and macrovascular complications^[6]. Remarkably, there has been proposed that Latin American population has an increased vulnerability for developing macrovascular diseases at glycemia levels lower than the internationally established cut points for DM2^[7,8]. Thus, it is clinically important to do an early detection of undiagnosed DM2 patients, and to identify individuals at risk for developing DM2 to implement intensive preventive interventions.

The diagnosis of DM2 is obtained by increased values of fasting plasma glucose (FPG), oral glucose tolerance test (OGTT) and/or glycated hemoglobin (HbA1C)^[9], but the determination of biochemical variables is costly, and this impairs its use as a large scale screening tool. Conversely, simple risk-scoring questionnaires are useful and constitute a more cost-effective DM2 screening approach^[10]. Hence, the international diabetes federation has recommended performing a blood test to detect possible diabetes when a high risk score has been obtained^[11].

A number of predictive risk-scoring models for DM2 are currently available for clinical use^[12-14]. But, most require specific blood test results and this limits its widespread use from a public health perspective. Meanwhile, the Finnish Diabetes Risk Score (FINDRISC)^[15] has demonstrated to be a simple, fast, inexpensive, noninvasive, and reliable tool to identify individuals at high risk for DM2. Therefore, the FINDRISC has been internationally assessed in several countries, including Colombia^[16-20]. However, in our country the ability of FINDRISC to predict the development of incident DM2 was not evaluated, and the specific risk scores for predicting undiagnosed and incident DM2 in our population were not established. Hence, this study aims to assess the performance of the FINDRISC score in detecting undiagnosed DM2 in the general population and to predict incident DM2 among prediabetics. Moreover, the study aims to establish the specific cutoff values for identifying increased risk of undiagnosed DM2 in the general population and incident DM2 among prediabetics in Colombia.

MATERIALS AND METHODS

A longitudinal observational study has been conducted. Between June 1, 2012 and October 31, 2012 adult subjects (age ≥ 35 years) who attended the general practitioner for any reason at the ambulatory service of the Ophthalmological Foundation of Santander - FOSCAL in Floridablanca, Colombia, were involved in the screening. People with known diabetes mellitus (type 1 or 2) were not recruited. Any acute illness, pregnancy in women, and currently use of metformin or other glucose-modifying prescription drugs, were also considered as exclusion criteria.

The subjects were asked to complete a modified version of the FINDRISC score^[15], which evaluates eight variables that are clearly correlated with the risk of DM2: Age, body mass index (BMI), waist circumference (WC), current antihypertensive medication, frequency of fruit and vegetable consumption, physical activity, personal history of high blood glucose, and family history of DM2. Variables are scored according to the risk that they may confer, resulting in a range of 0-26 total points. We adjusted the WC cutoffs points according to the previously described values that confer an increased cardio-metabolic risk in the Colombian population^[21]. As a result, the WC has been scored as follows: Men: < 90 cm, the score is 0; 90-98 cm, the score is 3; > 98 cm, the score is 4. Women: < 80 cm, the score is 0; 80-88 cm, the score is 3; > 88 cm, the score is 4.

General practitioners performed the anthropometric measurements. Weight and height were measured with light clothing and no shoes with calibrated scales and a wall-mounted stadiometers, respectively, while participants were asked to stand erect with their head positioned in the Frankfort horizontal plane. WC was measured midway between the lowest rib and the iliac crest using an anthropometric tape. BMI was calculated by dividing body weight by the square of height [BMI = weight (kg)/height (m)²].

Laboratory tests (FPG, OGTT and HbA1C) were collected directly from the hospital's database. Only those tests taken within the two months previous or after the survey were valid for the study. At least one of the tests: FPG, OGTT or HbA1c, should be present to consider the patient as suitable for the study. Classification of glucometabolic state was based on the American Diabetes Association (ADA) criteria^[9]. The diagnosis of DM2 was established when FPG ≥ 126 mg/dL, OGTT ≥ 200 mg/dL and/or HbA1c $\geq 6.5\%$. Prediabetes was diagnosed by the presence of impaired FPG (≥ 100 mg/dL to < 126 mg/dL), impaired OGTT (≥ 140 mg/dL to < 200 mg/dL) and/or impaired HbA1c ($\geq 5.7\%$ to $\leq 6.4\%$).

Thereafter, the subsample of baseline prediabetic participants was followed for diabetes incidence in real life settings. The updated glucometabolic tests (FPG, OGTT and HbA1C) were also collected from the hospital's database, and DM2 incident cases were diagnosed according to the ADA criteria^[9].

Ethics statement

The study protocol was in accordance with the Declaration of Helsinki, and the health research ethics board of the Ophthalmological Foundation of Santander - FOSCAL approved all study procedures. The subjects expressed their interest in participating in the study before they were included. As there were no interventions directly related to the study written informed consent was not required.

Statistical analysis

Descriptive statistics were computed for variables of interests, and included mean values and standard deviations of continuous variables and absolute and relative frequencies of categorical factors. Normality of distribution was checked for continuous variables using the Shapiro-Wilk test and by graphical methods. Wilcoxon Rank Sum test was used to investigate the differences in continuous variables. Testing for differences in categorical variables was accomplished using the Pearson's χ^2 test.

Moreover, we used unconditional multivariate logistic regression models to assess the associations between the FINDRISC score and undiagnosed and incident DM2. These analyses were adjusted for potential confounders, such as gender and age. We re-coded the FINDRISC into tertiles and compared the risk of DM2 in each tertile with the lowest category of risk (reference group).

To assess performance of the FINDRISC score with respect to undiagnosed and incident DM2, receiver operating characteristics (ROC) curves, sensitivity, specificity, positive predictive values and negative predictive values (NPV) were calculated. The maximum values of the Youden's index^[22] were used as a criterion for selecting the optimum cut-off points. All statistical analysis was carried out by a biomedical statistician using Stata statistical software, release 12.0 (Stata Corporation, College Station, TX, United States). A $P < 0.05$ was considered statistically significant.

RESULTS

A total of 772 subjects were suitable for the study, of which 544 (70.47%) were women. The overall mean age was 58.34 ± 12.07 years, the overall prevalence of undiagnosed DM2 was 2.59% (95%CI: 1.46-3.71), and the prevalence of prediabetes was 24.09% (95%CI: 21.06-27.11) (Table 1). Baseline demographic, anthropometric and metabolic characteristics of the study population are presented in Table 1, and the prevalences of FINDRISC questionnaire components are presented in Table 2.

The FINDRISC score was positively associated with undiagnosed DM2 (Table 3). The risk of DM2 increased with increasing tertiles of FINDRISC. Compared with participants in the lowest tertile, the risk of DM2 was 5.69 times higher for those in the highest tertile (OR = 5.69, 95%CI: 1.56-20.67).

Table 1 Baseline characteristics of the study population according to gender

Variables	Total (n = 772)	Men (n = 228)	Women (n = 544)
¹ Age, yr	58.34 (12.07)	58.63 (12.67)	58.22 (11.82)
¹ BMI, kg/m ²	27.36 (4.56)	27.08 (4.56)	27.47 (4.56)
¹ Waist circumference, cm	91.91 (10.47)	97.05 (10.88)	89.78 (9.52) ²
¹ Fasting plasma glucose, mg/dL	94.41 (12.60)	96.17 (13.94)	93.68 (11.94) ²
^{1,4} Oral glucose tolerance test, mg/dL	107.89 (32.82)	111.11 (36.55)	106.23 (30.75)
^{1,4} HbA1c, %	6.49 (1.27)	6.39 (1.24)	6.56 (1.31)
Undiagnosed DM2, n(%)	20 (2.59)	6 (2.63)	14 (2.57)
Prediabetes, n(%)	186 (24.09)	61 (26.75)	125 (22.98)
FINDRISC score	11.84 (4.80)	11.00 (4.71)	12.18 (4.80) ³

¹Data are presented as mean ± SD for continuous variables; ²Wilcoxon Rank Sum test $P < 0.05$;

³Wilcoxon Rank Sum test $P < 0.005$; ⁴Variable with missing values. BMI: Body mass index; DM2: Type 2 diabetes mellitus; FINDRISC: Finnish Diabetes Risk Score; HbA1c: Hemoglobin A1c.

Table 2 Prevalence of components of the Finnish Diabetes Risk Score according to gender n (%)

Variables	Total (n = 772)	Men (n = 228)	Women (n = 544)
Age (yr)			
< 45	106 (13.73)	35 (15.35)	71 (13.05)
45-54	207 (26.81)	59 (25.88)	148 (27.21)
55-64	203 (26.30)	51 (22.37)	152 (27.94)
> 64	256 (33.16)	83 (36.40)	173 (31.80)
BMI (kg/m ²)			
< 25	250 (32.38)	75 (32.89)	175 (32.17)
25-30	331 (42.88)	105 (46.05)	226 (41.54)
> 30	191 (24.74)	48 (21.05)	143 (26.29)
WC (cm)			
M: < 90; W: < 80	121 (15.67)	50 (21.93)	71 (13.05) ²
M: 90-98; W: 80-88	272 (35.23)	84 (36.84)	188 (34.56)
M: > 98; W: > 88	379 (49.09)	94 (41.23)	285 (52.39)
PA (30 min/d)			
Yes	362 (46.95)	112 (49.12)	250 (46.04)
No	409 (53.05)	116 (50.88)	293 (53.96)
Vegetables - fruits			
Daily	433 (56.09)	112 (49.12)	321 (59.01) ¹
No daily	339 (43.91)	116 (50.88)	223 (40.99)
Hypertension			
Without medication	442 (57.25)	136 (59.65)	306 (56.25)
With medication	330 (42.75)	92 (40.35)	238 (43.75)
Hyperglycemia antecedent			
No	634 (82.12)	199 (87.28)	435 (79.96) ¹
Yes	138 (17.88)	29 (12.72)	109 (20.04)
Familiar antecedents DM2			
No	473 (61.27)	147 (64.47)	326 (59.93)
Grandparents	72 (9.33)	26 (11.40)	46 (8.46)
Parents	227 (29.40)	55 (24.12)	172 (31.62)

¹Pearson's χ^2 test (χ^2) $P < 0.05$; ²Pearson's χ^2 test (χ^2) $P < 0.005$. DM2: Type 2 diabetes mellitus; BMI: Body mass index; WC: Waist circumference; PA: Physical activity; M: Male; F: Female.

Table 3 Odds ratios of undiagnosed type 2 diabetes mellitus according to Finnish Diabetes Risk Score tertiles at baseline

	Undiagnosed DM2 (FPG \geq 126 mg/dL and/or OGTT \geq 200 mg/dL and/or HbA1c \geq 6.5%)								
	1 st tertile			2 nd tertile			3 rd tertile		
	OR	95%CI	P value	OR	95%CI	P value	OR	95%CI	P value
Unadjusted	1.0	-	-	2.45	0.60-9.92	0.207	5.69	1.56-20.67	0.008
Gender-adjusted	1.0	-	-	2.49	0.61-10.10	0.199	5.88	1.60-21.54	0.007
Age- and gender-adjusted	1.0	-	-	2.28	0.55-9.35	0.252	4.93	1.28-18.92	0.020

1st tertile: ≤ 10 ; 2nd tertile: 11-14; 3rd tertile: ≥ 15 . OR: Odds ratio; DM2: Type 2 diabetes mellitus; FPG: Fasting plasma glucose; HbA1c: Hemoglobin A1c; OGTT: Oral glucose tolerance test.

Table 4 Hazard ratios of incident type 2 diabetes mellitus according to Finnish Diabetes Risk Score tertiles at baseline, during follow-up and among the subsample of prediabetic patients

	Incident DM2 (FPG \geq 126 mg/dL and/or OGTT \geq 200 mg/dL and/or HbA1c \geq 6.5%)								
	1 st tertile			2 nd tertile			3 rd tertile		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
Unadjusted	1.00	-	-	3.67	0.77-17.36	0.101	5.31	1.15-24.43	0.032
Gender-adjusted	1.00	-	-	3.93	0.81-18.89	0.087	5.75	1.22-27.03	0.027
Age- and gender-adjusted	1.00	-	-	3.52	0.70-17.53	0.124	4.81	0.93-24.85	0.061

1st tertile: \leq 12; 2nd tertile: 13-16; 3rd tertile: \geq 17. HR: Hazard ratio; DM2: Type 2 diabetes mellitus; FPG: Fasting plasma glucose; HbA1c: Hemoglobin A1c; OGTT: Oral glucose tolerance test.

Table 5 Finnish Diabetes Risk Score to identify undiagnosed type 2 diabetes mellitus by gender

FINDRISC score	Undiagnosed DM2 (FPG \geq 126 mg/dL and/or OGTT \geq 200 mg/dL and/or HbA1c \geq 6.5%)				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
Cutoff value \geq 11					
Men	83.3	49.1	4.2	99.1	0.324
Women	85.7	37.2	3.5	99.0	0.228
Cutoff value \geq 12					
Men	66.7	56.8	4.0	98.4	0.234
Women	85.7	44.9	3.9	99.2	0.306
Cutoff value \geq 13					
Men	66.7	66.2	5.1	98.7	0.328
Women	78.6	54.3	4.4	99.0	0.329
Cutoff value \geq 14					
Men	66.7	75.2	6.8	98.8	0.419
Women	71.4	62.6	4.8	98.8	0.340
Cutoff value \geq 15					
Men	50.0	81.1	6.7	98.4	0.310
Women	57.1	70.7	4.9	98.4	0.278
Cutoff value \geq 16					
Men	33.3	86.0	6.1	98.0	0.193
Women	50.0	76.2	5.3	98.3	0.262
Cutoff value \geq 17					
Men	33.3	88.3	7.1	98.0	0.216
Women	50.0	81.9	6.8	98.4	0.318

DM2: Type 2 diabetes mellitus; FPG: Fasting plasma glucose; OGTT: Oral glucose tolerance test; HbA1c: Hemoglobin A1c; PPV: Positive predictive value; NPV: Negative predictive value; FINDRISC: Finnish Diabetes Risk Score.

A total of 20 incident cases of DM2 (10.75%, 7 men and 13 women) among baseline prediabetic individuals occurred during 265.257 (82.234 men and 182.923 women) person-years of follow-up. The overall incidence of subsequent DM2 development was 7.5 (95%CI: 4.9-11.7) per 100 person-years. The incidence rates were slightly higher in men (8.5, 95%CI: 4.0-17.8, per 100 person-years) than in women (7.1, 95%CI: 4.1-12.2, per 100 person-years), but the difference was not statistically significant. Likewise, compared with participants in the lowest tertile of FINDRISC score at baseline, the risk of incident DM2 was 5.31 times higher for those in the highest tertile (HR = 5.31, 95%CI: 1.15-24.43) (Table 4).

The area under ROC curve (AUROC) of the FINDRISC score for detecting undiagnosed DM2 in both men and women was 0.7477 (95%CI: 0.5722-0.9232) and 0.7175 (95%CI: 0.5868-0.8481), respectively (Figure 1). The performance assessment of the FINDRISC score for identifying individuals at risk of undiagnosed DM2 is shown in Table 5. At the cutoff value of 14 in both

men (sensitivity = 66.7%; NPV = 98.8%) and women (sensitivity = 71.4%; NPV = 98.8%), the Youden's index was the highest for undiagnosed DM2 (0.419 in men and 0.340 in women).

The ROC curve for the incidence of DM2 among the prediabetic subsample by FINDRISC is shown in Figure 2. The AUROC curve were 0.7199 (95%CI: 0.5355-0.9043) in men and 0.6774 (95%CI: 0.5401-0.8146) in women. Given a Youden's index of 0.383 in men and 0.305 in women, FINDRISC cutoff values for incident DM2 were calculated to be 13 (sensitivity = 85.7%; NPV = 95.2%) in men and 16 (sensitivity = 69.2%; NPV = 93.4%) in women (Table 6).

DISCUSSION

Key findings

The current study assessed the performance of the FINDRISC questionnaire, and demonstrates that this can work reasonably well as screening tool, detecting cross-sectionally undiagnosed DM2 in the general population,

Table 6 Finnish Diabetes Risk Score to predict incident type 2 diabetes mellitus by gender, during follow-up and among the subsample of prediabetic patients

FINDRISC score	Incident DM2 (FPG \geq 126 mg/dL and/or OGTT \geq 200 mg/dL and/or HbA1c \geq 6.5%)				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
Cutoff value \geq 12					
Men	85.7	44.7	22.2	94.4	0.304
Women	92.3	28.0	15.2	96.3	0.202
Cutoff value \geq 13					
Men	85.7	52.6	25.0	95.2	0.383
Women	92.3	34.4	16.4	97.0	0.267
Cutoff value \geq 14					
Men	71.4	60.5	25.0	92.0	0.319
Women	84.6	39.8	16.4	94.9	0.244
Cutoff value \geq 15					
Men	57.1	65.8	23.5	89.3	0.229
Women	76.9	49.5	17.5	93.9	0.263
Cutoff value \geq 16					
Men	42.9	68.4	20.0	86.7	0.112
Women	69.2	61.3	20.0	93.4	0.305
Cutoff value \geq 17					
Men	42.9	73.7	23.0	87.5	0.165
Women	53.9	66.7	18.4	91.2	0.205
Cutoff value \geq 18					
Men	42.9	79.0	27.3	88.2	0.218
Women	46.2	76.3	21.4	91.0	0.224

DM2: Type 2 diabetes mellitus; FPG: Fasting plasma glucose; OGTT: Oral glucose tolerance test; HbA1c: Hemoglobin A1c; PPV: Positive predictive value; NPV: Negative predictive value; FINDRISC: Finnish Diabetes Risk Score.

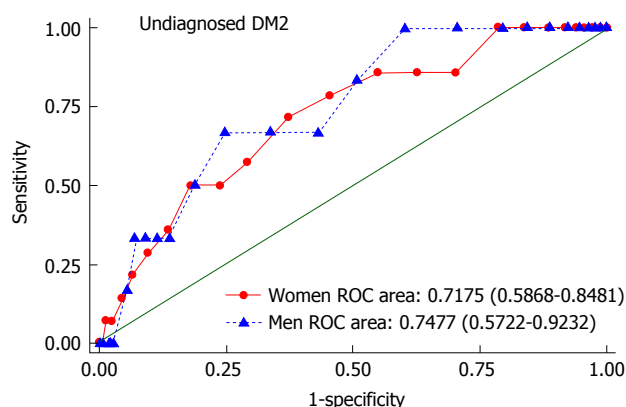


Figure 1 Receiver operating characteristics curves for the prevalence of undiagnosed type 2 diabetes mellitus by gender and Finnish Diabetes Risk Scores. DM2: Type 2 diabetes mellitus; ROC: Receiver operating characteristics.

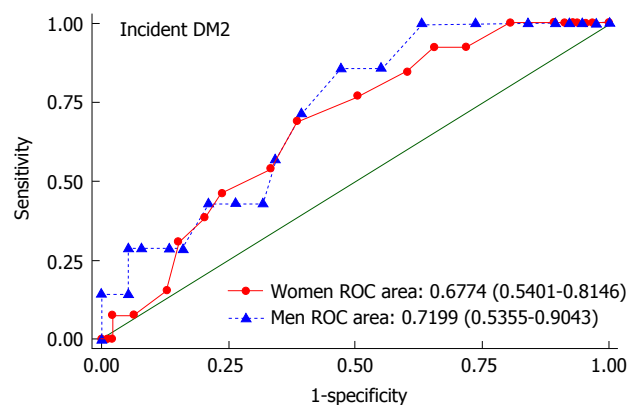


Figure 2 Receiver operating characteristics curves for the incidence of type 2 diabetes mellitus by gender and Finnish Diabetes Risk Scores, during follow-up and among the subsample of prediabetic patients. DM2: Type 2 diabetes mellitus; ROC: Receiver operating characteristics.

and longitudinally incident DM2 among individuals with prediabetes in Colombia. Our findings show a positive association between the FINDRISC score, undiagnosed DM2 in the general population and incident DM2 among prediabetic subjects. Moreover, in our population the FINDRISC score had an AUROC of 0.7477 in men and 0.7175 in women for identifying individuals at increased risk of undiagnosed DM2, and an AUROC of 0.7199 in men and 0.6774 in women for predicting incident DM2, which is comparable to that obtained in other similar studies^[18,19]. Using an optimal cutoff value of greater or equal to 14 both in men and women, this screening tool had good performance in identifying undiagnosed DM2. Meanwhile, FINDRISC cutoff values of 13 in men and 16 in women at baseline were defined as optimal to predict

DM2 in the subsample of prediabetic individuals.

DM2 is a strong, independent risk factor for cardiovascular disease and death^[3,23], and many epidemiologic analyses have identified a progressive relationship between prediabetes and these outcomes^[24,25]. Therefore, identifying individuals with undiagnosed DM2 and detecting individuals at risk for developing DM2 is essential to lead this target population the preventive actions, minimizing human and economic costs of diabetic complications^[9,11]. However, there are almost no symptoms of prediabetes or DM2 and as a consequence its detection is often delayed and at the time of diagnosis advanced complications are frequently present. It is estimated that approximately one-third of all people

with DM2 may be undiagnosed^[1,2]. Hence, using a simple and valid questionnaire, such as the FINDRISC score, as a preliminary screening method followed with more invasive and accurate diagnosis in primary care constitute a cost-effective and practical method with a potentially high national impact in terms of public health.

The efficiency of risk scores may vary between populations, and therefore, these should be validated in each population before use. The FINDRISC questionnaire was developed in Finland^[15], and has been validated in other populations studied so far^[16-20], demonstrating to be a simple and inexpensive tool that can identify those at high risk of having abnormalities in the glucose metabolism. Certainly, a previous study had already demonstrated the usefulness of FINDRISC to identify cross-sectionally people with glucose metabolism disorders in Colombia^[20], however in that study the cutoff level to identify patients at risk was chosen arbitrary. In that study, people with 13 or more FINDRISC points were screened with an OGTT, and they found this cutoff point to be useful to identify people with glucose metabolism disorders. Furthermore, this previous study did not assess the ability of FINDRISC in predicting incident DM2.

It is worth highlighting that there are several methodological differences between the several studies that have validated the FINDRISC questionnaire; modified or shortened versions have been used, in some studies the plasmatic glycemia tests have been only performed in subjects with a particular score, and in other cases prediabetes or metabolic syndrome has been also considered as an outcome. Therefore, the present study should be compared to these previously conducted studies with caution.

An important aspect of the present study is the assessment of the capacity of FINDRISC in the short-term prediction of incident DM2 among prediabetic individuals. To our knowledge, this is the first longitudinal validation of the FINDRISC questionnaire conducted in Latin America, while just other one study of similar characteristics was conducted in Europe. Soriguer *et al.*^[19] reported that in Spanish subjects with prediabetes the best prediction of risk of incident DM2 was found in those subjects with a FINDRISC score ≥ 9 . It should be noted that gender-specific cutoff values were not estimated in this previous study making thus comparisons difficult with our study.

Strengths and weaknesses of the study

There are some study limitations that warrant consideration. First, the participants were drawn from a unique healthcare center in Floridablanca and, thus, the results may not be applicable to the rest of Colombia. Second, the number of participants was relatively small, although the results were similar to those of other studies in different populations and larger cohorts^[16-20], which supports the validity of our present findings. Third, in most cases the diagnosis of DM2 was based on only one plasmatic value (FPG, OGTT or HbA1c

when performed), not two as recommended, and this may cause a serious bias in respect the definition of the main outcome event underestimating the true prevalence/incidence of DM2. However, diagnosis based on a single test result is accepted in epidemiological studies. In addition, it is noteworthy that this study was conducted in real life conditions, and the Colombian health system restricts the use of OGTT and HbA1c in patients with FPG < 100 mg/dL. Fourth, in our study most respondents were women, and this situation may partly be explained by behavioral habits, women are more likely to participate in completing questionnaires, they visit their physician more often, and usually pay more attention to their health.

This study has also considerable strengths. First, the diagnosis of DM2 was not self-reported. Second, all the analyzed subjects have available at least one plasmatic glycemia value. Third, the age distribution of the participants was wide and included the vast majority of the high-risk population.

In conclusion, the present study has assessed the performance of the FINDRISC questionnaire, demonstrating this as a useful screening tool to identify unknown DM2 in a cross-section of the Colombian population, and to predict incident DM2 among prediabetics. Moreover, we have demonstrated that a cutoff value greater or equal to 14 is the more appropriate detecting any previously undiagnosed DM2 in our population, both in men and women. Meanwhile, the optimal cutoff values for predict incident DM2 in prediabetic individuals were 13 in men and 16 in women.

COMMENTS

Background

The prevalence of type 2 diabetes mellitus (DM2) is increasing worldwide, but unfortunately its diagnosis is being made, in a high percentage, when vascular complications are already present. Therefore, the International Diabetes Federation recommends identification of persons at risk using simple risk-scoring questionnaires. Thus, this study aims to assess the performance of the Finnish Diabetes Risk Score (FINDRISC) questionnaire for detecting and predicting DM2 in a Colombian population.

Research frontiers

The FINDRISC questionnaire has been assessed and validated in several countries, including Colombia. However, in the authors' country the ability of FINDRISC to predict the development of incident DM2 has not been evaluated.

Innovations and breakthroughs

In the present study, the FINDRISC score demonstrated to be a useful screening tool to identify unknown DM2 in a cross-section of the Colombian population, and to predict incident DM2 among prediabetics.

Applications

Using a simple and valid questionnaire, such as the FINDRISC score, as a preliminary screening method for DM2 followed by more invasive and accurate diagnostic exams in primary care, constitute a cost-effective and practical method with a potentially high national impact in terms of public health.

Terminology

The FINDRISC is a simple, fast, inexpensive, noninvasive, and reliable tool to identify individuals at high risk for DM2.

Peer-review

This is a good observational study. Authors assessed the performance of the FINDRISC questionnaire in a Colombian population to detect undiagnosed DM2 in the general population and to predict incident DM2 among prediabetics. The study design is clear and the results are well to present. It demonstrated that the modified FINDRISC questionnaire is a good tool to detect undiagnosed DM2 and to predict incident DM2 in Colombian population.

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We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

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Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

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Osteocalcin as a hormone regulating glucose metabolism

Ippei Kanazawa

Ippei Kanazawa, Department of Internal Medicine 1, Shimane University Faculty of Medicine, Shimane 693-8501, Japan

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Correspondence to: Ippei Kanazawa, MD, PhD, Department of Internal Medicine 1, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan. ippeik@med.shimane-u.ac.jp
Telephone: +81-853-202183
Fax: +81-853-238650

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Abstract

The number of patients with osteoporosis and diabetes is rapidly increasing all over the world. Bone is recently recognized as an endocrine organ. Accumulating evidence has shown that osteocalcin, which is specifically expressed in osteoblasts and secreted into the circulation, regulates glucose homeostasis by stimulating insulin expression in pancreas and adiponectin expression in adipocytes, resulting in improving glucose intolerance. On the other hand, insulin and adiponectin stimulate osteocalcin expression in osteoblasts, suggesting that

positive feedforward loops exist among bone, pancreas, and adipose tissue. In addition, recent studies have shown that osteocalcin enhances insulin sensitivity and the differentiation in muscle, while secreted factors from muscle, myokines, regulate bone metabolism. These findings suggest that bone metabolism and glucose metabolism are associated with each other through the action of osteocalcin. In this review, I describe the role of osteocalcin in the interaction among bone, pancreas, brain, adipose tissue, and muscle.

Key words: Osteocalcin; Undercarboxylated osteocalcin; Glucose; Insulin; Adiponectin; Glucagon-like peptide-1; Diabetes mellitus

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Core tip: Osteocalcin, especially undercarboxylated form of osteocalcin, has an endocrine function to regulate glucose metabolism. Osteocalcin directly stimulates insulin secretion in pancreas and indirectly *via* increasing glucagon-like peptide-1 secretion in small intestine as well as adiponectin secretion in adipose tissue, and enhances insulin sensitivity in muscle. Therefore, osteocalcin may be an important factor linking between bone and glucose homeostasis.

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INTRODUCTION

The incidence of osteoporosis and type 2 diabetes mellitus is known to increase in prevalence with aging. However, both diseases were traditionally viewed as separate entities. Previous studies have shown that patients with type 1 and type 2 diabetes have an increased risk of fractures, and that hyperglycemia and

insulin affects bone metabolism. In addition, there is a positive association between bone mineral density (BMD) and fat mass^[1,2], suggesting that accumulation of fat mass influences bone metabolism. Adipose tissue secretes a variety of biological active molecules such as leptin, adiponectin, and resistin, all of which play important roles in glucose metabolism. Previously, these adipokines are reported to regulate bone metabolism^[3-5].

On the other hand, a previous excellent study using gene mutant mice has shown that osteocalcin, which is one of the osteoblast-specific proteins and has several hormonal features, regulates glucose metabolism^[6]. *Osteocalcin* knockout (*Ocn*^{-/-}) mice were previously generated to examine the role of osteocalcin in bone tissue^[7]. Although it was not reported at that time, the authors found that *Ocn*^{-/-} mice were obese and had an abnormal accumulation of visceral fat. In 2007, it was reported that *Ocn*^{-/-} mice displayed hyperglycemia and impaired glucose tolerance due to insulin insufficiency and resistance^[6]. In the mice, pancreatic β -cell proliferation and insulin secretion were significantly decreased, and insulin resistance by reduced adiponectin expression in adipocytes was observed. Osteocalcin has 46-50 amino acids and undergoes γ -carboxylation of glutamyl residues at three positions 17, 21, and 24, which facilitates binding of osteocalcin to hydroxyapatite in bone matrix. Further examinations have shown that undercarboxylated form of osteocalcin (ucOC) is an active form in glucose metabolism^[6,8]. In this review, I describe the role of osteocalcin in glucose metabolism and the association between serum osteocalcin level and parameters of glucose homeostasis in humans.

OSTEOCALCIN REGULATES GLUCOSE METABOLISM

The Karsenty group reported for the first time an interesting and excellent animal study using gene mutant mice models showing that osteocalcin secreted from bone might be involved in whole body glucose homeostasis (Figure 1)^[6]. In the study, *Ocn*^{-/-} mice and *Esp* knockout (*Esp*^{-/-}) mice were used to examine the function of osteocalcin in β -cell and adipocytes. *Esp* encodes osteotesticular protein tyrosine phosphatase (OST-PTP), which is restricted to osteoblasts, sertoli cells and embryonic stem cells^[9]. OST-PTP is a transmembrane tyrosine phosphatase and can not directly affect distant tissues. Since it stimulates carboxylation of osteocalcin and decreases osteocalcin bioactivity, *Esp*^{-/-} mice was examined as a model of gain of osteocalcin bioactivity^[6]. *Ocn*^{-/-} mice showed hyperglycemia and glucose intolerance, decreased β -cell and insulin secretion, decreased insulin sensitivity and adiponectin expression, and increased fat mass and serum triglyceride level. Moreover, when *Ocn* expression vector-transfected COS cells were cocultured with islets or adipocytes, the expression of insulin and adiponectin was significantly increased. In addition, recombinant osteocalcin injection improved

the glucose intolerance and increased insulin expression in β -cells. In contrast to *Ocn*^{-/-} mice, *Esp*^{-/-} mice showed hypoglycemia and low blood glucose level after glucose injection, increased insulin expression and secretion, as well as increased insulin sensitivity and adiponectin expression in adipose tissue. Furthermore, *Esp*^{-/-} mice displayed decreased fat mass and serum triglyceride level, a resistance of high fat diet-induced obesity and diabetes, as well as resistance to streptozotocin-induced diabetes. Namely, the metabolic phenotype of *Ocn*^{-/-} mice is the mirror image of the one seen in *Esp*^{-/-} mice. To examine whether metabolic abnormalities of *Esp*^{-/-} mice could be corrected by inhibition of osteocalcin expression, *Esp*^{-/-} mice were crossed with *Ocn*^{+/-} mice. In *Esp*^{-/-};*Ocn*^{+/-} mice, the metabolic abnormalities such as hypoglycemia, hyperinsulinemia and increased serum adiponectin level were completely reversed. Several *in vitro* experiments were performed by using carboxylated osteocalcin (cOC) and ucOC. UcOC significantly stimulated the expression of cyclin D1 and insulin in islets as well as of adiponectin in adipocytes, whereas cOC showed no effects on them. However, studies by other groups have suggested that both cOC and ucOC can stimulate the response to insulin in adipocytes and myoblasts^[10].

In addition to the direct effect of osteocalcin on insulin secretion, it has been shown that osteocalcin indirectly stimulates it through increasing the secretion of glucagon-like peptide-1 (GLP-1), an incretin released by intestinal endocrine cells^[11,12]. Mizokami *et al*^[11,12] demonstrated that treatment with ucOC significantly increased GLP-1 expression in STC-1 enteroendocrine cells *in vitro*, and that administration of ucOC increased serum GLP-1 and insulin levels in mice. These effects were potentiated by an inhibitor of dipeptidyl peptidase-4 and blocked by a GLP-1 receptor antagonist, suggesting that ucOC increases insulin secretion through GLP-1 secretion from intestinal endocrine cells. In contrast, cOC did not affect GLP-1 or insulin secretion.

From the Karsenty group, the effects of recombinant osteocalcin injection on glucose metabolism in wild-type (WT) mice were reported^[6]. Continuous intraperitoneal injection of low dose recombinant osteocalcin increased insulin secretion, β -cell proliferation, insulin sensitivity and adiponectin expression as well as decreased fat mass in WT mice. Moreover, recombinant osteocalcin injection prevented high fat diet-induced obesity and diabetes. Further, therapeutic potential of intermittent administration of recombinant osteocalcin was also tested^[13]. Daily injection of osteocalcin significantly improved glucose intolerance and insulin resistance in mice fed not only normal diet but also high fat diet. In addition, hepatic steatosis induced by high fat diet was completely recovered in mice treated with osteocalcin daily injection. Of interest, it is reported that oral administration of osteocalcin also improved impaired glucose tolerance *in vivo*^[11,12,14]. Oral administration of ucOC reached small intestine and remained there for at least 24 h as well as entered the general circulation. Daily

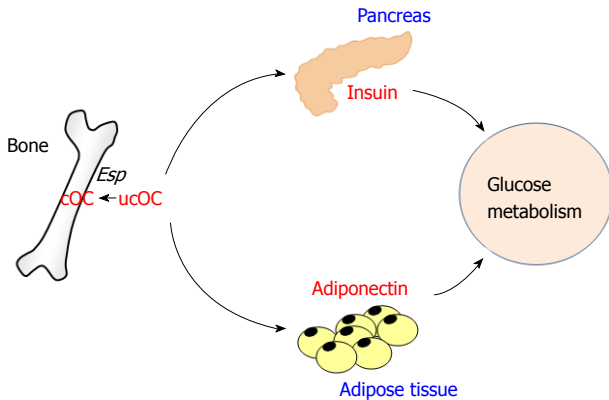


Figure 1 The effects of osteocalcin on pancreas and adipose tissue. Undercarboxylated osteocalcin (ucOC) is an active form regulating glucose metabolism. Esp inactivate osteocalcin by carboxylating ucOC. ucOC increases insulin expression in pancreas and adiponectin expression in adipose tissue.

and long-term (13 wk) intermittent oral administration of ucOC significantly reduced fasting blood glucose level and improved glucose tolerance in mice without affecting insulin sensitivity. Oral administration also increased fasting serum insulin level and β -cell area in pancreas. The serum GLP-1 level was increased in accordance with the presence of ucOC in the intestine and systemic circulation. Taken together, these findings suggest that the intermittent injection and oral administration of recombinant osteocalcin may be useful for treatment of type 2 diabetes and obesity. However, it is reported that oral administration of osteocalcin did not affect insulin sensitivity^[11,12]; thus further studies are necessary to define the difference between oral administration and intraperitoneal injection of osteocalcin.

AN ENDOCRINE FEEDFORWARD LOOP BETWEEN BONE AND PANCREAS

Previous studies have shown that patients with type 1 diabetes mellitus, which is caused by autoimmune destruction of insulin-producing β -cells, have a significant reduction in BMD with decreased bone formation and an increased risk of fragility fractures^[15-17]. These clinical features suggest that insulin signal in osteoblasts has pivotal roles in bone formation and bone development. Osteoblasts have a functional insulin receptor and that treatment with insulin stimulates the proliferation and differentiation of osteoblasts^[18,19]. In addition, osteoblast-specific insulin receptor knockout (*Ob-IR*^{-/-}) mice displayed reduced bone accumulation due to decreased bone formation and deficient number of osteoblasts, and deletion of insulin receptor in osteoblasts induced decreases in alkaline phosphatase (ALP) activity and osteocalcin expression by inhibiting a Runx2 inhibitor, Twist2^[20]. These findings indicate that insulin signaling may be an anabolic factor for bone formation; however, the detail mechanisms are still unclear. FoxO1, which belongs to the Forkhead family of transcription factors, is

a major transcriptional mediator of insulin signaling and insulin transmits its signal by inhibiting FoxO1 activity in various cells^[21]. Previous *in vitro* studies showed that FoxO1 physically interacts with Runx2 *via* its C-terminal region and inhibits Runx2-dependent transcriptional activity as well as osteocalcin expression in osteoblasts, and that insulin and insulin-like growth factor-I signals prevent FoxO1 from inhibiting Runx2 activity by promoting FoxO1 phosphorylation and nuclear exclusion^[22]. In contrast, osteoblast-specific FoxO1 knockout (*Ob-FoxO1*^{-/-}) mice showed marked reduction of bone mass^[23]. Moreover, FoxO1 is known to protect osteoblast function against oxidative stress. Therefore, further studies are necessary to understand the underlying mechanism of insulin in osteoblasts.

Based on the action of insulin in bone, with regard to the hormonal loop networks, it is rational to suggest that signals derived from osteoblasts might affect insulin expression and secretion in β -cells. Previously, Fulzele *et al*^[20] and Ferron *et al*^[24] reported that insulin signaling in osteoblasts contributes to whole-body glucose homeostasis by increasing β -cell proliferation and insulin secretion by using *Ob-IR*^{-/-} mice, indicating a feedforward loop between bone and pancreas. *Ob-IR*^{-/-} mice developed marked peripheral adiposity and hyperglycemia accompanied by severe glucose intolerance and insulin resistance. Fat mass was significantly greater in *Ob-IR*^{-/-} mice than that in control mice, and examination of body composition revealed a 40% increase in fat mass and an 8% decrease in lean mass in *Ob-IR*^{-/-} mice. Moreover, glucose tolerance tests showed that plasma glucose levels after glucose injection was significantly higher in *Ob-IR*^{-/-} mice than controls, whereas serum insulin level was significantly decreased in *Ob-IR*^{-/-} mice. Pancreatic β -cell mass and insulin expression were significantly decreased in *Ob-IR*^{-/-} mice, and insulin tolerance tests and gene expression analysis showed that *Ob-IR*^{-/-} mice had a severe insulin resistance. Furthermore, *Ob-IR*^{-/-} mice had decreased rates of oxygen consumption and energy expenditure compared with controls. Serum ucOC level was decreased in *Ob-IR*^{-/-} mice, and its infusion reversed the glucose intolerance seen in *Ob-IR*^{-/-} mice, suggesting that an endocrine loop through insulin and ucOC exists between bone and pancreas. On the other hand, *Ob-FoxO1*^{-/-} mice showed hypoglycemia and hyperinsulinemia with an increase in β -cell mass^[25]. *Ob-FoxO1*^{-/-} mice have a phenotype that mirrors the metabolic phenotype of *Ocn*^{-/-} mice, thus suggesting a gain of osteocalcin activity in *Ob-FoxO1*^{-/-} mice. Ferron *et al*^[24] showed that FoxO1 haploinsufficiency rescued the phenotype of *Ob-IR*^{-/-} mice. These findings suggest that FoxO1 inactivation may be involved in the effects of insulin signal in osteoblasts on systemic glucose homeostasis. Previous studies showed that an interaction of FoxO1 and ATF4 stimulated osteocalcin expression by luciferase assay using osteocalcin promoter gene^[23], whereas their interaction inactivated osteocalcin by reducing undercarboxylated form, resulting in glucose intolerance^[26].

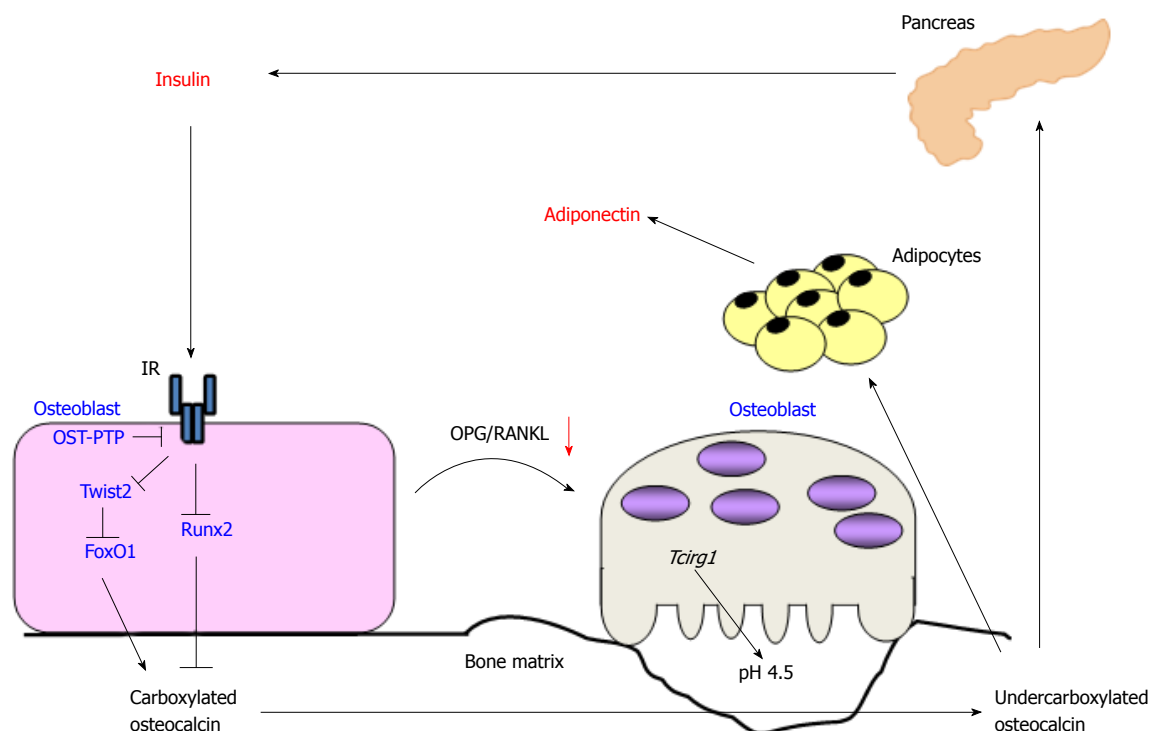


Figure 2 Schematic representation of the endocrine loops between bone and pancreas. Insulin signaling stimulates the expression of osteocalcin and osteoblastic differentiation via inhibiting Twist2, an inhibitor of Runx2, as well as FoxO1, resulting in accumulation of carboxylated osteocalcin in bone matrix. Conversely, insulin activates osteoclasts and accelerate bone turnover via increasing the ratio of osteoprotegerin/receptor activator of nuclear factor kappa-B ligand. Activated osteoclasts decarboxylate bone matrix-embedded osteocalcin, and then undercarboxylated osteocalcin (ucOC) is released into the circulation. ucOC stimulates the expression of insulin in pancreas and of adiponectin in adipose tissue. OST-PTP: Osteotesticular protein tyrosine phosphatase; AMPK: AMP-activated protein kinase; OPG/RANKL: Osteoprotegerin/receptor activator of nuclear factor kappa-B ligand; IR: Insulin receptor.

OSTEOCLASTS REGULATE GLUCOSE METABOLISM BY DECARBOXYLATION OF OSTEOCALCIN

Osteoblasts produce osteocalcin and carboxylate it dependently on vitamin K, resulting in accumulation of bone matrix-embedded cOC. For the function of osteocalcin in glucose metabolism, osteoclasts are reported to be necessary. Ferron *et al*^[24] noticed that serum level of CTx, a marker of bone resorption, was markedly decreased in *Ob-IR*^{-/-} mice. Co-culture with osteoclast precursor cells and *IR* null osteoblasts showed that the area covered by resorption pits was significantly decreased. The expression of osteoprotegerin (OPG), a decoy receptor for receptor activator of nuclear factor kappa-B ligand (RANKL), was significantly increased in *IR* null osteoblasts, and insulin treatment decreased OPG expression and secretion in WT osteoblasts, but not *IR* null osteoblasts. In addition, the expression of CathepsinK (CtsK) and *Tcirg1*, both of which play pivotal roles in bone resorption, was decreased in *Ob-IR*^{-/-} bone. The expression of CtsK and *Tcirg1* was also decreased in osteoclast precursor cells cocultured with *IR* null osteoblasts. These findings suggest that insulin signaling in osteoblasts favors not only osteoblastic differentiation and osteocalcin expression but also the activation of osteoclasts and bone resorption by inhibiting OPG

expression (Figure 2).

The gene *Tcirg1* encodes a vacuolar proton pump subunit essential for acidification of bone matrix, which is essential for bone resorption^[27]. Because acidification decarboxylates proteins^[28], it was assumed that insulin signaling in osteoblasts may regulate glucose metabolism by decarboxylation of osteocalcin in bone matrix following activation of osteoclasts. To examine whether or not the regulation of glucose metabolism by insulin signaling in osteoblasts depends on the activation of osteoclasts, *Ob-IR*^{-/-} mice were crossed with *oc/oc* mice, a model of loss-of-function in *Tcirg1*^[29]. *Ob-IR*^{-/-}; *oc/+* mice showed a significant reduction in insulin secretion as well as impaired glucose tolerance although *Ob-IR*^{+/-} or *oc/+* mice had no phenotype of glucose intolerance. Moreover, *Esp*^{-/-}; *oc/+* mice showed normal bone resorption, normal osteocalcin carboxylation, blood glucose, insulin secretion, and insulin sensitivity, while *Esp*^{-/-} mice showed hypoglycemia and low blood glucose level after glucose injection, increased insulin expression and secretion, as well as increased insulin sensitivity and adiponectin expression in adipose tissue. Furthermore, treatment with alendronate, a bisphosphonate, in *Esp*^{-/-} mice showed that the phenotype of glucose abnormality was completely normalized. On the contrary, RANKL treatment induced bone resorption and increased serum level of ucOC, resulting in less glucose intolerance and less fat mass in WT mice fed a high-fat diet than controls.

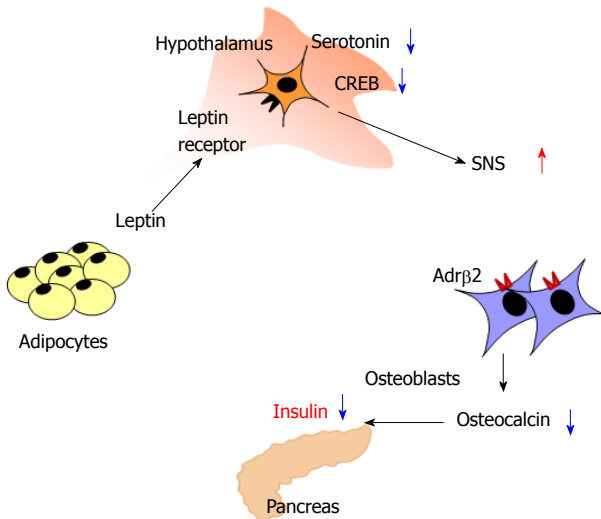


Figure 3 Schematic representation of the regulation of insulin secretion by leptin. Leptin directly inhibits insulin expression in pancreas. Leptin activates SNS by decreasing serotonin and cAMP response element binding protein in hypothalamus, and then enhanced SNS suppresses osteoblast differentiation and osteocalcin expression through the $\text{Adr}\beta 2$ in osteoblasts. Thus, leptin indirectly inhibits insulin expression via central nervous system and bone. SNS: Sympathetic nervous system.

Taken altogether, these findings indicate that bone resorption is essential to activate osteocalcin and regulate glucose homeostasis by bone.

THE CROSS RELATIONSHIPS BETWEEN BONE AND ADIPOSE TISSUE THROUGH OSTEOCALCIN AND ADIPOKINES

Previous studies have shown that adipose tissue is involved in bone metabolism. Adipocyte is recently known to not only be an energy-storing organ but also secrete a variety of biological active molecules, which are named adipokines^[30]. Leptin is known to suppress appetite and regulate energy expenditure through its receptor presented in hypothalamus. In leptin-deficient (*ob/ob*) and leptin receptor-deficient (*db/db*) mice, high bone mass with a massive increase in bone formation was observed^[31]. Although *ob/ob* mice had no leptin signal in osteoblasts, intracerebroventricular infusion of leptin decreased bone mass, suggesting that leptin is a potent inhibitor of bone formation acting through the central nervous system. Ultimately, leptin inhibits the production and release of serotonin in brainstem neurons^[32], and this decrease in serotonin leads to reduced CREB signaling in the hypothalamus, resulting in an increased activation of the sympathetic nervous system (SNS)^[33]. Neurons from the SNS are present in bones and activation of SNS inhibits the proliferation and differentiation of osteoblasts through the $\beta 2$ -adrenergic receptor ($\text{Adr}\beta 2$)^[34]. Of interest, it has been shown that leptin regulates insulin secretion through direct and indirect mechanisms. Morioka *et al*^[35] reported that pancreas-specific leptin receptor knockout mice showed

improved glucose tolerance due to enhanced early-phase insulin secretion. Upregulation of SNS by leptin has been shown to indirectly suppress insulin secretion by inhibiting the production of uOC. Hinoi *et al*^[36] reported that *ob/ob* and osteoblast-specific deletion of $\text{Adr}\beta 2$ (*Ob-Adr\beta 2*^{-/-}) mice showed increases in serum insulin level and decreases in blood glucose level compared to WT mice. *Ob/+; Ob-Adr\beta 2*^{+/-} mice showed an increase in serum insulin level and a decrease in blood glucose level although serum insulin and blood glucose levels were not changed in *ob/+* or *Ob-Adr\beta 2*^{+/-} mice. Moreover, when *ob/ob* mice were crossed with *Ocn*^{-/-} mice, *ob/ob; Ocn*^{-/-} mice reversed the glucose abnormalities of *ob/ob* mice. Because insulin is adipogenic, increases body fat mass, and stimulates the production and secretion of leptin^[37], it is suggested that negative feedback loops may exist between pancreas, adipose tissue, brain, and bone (Figure 3).

On the other hand, resistin acts in insulin target organs such as skeletal muscle, liver, and adipose tissue and reduces insulin sensitivity there^[38]; thus, it is suggested to be a molecule linking obesity to type 2 diabetes^[39]. Resistin is previously reported to be expressed in osteoblasts and osteoclasts and to increase the number of differentiated osteoclasts as well as the proliferation of osteoblastic cells^[40], suggesting that resistin may be involved in bone metabolism. However, previous studies showed that osteocalcin did not affect the expression of leptin or resistin in adipose tissue^[6,25]. Therefore, these two adipokines may affect the function of osteocalcin in glucose metabolism but may not have direct cross relationships with osteocalcin.

It has been shown that osteoblast has an adiponectin receptor and adiponectin signaling stimulates the differentiation and osteocalcin expression in osteoblasts. Previously, Luo *et al*^[41] showed that recombinant adiponectin increased ALP activity and osteocalcin expression in human osteoblasts. Furthermore, we demonstrated that adiponectin activated AMP-activated protein kinase (AMPK), and that a knockdown of adiponectin receptor by using siRNA induced an inhibition of ALP activity as well as of osteocalcin expression in mouse osteoblastic MC3T3-E1 cells^[42]. These findings suggest that adiponectin directly stimulates osteoblastic differentiation and osteocalcin expression *in vitro*. Moreover, it is also reported that adiponectin stimulated osteoclast activity by increasing RANKL expression and decreasing OPG expression in osteoblasts although adiponectin had no direct effects on osteoclasts^[43]. Because adiponectin stimulates osteocalcin expression in osteoblasts and the differentiation of osteoclasts as well as osteocalcin alternatively stimulates the expression of adiponectin in adipocytes, it is reasonable to assume that there is an endocrine loop between bone and adipose tissue through adiponectin and osteocalcin (Figure 4). However, there is no direct evidence showing the endocrine loop so far.

Taken together, leptin negatively regulates insulin secretion from pancreas directly and indirectly through hypothalamus-SNS and bone, while adiponectin posi-

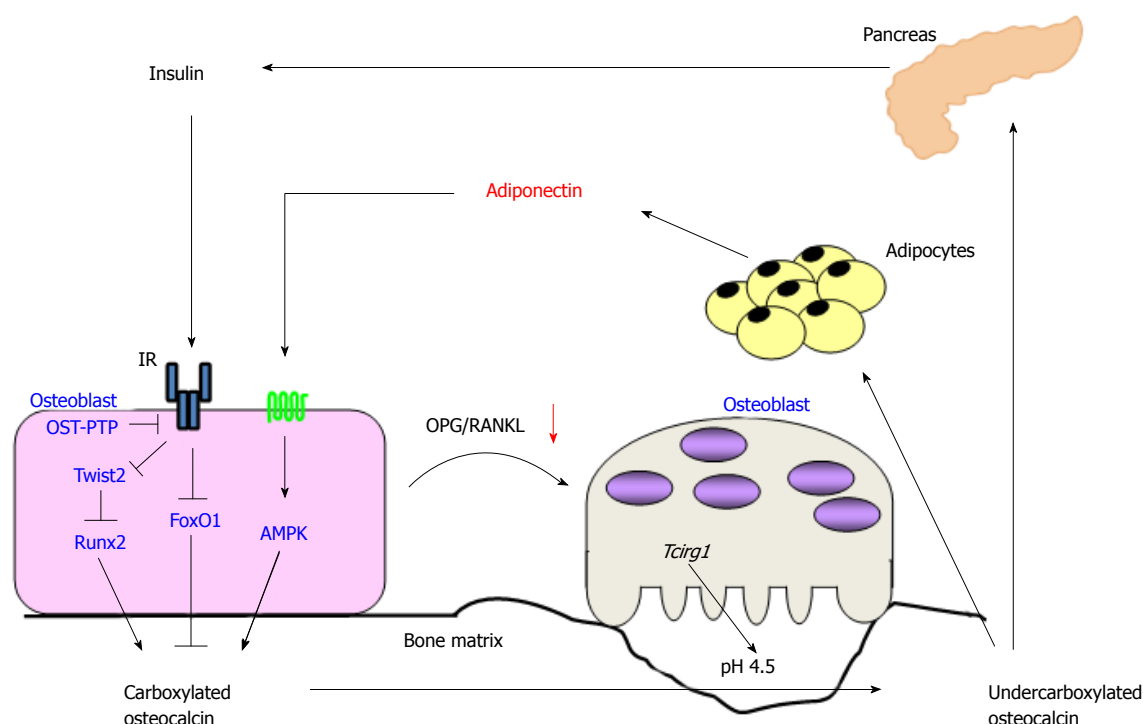


Figure 4 Schematic representation of the endocrine loops between bone and pancreas, and adipose tissue. Insulin signaling stimulates the expression of osteocalcin and osteoblastic differentiation via inhibiting Twist2, an inhibitor of Runx2, as well as FoxO1, resulting in accumulation of carboxylated osteocalcin in bone matrix. Adiponectin also stimulates osteocalcin expression and the differentiation of osteoblasts via AMP-activated protein kinase (AMPK) signaling pathway. Both insulin and adiponectin activate osteoclasts and accelerate bone turnover via increasing the ratio of RANKL/OPG. Activated osteoclasts decarboxylate bone matrix-embedded osteocalcin, and then undercarboxylated osteocalcin (ucOC) is released into the circulation. UcOC stimulates the expression of insulin in pancreas and of adiponectin in adipose tissue. OST-PTP: Osteotesticular protein tyrosine phosphatase; IR: Insulin resistance.

tively regulates insulin secretion through bone. In patients with obesity and metabolic syndrome, increased serum leptin and decreased serum adiponectin levels may lead to a reduction in residual insulin secretion from pancreas and impaired glucose tolerance in part through bone.

THE EFFECTS OF OSTEOCALCIN ON MUSCLE

Previous studies have shown that osteocalcin affects muscle function and myoblastic differentiation. Tsuka *et al*^[44] reported that ucOC treatment activated ERK signaling in a dose-dependent manner in C2C12 myotubes, and that ucOC significantly increased insulin-induced glucose uptake by activating ERK signaling in the cells. Shen *et al*^[45] showed that osteocalcin expression was significantly decreased in osteoblast/osteocyte-specific Connexin43 knockout mice (*Ob/Oc-Cx43*^{-/-}). Of note, muscle volume and muscle power were significantly reduced in *Ob/Oc-Cx43*^{-/-} mice compared to WT mice. Treatment with ucOC significantly increased fusion rate of C2C12 cells, and injection of ucOC to *Ob/Oc-Cx43*^{-/-} mice significantly increased muscle volume and grip strength. On the other hand, recent several studies have shown that muscle-derived factors named myokines affect bone metabolism. For example, Tanaka *et al*^[46] reported that osteoglycin secreted from myoblasts regulated osteoblastic differentiation and osteocalcin expression.

Stable overexpression of osteoglycin significantly enhanced the expression of osteocalcin in osteoblastic MC3T3-E1 cells, while a reduction in endogenous osteoglycin decreased it. Treatments with the conditioned medium from osteoglycin-overexpressed or -suppressed myoblastic cells showed the same results mentioned above. Family with sequence similarity 5, member C (FAM5C) is also secreted from muscle, and FAM5C is reported to stimulate the differentiation and osteocalcin expression in osteoblastic cells^[47]. Therefore, there may be an endocrine cross relationship between bone and muscle through osteocalcin.

A RECEPTOR FOR OSTEOCALCIN AND ITS SIGNAL TRANSDUCTION

Previous studies suggest that G-protein coupled receptor family C group 6 member A (GPRC6A) is a candidate or mediating the response to osteocalcin in β -cells^[48]. GPRC6A is orphan receptor belonging to the G protein-coupled receptors, which is known as seven-transmembrane domain receptors, and is ubiquitously expressed and sense amino acids and extracellular calcium^[49,50]. It is reported that GPRC6A knockout mice showed osteopenia, hyperglycemia, impaired glucose tolerance, insulin resistance, and hepatic steatosis^[51], suggesting that GPRC6A may participate in the anabolic response of multiple tissues. Based on the metabolic abnormalities

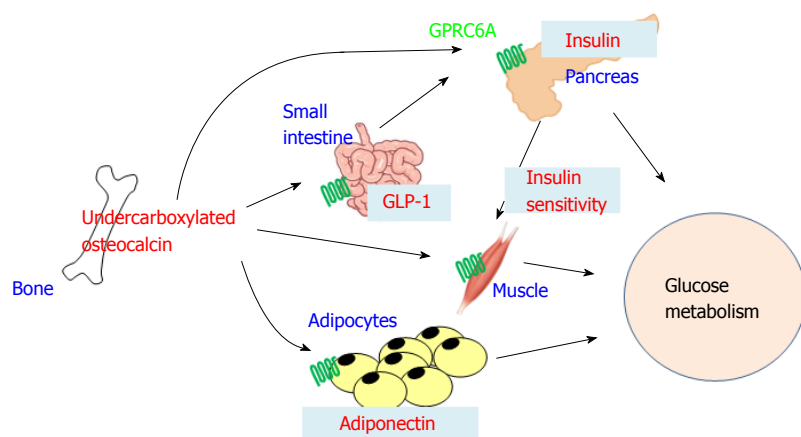


Figure 5 Schematic representation of the mechanisms regulating glucose metabolism by bone. Undercarboxylated osteocalcin (ucOC) secreted from bone directly stimulates insulin secretion from pancreas and indirectly through increasing the secretion of glucagon-like peptide-1 (GLP-1) from small intestine. UcOC stimulates the expression of adiponectin in adipose tissue, resulting in increasing insulin sensitivity. UcOC also enhances insulin signaling in muscle. G-protein coupled receptor family C group 6 member A (GPRC6A) is a receptor for ucOC and expressed in pancreas β -cells, epithelial cells of small intestine, adipocytes, and myotubes.

of the GPRC6A knockout mice, Pi *et al*^[51] hypothesized that GPRC6A might be involved in the function of ucOC in glucose homeostasis. To examine the role of GPRC6A in osteocalcin function, the effects of osteocalcin on GPRC6A-expressed cells were investigated. Recombinant osteocalcin stimulated ERK activity in HEK-293 cells overexpressing GPRC6A in a dose-dependent manner, while it did not affect untransfected control cells. It was confirmed that mouse pancreatic β -cell TC-6 cell line and pancreas isolated from WT mice expressed GPRC6A, and that recombinant osteocalcin treatment stimulated ERK activity *in vitro* and *in vivo*. Moreover, administration of recombinant osteocalcin induced significant increases in insulin expression in pancreas as well as in serum insulin level in WT mice, but not GPRC6A knockout mice. In 3T3 adipocytes, ucOC activated adenylate cyclase to produce cAMP and ERK signaling through GPRC6A, resulting in the expression of adiponectin^[14]. On the other hand, it is reported that ucOC did not increase cytosolic cAMP in C2C2 myotubes, and that the increased glucose uptake by ucOC was not blocked by an inhibitor of PKA although GPRC6A was expressed in C2C12^[44]. Further, knockdown of GPRC6A using RNA interference did not affect the action of ucOC in the cells. GPRC6A is also expressed in epithelial cells of the small intestine, and colocalized with GLP-1 in the cells^[12], suggesting that ucOC may stimulate GLP-1 expression *via* GPRC6A although there is no direct evidence thus far.

However, there are still several issues on the signaling pathway of osteocalcin to be clarified. No studies on whether ucOC, not cOC, directly binds to GPRC6A are reported thus far. It is also still unclear whether the effects of ucOC are solely mediated by GPRC6A. It is reported that the response of GPRC6A to ucOC was similar to that of calcium and arginine which are known as GPRC6A ligands. It is thus speculated that GPRC6A could sense both nutrient derived factors, such as calcium and amino acids, as well as ucOC and may not only participate in the endocrine function of ucOC. In addition, GPRC6A is widely expressed in multiple tissues and GPRC6A knockout results in multiple metabolic abnormalities. Additional examination using conditional deletion of GPRC6A in specific tissue will be necessary to

establish the tissue-specific functions of GPRC6A.

ASSOCIATION BETWEEN OSTEOCALCIN AND GLUCOSE METABOLISM IN HUMANS

Since *in vitro* and *in vivo* studies described above have shown that osteocalcin plays crucial roles in glucose metabolism, of particular interest is whether osteocalcin level in the circulation is associated with glucose metabolism in humans. Indeed, the size and some amino acids of osteocalcin are different between mice and humans, and osteocalcin is encoded by a single gene in humans that is highly conserved across species, while mice contain a cluster of three osteocalcin genes^[51,52]. Thus, it is quite important to examine the role of osteocalcin in glucose metabolism also in humans. Kindblom *et al*^[53] showed that total osteocalcin level was inversely correlated with plasma glucose level and fat mass in elderly non-diabetic subjects. Fernandez-Real *et al*^[54] also demonstrated that serum total osteocalcin level was associated with insulin sensitivity in non-diabetes subjects. Pittas *et al*^[55] reported cross-sectional and longitudinal studies showing that serum total osteocalcin level was inversely associated with fasting plasma glucose, fasting insulin, a parameter of insulin resistance [homeostasis model assessment for insulin resistance (HOMA-IR)], and fat mass in a cross-sectional analysis, and that total osteocalcin level was associated with changes in fasting plasma glucose in a prospective analysis. We also previously showed that total serum osteocalcin was inversely associated with glucose and visceral fat mass and positively with serum adiponectin level, parameters of insulin secretion and its sensitivity in patients with type 2 diabetes^[56,57]. In addition, we reported a longitudinal study showing that changes in osteocalcin was negatively correlated with changes in HbA1c during treatments of type 2 diabetes^[58].

To examine the association of ucOC with glucose metabolism, we previously measured serum ucOC levels by using electrochemiluminescence immunoassay and analyzed the association between ucOC and parameters

of glucose metabolism in diabetic patients. We firstly reported that serum ucOC level was negatively correlated with %Trunk fat and visceral/subcutaneous fat ratio as well as fasting plasma glucose and HbA1c independent of various confounding factors^[59]. However, the correlations of ucOC with the parameters were almost same as those of total osteocalcin. Hwang *et al*^[60] also reported that elevated levels of both cOC and ucOC were associated with improved glucose tolerance and that ucOC was associated with enhanced β -cell function, and that cOC was associated with improved insulin sensitivity in middle-age male healthy subjects. On the contrary, Iki *et al*^[61] showed that ucOC was significantly and inversely correlated with fasting plasma glucose, HbA1c and HOMA-IR after adjusting for total osteocalcin, while total osteocalcin was not associated with these parameters after adjusting for ucOC. These findings suggest that osteocalcin is involved in glucose metabolism not only in rodents but also in humans. However, because it is still controversial whether ucOC is the active form of endocrine factor in humans, further large-scale studies and meta-analysis were necessary in future.

CONCLUSION

Emerging evidence from epidemiological, clinical, and experimental studies have shown that bone interacts with glucose metabolism by regulated insulin secretion from pancreas as well as adipokines from adipose tissue, and that bone is an active tissue involved in energy homeostasis (Figure 5). Previous *in vitro* and *in vivo* studies have shown that osteocalcin has an endocrine function regulating systemic glucose homeostasis and plays important roles in the interaction among bone, pancreas, and adipose tissues. Although several clinical studies suggested that osteocalcin might be involved in systemic glucose homeostasis, there are no direct evidence that osteocalcin regulate glucose metabolism in human. This relatively new topic should further be explored to understand the pathophysiology of glucose tolerance and diabetes-related bone disease and develop a new therapy of metabolic syndrome and diabetes mellitus.

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Randomized Clinical Trial

Buddy Study: Partners for better health in adolescents with type 2 diabetes

Allison C Sylvetsky, Radha Nandagopal, Tammy T Nguyen, Marisa R Abegg, Mahathi Nagarur, Paul Kaplowitz, Kristina I Rother

Allison C Sylvetsky, Radha Nandagopal, Tammy T Nguyen, Marisa R Abegg, Mahathi Nagarur, Kristina I Rother, Section on Pediatric Diabetes and Metabolism, NIDDK, National Institutes of Health, Bethesda, MD 20892, United States

Allison C Sylvetsky, Department of Exercise and Nutrition Sciences, the George Washington University, Washington, DC 20037, United States

Radha Nandagopal, Paul Kaplowitz, Division of Endocrinology, Children's National Medical Center, Washington, DC 20310, United States

Author contributions: Nandagopal R, Kaplowitz P and Rother KI designed this research; Nandagopal R, Nguyen TT and Abegg MR collected the data; Sylvetsky AC, Nagarur M and Rother KI analyzed and interpreted the data; Sylvetsky AC wrote the first draft of the article and all of the authors intellectually contributed to drafting, revising, and writing of the final manuscript; all authors approve of the submission of this manuscript.

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Clinical trial registration statement: This study is registered at ClinicalTrials.gov. The registration identification number is NCT01007266.

Informed consent statement: Informed written consent and assent (in individuals < 18 years of age) were obtained prior to enrollment.

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Correspondence to: Kristina I Rother, MD, MHSc, Section on Pediatric Diabetes and Metabolism, NIDDK, National Institutes of Health, 9000 Rockville Pike, Building 10, Room 8C432A, Bethesda, MD 20892, United States. kr58q@nih.gov
Telephone: +1-301-4354639
Fax: +1-301-4808277

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Abstract

AIM: To investigate whether assigning young, healthy and motivated lay volunteer partners ("buddies") to adolescents with type 2 diabetes improves hemoglobin A1c (HbA1c).

METHODS: Adolescents with type 2 diabetes were

randomized to partnering with a “buddy” or to conventional treatment. During the initial screening visit, which coincided with a routine outpatient diabetes clinic visit, patients with type 2 diabetes underwent a physical examination, detailed medical history, laboratory measurement of HbA1c, and completed two questionnaires (Pediatric Quality of Life Inventory and Children’s Depression Inventory) to assess their overall quality of life and the presence of depressive symptoms. Patients were then randomized to the intervention (the buddy system) or conventional treatment (standard care). All patients were scheduled to return for follow-up at 3- and 6-mo after their initial visit. HbA1c was determined at all visits (*i.e.*, at screening and at the 3- and 6-mo follow-up visits) and quality of life and depressive symptoms were evaluated at the screening visit and were reassessed at the 6-mo visit.

RESULTS: Ten adolescents, recruited from a pool of approximately 200 adolescents, enrolled over a two-year time period, leading to premature termination of the study. In contrast, we easily recruited motivated lay volunteers. We found no change in HbA1c from the initial to the 6-mo visit in either group, yet our small sample size limited systematic assessment of this outcome. Participants repeatedly missed clinic appointments, failed to conduct self-glucose-monitoring and rarely brought their glucometers to clinic visits. Total quality of life scores (72.6 ± 6.06) at screening were similar to previously reported scores in adolescents with type 2 diabetes (75.7 ± 15.0) and lower than scores reported in normal-weight (81.2 ± 0.9), overweight (83.5 ± 1.8), and obese youths without diabetes (78.5 ± 1.8) or in adolescents with type 1 diabetes (80.5 ± 13.1). Among adolescents who returned for their 6-mo visit, there were no differences in total quality of life scores (70.2 ± 9.18) between screening and follow-up.

CONCLUSION: Our approach, effective in adults with type 2 diabetes, was unsuccessful among adolescents and emphasizes the need for innovative strategies for diabetes treatment in adolescent patients.

Key words: Diabetes mellitus type 2; Quality of life; Adolescent; Hemoglobin A1c; Social support

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Core tip: Our manuscript details results and challenges during a simple psychosocial intervention trial where young, healthy and motivated lay volunteer partners (“buddies”) were assigned to adolescents with type 2 diabetes. We experienced difficulty in the recruitment and retention of adolescent patients, which ultimately led to premature study termination. Despite our negative findings, our manuscript calls attention to the fact that psychosocial approaches shown to be effective in adults with type 2 diabetes may not translate in adolescent patients and conveys a unique and important message to other investigators who may wish to attempt similar

interventions among adolescents with type 2 diabetes.

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INTRODUCTION

Type 2 diabetes in adolescence is generally associated with obesity, a positive family history of type 2 diabetes, and a low-income minority background^[1,2]. Beta cell failure in adolescents progresses more rapidly than in adults and responds less to medical treatment as was shown in the recently completed TODAY trial (Treatment Options for Type 2 Diabetes in Adolescents and Youth)^[3]. Because the progression of diabetes.... and ending with: Critical in this patient population. This trial is the only existing large-scale intervention study in youth with type 2 diabetes, which is in part due to difficult recruitment of these individuals^[4]. Obesity related co-morbidities together with potentially long-lasting diabetes, dramatically increase the risk of macrovascular disease later in life. Microvascular complications including peripheral neuropathy and retinopathy have also been shown to occur, even at such a young age^[5,6].

Guidelines from the American Academy of Pediatrics recommend that clinicians combine weight management counseling focused on improving diet, increasing physical activity, and reducing television and computer screen time along with metformin administration at the time of diabetes diagnosis^[1]. It is well known, however, that adolescent patients^[7,8] and those from low-income minority groups^[8] often have difficulties in adhering to these recommended life-style changes and medical treatments. Even among adults who historically exhibit better compliance compared to adolescents, non-adherence is one of the most important barriers to successful treatment^[9]. Psychosocial interventions in adults with type 2 diabetes have shown promise in increasing adherence to treatment^[10-15] and/or improving hemoglobin A1c (HbA1c)^[16-22]. For example, two interventions^[16,17], in which adults with type 2 diabetes were paired with age- and gender- matched lay peer mentors (who also had diabetes), were effective in improving blood glucose control. Other interventions involving diabetes self-management education conducted in a group setting^[18-21] have also led to better glycemia, while diabetes support delivered *via* online^[23], telephone^[10,14,22], or text messaging^[15] programs has improved treatment adherence. Educational and psychosocial interventions have also been effective in improving both HbA1c and psychological health in adolescents with type 1 diabetes^[24], yet to our knowledge, similar studies have not been conducted in adolescents with type 2 diabetes. The objectives of this study were to test whether a low-cost

intervention in which a young, healthy and motivated lay volunteer partner is assigned to an adolescent with type 2 diabetes, can improve HbA1c, adherence to treatment, and quality of life.

MATERIALS AND METHODS

Participants

Adolescents (aged 12-20 years) with type 2 diabetes received information about the "Buddy Study" from their pediatric endocrinologists during routine outpatient diabetes clinic visits at Children's National Medical Center (CNMC) in Washington, DC and at the National Institutes of Health Clinical Center (NIH CC) in Bethesda, MD. Whenever possible, interested patients and their caregivers also met with a trained research assistant to learn more about the study immediately after their clinic appointment. Recruitment occurred between January 2010 and November 2011. The diagnosis of type 2 diabetes was based on their primary physician's assessment^[25]. For study inclusion, patients had to have a documented HbA1c $\geq 7\%$ (≥ 53 mmol/mol). Individuals were excluded if they had a significant comorbidity or psychological disorder that would interfere with their ability to participate (e.g., a history of violent behavior, which could pose a risk to the lay volunteers), or if they were pregnant or planning to become pregnant within six months of the initial visit. Informed written consent and assent (in individuals < 18 years of age) were obtained prior to enrollment. The study protocol, consents and all study procedures were approved by the Institutional Review Boards at the CNMC and the NIH CC and were in accordance with the Declaration of Helsinki.

Lay volunteers, or "buddies", between 18 and 25 years of age were recruited from a pool of research assistants at the National Institutes of Health (NIH). Volunteers were screened and selected by the study physicians and were matched by gender with an adolescent patient. This was deemed necessary to facilitate the home visits. Further matching was not conducted (e.g., by race, ethnicity, body mass index or education) for practical reasons due to the known demographic characteristics of the NIH research assistants. The lay volunteers did not have type 2 diabetes. All volunteers underwent standardized training and criminal background check in collaboration with the NIH Volunteer Services office and received specific training about the management of home visits from a NIH social worker.

Study design

The "Buddy Study" was a randomized, parallel-group study of six months duration conducted at CNMC in Washington, DC and the NIH CC in Bethesda, MD. The NIH CC depends on physician-referred or self-referred research participants while CNMC is a tertiary medical center in which approximately 120 youths with type 2 diabetes (new and established disease) are seen annually. During the initial screening visit, which coincided with a routine outpatient diabetes clinic visit, patients

with type 2 diabetes underwent a physical examination, detailed medical history, laboratory measurement of HbA1c, and completed two questionnaires (Pediatric Quality of Life Inventory (PedsQL)^[26] and Children's Depression Inventory (CDI)^[27] to assess their overall quality of life and the presence of depressive symptoms. Patients were then randomized to the intervention (the buddy system) or conventional treatment (standard care). All patients were scheduled to return for follow-up at 3- and 6-mo after their initial visit. Participants received modest financial compensation for their time and inconvenience (\$100).

The intervention arm (buddy group) was designed to receive weekly telephone calls from their assigned buddies and one home visit per month (lasting 30-60 min) to encourage "bonding" in a comfortable environment. Meetings between patients and buddies took place at locations of the patient's choice (preferably at their home), and contacts were made *via* phone, cell phone, and e-mail. Alternative buddy-patient meeting places included schools, coffee-shops, or libraries chosen by both parties at a mutually convenient time if home visits were declined by the participant or his/her family. Buddies were encouraged to not only ask the patient about diabetes management and provide telephone reminders for diabetes follow-up appointments, but also to discuss the patient's home and social life in order to promote a nurturing and motivating relationship. Buddies were strictly prohibited from providing medical advice and were told to contact the Principal Investigator should a need for medical advice arise. Details of the study procedures are shown in Figure 1.

Measures

The primary outcome was the effect of the intervention on hemoglobin A1c (HbA1c), which was measured using the Siemens-Bayer DCA 2000+. At all visits, HbA1c, height and weight were measured, and body mass index (BMI) was calculated. Change in HbA1c for the intervention arm (buddy group) vs the conventional treatment group was compared using the Student's *t*-test. Socio-demographic and clinically relevant information including self-reported race/ethnicity, family history of diabetes and patient medication use was also collected. All clinical information and laboratory data were compiled in the *eSphere* Clinical Trials Data Management System (Espirit Health, Chicago, IL).

Adolescents' quality of life and depressive symptoms were evaluated at the screening visit and were reassessed at the 6-mo visit using the PedsQL^[26], a validated 23-item questionnaire to assess physical, emotional, social and school functioning and the CDI^[27], a validated 27-item self-report measure designed to determine the extent and severity of depressive symptoms in children (cut-off for depression score ≥ 13), respectively.

RESULTS

Forty adolescents with type 2 diabetes were screened

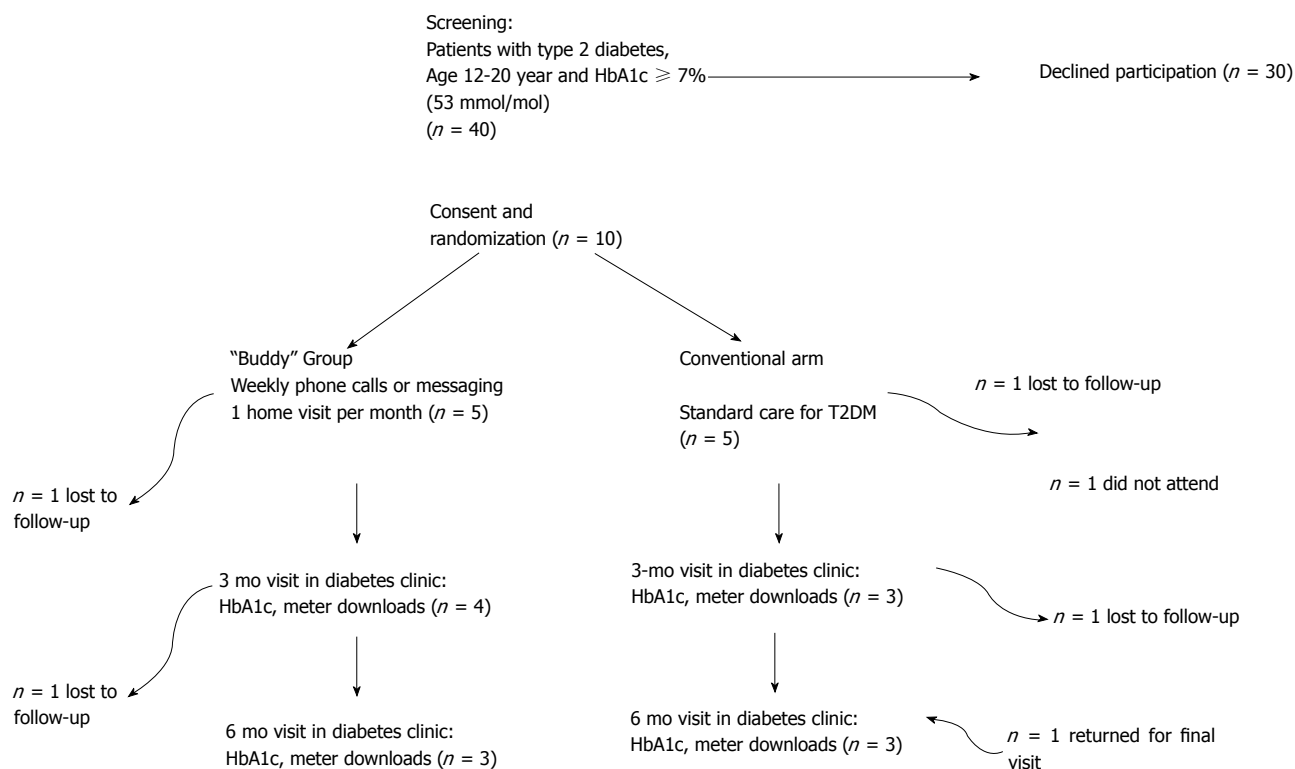


Figure 1 Forty adolescents with type 2 diabetes were screened and eligible for participation in the “Buddy Study”. Ten adolescents were enrolled in the study, of whom five were randomized to the intervention arm and paired with a buddy. The remaining five adolescents were randomized to the standard care group and were not paired with a buddy. Five adolescents (three randomized to the buddy group and two to the conventional arm) returned to the clinic for both 3- and 6-mo follow-up visits and six adolescents completed the six month study. HbA1C: Hemoglobin A1C.

and eligible. As shown in Figure 1, ten adolescents were enrolled in the “Buddy Study”, of whom five were randomized to the intervention arm and paired with a buddy. Five adolescents (three randomized to the buddy group and two to the conventional arm) returned to the clinic for both 3- and 6-mo follow-up visits. Baseline characteristics and a brief case description for each adolescent are shown in Table 1. The majority of our study participants were non-Hispanic Black, obese (mean BMI $37.0 \pm 13.7 \text{ kg/m}^2$) and all but one had a positive family history for type 2 diabetes. The average age was 15.8 ± 2.0 years, diabetes duration 22.1 ± 20.4 mo, and the starting HbA1c was $10.6\% \pm 3.0\%$ (92.4 mmol/mol) with all participants receiving metformin and four of ten receiving insulin. Diabetes and obesity related comorbidities were documented in 50%, but not all patients had undergone screening for retinopathy.

While early study termination prevented us from systematically assessing the primary outcome, HbA1c did not improve at 6 mo compared to screening in either group. Total quality of life scores (72.6 ± 6.06) at screening were similar to previously reported scores in adolescents with type 2 diabetes (75.7 ± 15.0)^[28] and lower than scores reported in normal-weight (81.2 ± 0.9), overweight (83.5 ± 1.8), and obese youths without diabetes (78.5 ± 1.8)^[29] or in adolescents with type 1 diabetes (80.5 ± 13.1)^[28]. Among adolescents who returned for their 6-month visit, there were no

differences in total quality of life scores (70.2 ± 9.18) between screening and follow-up. Using the CDI criteria for depression, three adolescents were depressed but none was suicidal at screening. No participant received treatment with antidepressants.

The average age of our lay volunteers (buddies) was 23.0 ± 0.71 years and four of the five volunteers were female, as adolescent patients and buddies were gender matched. The four female buddies all self-identified as non-Hispanic White, while the one male buddy self-identified as Asian.

DISCUSSION

In this study, we aimed to test whether a “buddy” intervention in adolescent patients with type 2 diabetes was effective in improving HbA1c, adherence to treatment, and quality of life. This particular approach has been shown to be promising in adults with type 2 diabetes and similar educational and psychosocial interventions have been successful in adolescents with type 1 diabetes^[24], but has not been tested in adolescents^[10,11].

Recruitment of adolescents with type 2 diabetes was difficult. Only ten adolescents, recruited from a pool of approximately 200 outpatients at CNMC, enrolled over a two-year time period, which led to premature termination of the study. In contrast, we easily recruited motivated lay volunteers. We found no change in HbA1c

Table 1 Socio-demographic characteristics and case descriptions of adolescents with type 2 diabetes mellitus in the Buddy Study

ID	Age (yr)	Diabetes duration (mo)	Sex	Ethnicity/race	Medications (hypoglycemic agents)	BMI (kg/m ²)	T2DM family history	Complications, comorbidities	Case description
1	14	22	Male	Non-hispanic black	Metformin, insulin	24.1	Yes	None	Control group. Poor medication and dietary compliance. Frequently consumed sugar-sweetened beverages and sneaked food late at night. Mother attributed behavior to depression and stress from a recent custody battle. Significant behavioral issues in school
2	19	42	Female	Non-hispanic white	Metformin	39.5	Yes	Pre-hypertension	Buddy group. Fairly compliant with oral medications but noncompliant with insulin administration or blood glucose monitoring. Improved dietary habits but not exercise
3	18	48	Male	Non-hispanic black	Metformin	39.5	Yes	Cataract	Control group. History of anorexia. Complicated relationship with food. Has developmental delay and is in special education classes at school. Motivated to change lifestyle. Poor compliance with medication and glucose monitoring
4	14	11	Female	Non-hispanic black	Metformin	42.9	Yes	Hypertension	Buddy group. Poor compliance with medication. Skipped breakfast and lunch. Snacked excessively after school and in the evening. Mother had limited ability to supervise because she was not often home
5	13	5	Female	Non-hispanic black	Metformin	71.5	Yes	Microalbuminuria	Control group. First seen in clinic for obesity at age 6, then lost to follow-up for 7 yr prior to entering study. Gained 109.4 kg during this period. Discontinued sodas and juices and signed up for an exercise class, however, was subsequently lost to follow-up
6	14	13	Female	Hispanic	Metformin	34.2	Yes	None	Buddy group. Unmotivated to initiate behavior change and non-compliant with medication and blood glucose monitoring. Unresponsive to communication attempts by assigned buddy. Did not report any exercise. No attempt to alter dietary habits. Lost to follow-up
7	16	3	Male	Asian/pacific islander	Metformin	32.7	Yes	None	Control group. Very motivated and successful at lifestyle modification. Reverted to poor diet and exercise following family emergency. Medications subsequently re-initiated but compliance remained poor
8	17	60	Female	Asian/pacific islander	Metformin, Insulin	24.7	Yes	None	Buddy group. Poor medication compliance. No exercise despite parental encouragement. Removed sugar-sweetened beverages from diet but struggled with portion control. Improved compliance with medication regimen following hospitalization
9	17	11	Male	Non-hispanic black	Metformin, Insulin	34.3	Yes	Microalbuminuria hypertension	Buddy group. Compliant with medication but not glucose monitoring or diet. Mother encouraged portion control with little success. Patient had developmental delay but appeared to understand importance of lifestyle modification and was motivated. However, lost to follow-up
10	16	6	Male	Non-hispanic black	Metformin, Insulin	26.8	No	None	Control group. Poor compliance with medication and blood glucose monitoring. Lost to follow-up
<i>n</i> = 10	15.8 ± 2.0	22.1 ± 20.4	50% F	60% Non-hispanic black	100% metformin 40% insulin	37.0 ± 13.7	90% yes	50% yes	

BMI: Body mass index; T2DM: Type 2 diabetes mellitus.

from the initial to the 6-mo visit in either group, yet our small sample size limited systematic assessment of this outcome. The early termination of the “Buddy Study” was particularly disappointing, as the scientific community

supported the “Buddy Study” as an important and worthwhile trial. One team member (RN) was awarded the 2010 Endocrine Fellows Foundation Marilyn Fishman Grant for Diabetes Research for designing the protocol.

Furthermore, the study was promoted by the Scientific Director of the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) as part of the “Healthy Moments” radio series^[30]. Our experience may serve to caution other investigators in attempting to implement similar strategies for diabetes management among adolescents. It is possible that others have conducted but not reported such experience, because bias against submission and publication of negative study findings is problematic in the medical literature^[31]. Our seemingly “unexciting” findings convey a unique message for other investigators^[32].

Challenges in the recruitment of adolescents into clinical research protocols have been well described^[4,33,34]. Similar to most adolescents, these youths with type 2 diabetes strive to fit in with peer norms and wish to conform to their perception of what is “normal”, posing a barrier to participation in research studies^[35]. Even in the TODAY trial, the largest and most resource-intensive randomized, controlled intervention trial to be conducted in adolescents with type 2 diabetes^[36], recruitment was difficult and the projected recruitment period had to be extended by two years^[37]. This emphasizes the need for improved recruitment strategies specifically targeting adolescents.

As reflected in our cohort, data from both TODAY and the “Search for Diabetes in Youth” (SEARCH) trials^[37,38] have demonstrated that type 2 diabetes disproportionately affects youth from racial/ethnic minority groups. In addition to facing difficulties with recruitment of individuals from minority groups^[39,40] and younger age groups^[41] into chronic disease prevention and treatment programs, epidemiologic data suggest that poor blood glucose control is most prevalent among these subgroups^[38]. In accordance with the emerging field of molecular pathological epidemiology (MPE)^[42], complex diseases including type 2 diabetes may comprise various subtypes involving heterogeneous subpopulations. Because the etiology underlying type 2 diabetes is multifactorial, different disease subtypes may be associated with different biological, social, and environmental determinants and diverse natural histories. Thus, diabetes may progress at different rates and respond differently to interventions and treatments in certain individuals^[43], as we observed in our study of adolescents with type 2 diabetes.

We observed low self-reported quality of life and frequent depressive symptoms, both of which are associated with exacerbated metabolic disturbance and poor glycemia control^[44]. Given the high rates of treatment failure on metformin among adolescents^[36], the implementation of a buddy system to encourage and sustain lifestyle changes and improve psychosocial health was a seemingly hopeful undertaking. However, even the best-designed programs cannot be effective if adolescents do not participate^[45] nor can they be successful if adolescents who do participate are not compliant with medications and study requirements. This is exemplified by the high frequency of missed clinic appointments, continued failure to conduct self-glucose

monitoring, and widespread non-compliance with medication and lifestyle recommendations. Of note, the “Buddy Study” was designed to place the burden and inconvenience of study participation on the research team rather than on the study participants (e.g., meetings between patients and buddies took place at locations of the patient’s choice, and contacts were made *via* phone, cell phone, and e-mail).

Several modifications to our study may have facilitated improved enrollment and/or enhanced compliance with treatment recommendations. First, pairing adolescents with peer volunteers who themselves have type 2 diabetes^[16] and had successfully improved their glycemia^[46] or with lay volunteers of the same race/ethnicity and/or socio-economic status^[46] may have been more effective in building trust between adolescents and their buddies^[47] and generating interest in study participation. Approaching adolescents at the time of their diabetes diagnosis may also have been helpful, as early intervention has shown promise in chronic disease management^[48]. Future efforts to raise adolescent understanding of the physiology of type 2 diabetes may also be worthwhile in enhancing participation^[49].

Another hurdle is the limited time a practicing physician can afford to spend on clinical trial recruitment. In our study, several patients were not informed about the study by the treating physician because the medical, psychological and/or psychosocial situation was so complicated that no further topics could be discussed in the short time of the clinic visit. Though we attempted to have a research assistant present at all times, logistically this was not feasible.

In summary, our study provides insight into the difficulties of translating an intervention effective in adults with type 2 diabetes into a successful approach in adolescents with the same condition. The challenges faced during the “Buddy Study” may serve as a caution to other investigators attempting to implement similar strategies for diabetes management among adolescents. Our findings emphasize the urgent need for improved recruitment strategies specifically targeting adolescents.

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COMMENTS

Background

Type 2 diabetes in adolescence is generally associated with obesity, a positive family history of type 2 diabetes, and a low-income minority background. Obesity related co-morbidities together with long-lasting diabetes dramatically

increase the risk of micro-and macro-vascular complications at a young age.

Research frontiers

Psychosocial interventions in adults with type 2 diabetes and in youth with type 1 diabetes have shown promise in increasing adherence to treatment, improving psychological health in adolescents with type 1 diabetes, and/or lowering hemoglobin A1c (HbA1c), yet similar studies have not been conducted in adolescents with type 2 diabetes.

Innovations and breakthroughs

The study tested an intervention shown to be effective in adults with type 2 diabetes in a cohort of adolescents with the same condition. The findings provide insight into the difficulties of translating an intervention effective in adults with type 2 diabetes into a successful approach in adolescents and highlight the need for innovative strategies to improve recruitment and retention of adolescents with type 2 diabetes into diabetes treatment programs.

Applications

Given the recruitment challenges faced, the authors' study may serve as a caution to other investigators attempting to implement similar strategies for diabetes management among adolescents. Based on their experience, additional practical considerations for designing interventions in adolescents may include pairing adolescents with peer volunteers who themselves have type 2 diabetes and had successfully improved their glycemia or with lay volunteers of the same race/ethnicity and/or socio-economic status. In addition, future efforts to raise adolescent understanding of the physiology of type 2 diabetes may also be worthwhile in motivating adolescents to participate in diabetes treatment programs.

Terminology

While they expect that the terminology in our manuscript is familiar to most readers, they wish to define two critical terms mentioned repeatedly in the manuscript: psychosocial intervention and (HbA1c). Psychosocial interventions are interventions that are designed to change behavior and have a direct focus on a person's social environment including interpersonal interactions and social support. This is in contrast to a medical approach, in which participants are prescribed medication or assigned to a specific diet. (HbA1c) is a commonly used indicator of glycemic control over a 3-4 mo period. (HbA1c) measures the percentage of one's hemoglobin (a protein in red blood cells) that is glycosylated or in other words, has sugar attached to it.

Peer-review

The study is an interesting analysis about the insight into the difficulties of translating an intervention effective in adults with type 2 diabetes into a successful approach in adolescents with the same disease.

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