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Update on biomarkers of glycemic control

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Abstract

Attaining and maintaining good glycemic control is a cornerstone of diabetes care. The monitoring of glycemic control is currently based on the self-monitoring of blood glucose (SMBG) and laboratory testing for hemoglobin A1c (HbA1c), which is a surrogate biochemical marker of the average glycemia level over the previous 2-3 mo period. Although hyperglycemia is a key biochemical feature of diabetes, both the level of and exposure to high glucose, as well as glycemic variability, contribute to the pathogenesis of diabetic complications and follow different patterns in type 1 and type 2 diabetes. HbA1c provides a valuable, standardized and evidence-based parameter that is relevant for clinical decision making, but several biological and analytical confounders limit its accuracy in reflecting true glycemia. It has become apparent in recent years that other glycated proteins such as fructosamine, glycated albumin, and the nutritional monosaccharide 1,5-anhydroglucitol, as well as integrated measures from direct glucose testing by an SMBG/continuous glucose monitoring system, may provide valuable complementary data, particularly in circumstances when HbA1c results may be unreliable or are insufficient to assess the risk of adverse outcomes. Long-term associations of these alternative biomarkers of glycemia with the risk of complications need to be investigated in order to provide clinically relevant cut-off values and to validate their utility in diverse populations of diabetes patients.

Key words: Diabetes mellitus; Hemoglobin A1c; Fructosamine; Glycated albumin; 1,5-anhydroglucitol; Plasma glucose; Glucose variability; Diabetic complications

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Core tip: Monitoring of glycemic control is currently based on the self-monitoring of blood glucose and laboratory testing for hemoglobin A1c (HbA1c), which is a surrogate marker of the average glycemia level over the past 2-3 mo. The severity of hyperglycemia and glycemic variability contribute to the pathogenesis of complications,

but the HbA1c measurement reflects only a piece of these important variables. In this review, we provide a critical update on the use of HbA1c and alternative biomarkers of glycemic control, with particular emphasis on the need for a personalized approach in utilizing and interpreting different tests in a clinically meaningful manner.

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INTRODUCTION

Attaining and maintaining good glycemic control is the cornerstone of diabetes care^[1]. The results of the seminal Diabetes Control and Complications Trial (DCCT) clearly evidenced that glycemic control is causatively related to microvascular complications in type 1 diabetes^[2]. A long-term follow-up in the Epidemiology of Diabetes Interventions and Complications Study (EDIC) confirmed that keeping glycemia as close as possible to its normal range with intensified insulin therapy ameliorated both microvascular and cardiovascular complications for 30 years in the same cohort of patients^[3].

Similar evidence of the beneficial effect of intensive glucose control practices in reducing the risk of diabetic complications, adverse cardiovascular outcomes and mortality were shown in type 2 diabetes patients in both the United Kingdom Prospective Diabetes Study (UKPDS) intervention and in follow-up trials^[4,5]. However, although additional intensification of glucose control in type 2 diabetes patients provided some benefits^[6,7], it was associated with serious adverse outcomes such as an increased overall mortality^[8] that was most likely due to severe hypoglycemia as a side-effect of a more aggressive antihyperglycemic therapy^[9]. These data indicated that a personalized approach to glycemic goals that uses clinically validated biomarkers rather than a “one-size-fits-all” concept may provide a valid rationale for optimal diabetes care.

The concept of glycemic control monitoring is currently based on self-monitoring of blood glucose (SMBG) and laboratory testing for hemoglobin A1c (HbA1c), which is a surrogate biochemical marker of the average glycemia level over the previous 2-3 mo period^[10]. HbA1c emerged as a key determinant of the risk cut-off for diabetic complications and as a setting point for optimal glycemic control in both DCCT and UKPDS trials, and it is considered to be a gold standard of diabetes care in contemporary clinical practice^[11]. HbA1c provides valuable, standardized and evidence-based information that is relevant for clinical decision-making; however, several biological and analytical interferences, as well as clinical conditions, limit its accuracy in reflecting the true glycemia level^[12,13]. Recent technological advances in the field of continuous glucose monitoring systems (CGMS) have revealed new insights in short-term glucose dynamics which are not reflected by HbA1c, although it seems to be relevant in assessing the risk of diabetic complications^[14,15].

Thus, alternative glycemic markers that provide reliable information about glycemic control in addition to and beyond HbA1c are needed to improve the quality of clinical care across a heterogeneous diabetes population^[16,17].

The aim of this narrative review is to provide a critical update on the use of HbA1c and alternative biomarkers of glycemic control, with a particular emphasis given to the need for a personalized approach in utilizing and interpreting different tests in a clinically meaningful manner.

HBA1C

HbA1c results from the posttranslational modification of hemoglobin A by the nonenzymatic covalent binding of glucose to the N-terminal valine of the β -globin chain^[10]. This reaction is termed glycation and affects all structural and circulating proteins with free amino-acid residues that are available for binding monosaccharides. The glycation of hemoglobin is a two-step chemical reaction whereby glucose covalently binds to the free amino-groups within globin chains^[18]. The first step of this process results in labile aldimine (a Schiff base), which can either

dissociate or further convert to a stabile ketoamine by an Amadori rearrangement, depending on the glucose concentration in the blood^[10]. HbA1c was first observed as a minor chromatographic fraction of adult hemoglobin in 1958 and was named according to its chromatographic column elution sequence^[19], but its relevance in diabetes was revealed in 1969 by Rahbar^[20], who observed significantly higher HbA1c values in diabetic patients. Since glycation is a nonenzymatic reaction, it complies with the law of mass action. Thus, assuming normal erythropoiesis and a stable hemoglobin concentration, HbA1c reflects the average glycemia level during one red blood cell life cycle (2-3 mo)^[21].

Considering the high biological variability, the dynamics of glucose, as well as the limitations of blood glucose monitoring technology, at that time, the possibility of obtaining an integrated average glycemia value by the measurement of a single biomarker elicited immense interest and provided a powerful tool in both diabetes research and clinical management. HbA1c testing was soon facilitated by the development of a new analytical methodology that was suitable for use in clinical laboratories.

Various analytical methods for HbA1c determination commonly utilize either of the two principles (Table 1): (1) HbA1c separation from other hemoglobin fractions that is based on charge differences using either chromatography or electrophoresis; or (2) the direct measurement of HbA1c by specific binding (immunochemistry or affinity) or enzymatic cleavage^[22]. Due to differences between these analytical methods in their use of different principles and a lack of standardization, HbA1c testing inherently suffers from a significant between-method variability which has seriously affected its clinical accuracy in the longitudinal monitoring of average glycemia with different methods and comparing the results of the DCCT- and UKPDS-derived targets. Heterogeneity of molecular entities that were measured by different methods significantly contributed to the analytical variability, as the glycation reaction involved not only β -N-terminal valine but also other accessible amino groups within the α and β -globin chains, and these results depended on the type of analyte that was captured by a particular method^[12]. Thus, the standardization of the HbA1c measurement and reporting that included a uniform definition of the analyte was shortly identified as one of the most important issues in diabetes care^[23,24].

Clinical harmonization was accomplished within the National Glycohemoglobin Standardization Program (NGSP), which was established by the American Diabetes Association (ADA) and the American Association of Clinical Chemistry (AACC). The goal of the NGSP was to harmonize the HbA1c results that were obtained by different methods with the highly reproducible but insufficiently specific method (ion-exchange chromatography) that was used in the DCCT and UKPDS trials, thereby enabling the traceability and comparability of results to the evidence-based clinical criteria^[25]. Almost simultaneously to the NGSP, the International Federation of Clinical Chemistry (IFCC) set up an HbA1c Standardization Program that was aimed at designing a comprehensive reference system with both reference methods and a primary reference standard for a structurally-defined analyte^[23,26,27]. The comparison between the two reference systems revealed an excellent linear correlation between the DCCT- and IFCC-reference systems but significantly lower HbA1c values with the latter, more specific method. This finding raised concerns regarding the risks of deterioration of the glycemetic control with the adoption of the new reference system, which had been reported previously^[28].

In 2010, a Global Consensus on HbA1c measurement and reporting was issued by an international committee representing the ADA, European Association for the Study of Diabetes (EASD), International Diabetes Federation (IDF), IFCC and International Society for the Pediatric Diabetes (ISPAD)^[29]. Briefly, the Global Consensus defined the IFCC reference as the only valid anchor for commercial methods calibration and a dual reporting of the HbA1c results as mmol/mol (IFCC-related units) and % (NGSP/DCCT-related units). A master equation describing the relationship between the two reference systems should be used for the interconversion of the results:

$$\text{HbA1c NGSP/DCCT (\%)} = 0.09148 \times \text{HbA1c IFCC (mmol/mol)} + 2.152$$

$$\text{HbA1c IFCC (mmol/mol)} = 10.93 \times \text{HbA1c NGSP/DCCT (\%)} - 23.50$$

Editors of scientific journals were encouraged to require both units of HbA1c reporting to promote the clarity and comparability of results between studies that used HbA1c as an outcome measure and to facilitate the combination of these results in meta-analyses. The Global Consensus definitely enabled the uniform traceability and improved analytical quality of HbA1c measurements^[12]; however, it failed to harmonize the reporting of these results, as different countries use different reporting units, which may thus complicate a direct comparison of results across the world^[30].

Today, the analytical procedures for HbA1c measurement are harmonized and the between-method/laboratory variabilities have been gradually reduced towards a

Table 1 Characteristics of the analytical methods for hemoglobin A1c measurement

Method	Advantages	Disadvantages
Ion exchange chromatography	DCCT method, high reproducibility	Lack of specificity; interference from hemoglobinopathies and HbF
Capillary electrophoresis	High reproducibility; specificity	Time-consuming, costly
Boronate affinity chromatography	Minimal interference from hemoglobinopathies	Analyte-related unspecificity (total GHb)
Immunoassay	Specificity	Some interference from HbF

DCCT: Diabetes Control and Complications Trial; HbF: Fetal hemoglobin; GHb: Total glycated hemoglobin.

desirable goal, which is a coefficient of variation (CV) < 3.5%^[12]. Regarding the within-laboratory imprecision, current guidelines recommend a CV < 2% for NGSP-HbA1c equivalents^[31], and this is achievable with almost all of the commercially available laboratory methods apart from point-of-care systems for HbA1c testing, which still need improvement^[22]. However, global harmonization and ongoing efforts to improve the analytical quality^[32] cannot obviate the limitations of HbA1c measurement due to the hemoglobin-related interferences.

It has long been recognized that hemoglobin variants interfere with HbA1c synthesis and measurement, and this interference depends on the nature of the congenital disorder afflicting hemoglobin synthesis and the analytical method that is used to measure HbA1c^[22]. Thalassemia traits, HbS, HbC, HbE and HbF are among the most abundant hemoglobin-related interferences^[33]. Additionally, other posttranslational modifications of hemoglobin such as carbamylation by uremic toxins in end-stage renal disease may significantly interfere with some HbA1c assays^[34]. It should be noted that the majority of interferences have been mitigated by improvements of analytical methodologies, and the remaining interferences have been depicted and rigorously scrutinized. A comprehensive list of HbA1c methods that have been characterized for their susceptibility to hemoglobin-related interferences is available and is continuously updated on the NGSP website^[35].

Biological confounders influencing the accuracy of HbA1c as a glycemic marker have emerged as a significant issue after analytical harmonization, despite the fact that a substantial intraindividual variability in HbA1c values was recognized long ago. Studies on the relationship between HbA1c measurements and average glycemia levels revealed a strong linear correlation with a wide interindividual variability, *e.g.*, an HbA1c of 7% (53 mmol/mol) could correspond to an average glucose concentration ranging from 6.8 to 10.3 mmol/L^[36]. Physiological factors such as age and ethnicity, as well as genetics, seem to be major determinants of this variability.

Age was found to be associated with a gradual increase of HbA1c levels in nondiabetic individuals independently of sex and level of glycemia, indicating that age-specific reference intervals/clinical cut-off points may improve the clinical accuracy of this test in both the diagnosis and management of diabetes^[37]. There are ethnic differences in HbA1c values even when glycemia levels are the same; a recent meta-analysis revealed that Caucasians have slightly lower HbA1c values in comparison to persons of other ethnic groups^[38]. While the clinical relevance of this finding needs to be further investigated, the authors concluded that a better understanding of the molecular mechanisms behind this observed between-race variability in HbA1c may improve its clinical applicability.

Recent genetic studies have revealed that multiple genomic loci are associated with HbA1c levels, and this could provide a plausible explanation for the physiological factors determining its variability and clinical utilization towards a more personalized approach^[39]. Among the 60 genetic variants that were found to influence HbA1c, 19 variants associated with glycemic pathways were identified, and among the rest of variants that were involved in nonglycemic pathways, 22 erythrocytic variants were found^[40]. Among these, a variant on the X chromosome coding for glucose-6-phosphate dehydrogenase (G6PD) was associated with a significantly higher HbA1c variability in populations of African ancestry when compared to other ethnic groups. This highly prevalent variant is associated with a shorter erythrocyte lifespan and, consequently, falsely decreased HbA1c levels, which may have serious impacts for diabetes care in afflicted individuals^[40].

Nonglycemic factors affecting HbA1c levels include erythropoiesis, hemoglobin synthesis and conditions influencing red blood cell survival. Deficiency anemias generally elicit falsely increased HbA1c levels due to the increased levels of aged erythrocytes that are found in patients with this disease, whereas falsely decreased HbA1c levels can be observed in hemolytic anemias of any cause^[41]. Nonhematological conditions influencing HbA1c values include pregnancy, chronic

renal failure and certain medications^[22]. Variability in the normal erythrocyte lifespan is another significant confounder of HbA1c accuracy. Malka *et al*^[42] recently proposed a mechanistic mathematical model integrating hemoglobin glycation and red blood cell kinetics that provided a personalized insight into average glucose levels and reduced the occurrence of diagnostic errors due to a misinterpretation of average glycemia (as reflected by HbA1c) by more than 50%. The applicability and clinical utility of the proposed model have yet to be determined.

Furthermore, part of the variability in HbA1c is considered to be a consequence of differences in glycation rate, which is a concept that was proposed as the “glycation gap” 15 years ago^[43]. The glycation gap hypothesis is based on the differences between the intra- and extracellular surrogate markers of average glycemia, *i.e.*, HbA1c and fructosamine, and it was proposed as an explanation to the commonly encountered clinical problem of discrepancy between various glycemia measures that cannot be attributed to any other confounding factor^[44]. In spite of subsequent evidence from a twin study that shows that the glycation gap may be a genetically determined characteristic of an individual^[45], this concept has been considered implausible by some authors due to the lack of validating data or supporting evidence of the underlying mechanism^[46]. Nevertheless, an accumulating body of evidence indicates that glycemetic variability, as assessed by either the glycation gap or another discordance measure called the hemoglobin glycation index^[47], is indeed associated with adverse diabetes-related outcomes such as mortality, micro- and macrovascular complications, and hypoglycemic episodes that are associated with intensive treatment^[48,49]. Interindividual heterogeneity in glucose transport across the erythrocyte membrane was proposed as a possible explanation for inconsistencies between HbA1c and other measures of glycemia^[50]. Genome-wide association studies also support the plausibility of the glycation gap concept since one of the identified loci, FN3K, encodes fructosamine-3-kinase, which is an enzyme that is involved in deglycation of glycated proteins^[39]. Dunmore *et al*^[51] recently reported a significant difference in the erythrocyte fructosamine-3-kinase activities between glycation gap categories and pinpointed FN3K both as a novel predictor of the risk for development of and as a potential target for the prevention of diabetic complications.

Current clinical guidelines recommend regular HbA1c testing twice a year in all diabetic patients who achieve their glycemetic targets, and they recommend an increased frequency of testing not to exceed four times a year for patients who have changed therapy and/or have not achieved their treatment goals^[1]. The general recommendation is to keep the HbA1c levels < 7% (53 mmol/mol); however, the target should be individualized for individual patients depending on the diabetes duration, age or life expectancy, CVD and other comorbidities, hypoglycemia unawareness and psychosocial factors^[52]. A reference change value of 0.5% (5 mmol/mol) in the longitudinal monitoring of an individual patient is considered to be clinically significant^[22].

The use of HbA1c as a diagnostic test for diabetes with a diagnostic cutoff set at an HbA1c level of 6.5% (48 mmol/mol) has recently been recommended by prominent professional organizations and by the World Health Organization^[53,54]. Low intraindividual biological variability, the stability of the analyte and the independence of results to the prandial status were the most pronounced advantages of HbA1c over plasma glucose, while higher costs and the limited availability of the test were considered as its disadvantages^[55]. However, the diagnostic accuracy of HbA1c at a given threshold was found to be poor in many studies^[56-58], as well as in a recent global surveillance on the prevalence and diagnosis of diabetes^[59], which is at least in part a consequence of numerous biological confounders^[38,60]. A comprehensive list of biological, (patho) physiological and pharmacological factors that may influence the synthesis, measurement and/or interpretation of HbA1c is presented in [Table 2](#).

GLYCATED PROTEINS

Fructosamine (1-amino-1-deoxy fructose) is a common term for all glycated plasma proteins. It is a ketoamine that is formed by the irreversible nonenzymatic binding of glucose to plasma proteins in a process called glycation. Glycation is a nonenzymatic process where a labile Schiff base (aldimine) is formed at an early stage and is subsequently rearranged to a stabile Amadori product (ketoamine) due to the covalent binding of glucose to the lysine, arginine and cysteine amino-group residues within protein molecules^[61].

Glycated albumin (GA) is formed in a similar reaction as fructosamine and is specific to albumin molecule^[62]. In conditions that are associated with high glucose levels, plasma proteins are exposed to greater glycation, which leads to increased

Table 2 Biological, (patho)physiological and pharmacological factors influencing hemoglobin A1c**Factor influencing HbA1c synthesis/measurement/interpretation**

Age, ethnicity
Genetic factors (e.g. Glucose-6-phosphate dehydrogenase variants)
Pregnancy
Red blood cell lifespan
Haemolytic anaemia
Iron deficiency anaemia
Haemoglobin variants
Accute haemorrhage
Splenomegaly
Splenectomy
Transfusion
Chronic liver disease
End-stage renal disease
Rheumatoid arthritis
Vitamin C
Drugs (aspirin, erythropoietin, dapsone, antiretroviral agents)
Endogenous interferents (high levels of bilirubin/triglycerides)

HbA1c: Hemoglobin A1c.

fructosamine and GA formation. Fructosamine and GA reflect the average blood glucose concentration during the lifetime of either total plasma proteins or albumin, both of which are within the range of two to three weeks^[63].

Despite the fact that albumin is a major constituent of plasma proteins, fructosamine and GA may not be considered as totally equal measures of glycemia due to their differences in analytical procedures and their currently established clinical performance. Fructosamine was identified long ago, but the lack of analytical standardization and problems with the assay's specificity and susceptibility to interference by hyperlipidemia limited its use in diabetes management. Additionally, there was insufficient evidence to correlate fructosamine and GA with long-term outcomes in patients with diabetes^[64].

However, over the years, the development and improvement of methods for determining fructosamine and GA have paved the way for many studies that focused on their analytical and clinical significance. Affinity chromatography^[65], ion-exchange chromatography^[66] and high-performance liquid affinity chromatography^[67] were all developed as methods for the direct measurement of GA along with liquid chromatography-tandem mass spectrometry (LC-MS/MS) as a "gold standard"^[68]. However, these methods are complicated and expensive and require dedicated equipment and expertise, and this has limited their routine use. Consequently, simpler and more affordable colorimetric and enzymatic methods, applicable on various automated analytical platforms, were developed for use in clinical laboratories^[69]. Enzymatic methods showed a better analytical performance and were free of colorimetric interferences (*e.g.*, bilirubin)^[70-72]. Various commercial kits are available for GA measurement depending on the type of enzyme that was used in the reaction and the units used to express the results ($\mu\text{mol/L}$, mmol/L or % GA fraction).

Currently, the method of choice for fructosamine determination is the second generation of the nitroblue tetrazolium colorimetric procedure, in which there is a separation of glycated from nonglycated proteins based on their differences in chemical reactivity^[73]. The assay itself is inexpensive, rapid, simple, highly specific and free of interferences from uric acid or polylysine. Nevertheless, despite many improvements, this method is still sensitive to rapid changes in ambient temperature and interferences from extremely high levels of some compounds with reducing properties, such as bilirubin and vitamin C^[64]. Still unresolved is the issue of whether the resulting fructosamine measurements should be corrected for either total protein or albumin concentrations. The results are relatively ambiguous^[74], but it was recently reported that correcting the fructosamine measurement for proteins may improve its correlation with HbA1c and its overall performance in detecting diabetes^[75].

Given the faster protein metabolic turnover, fructosamine and GA values reflect shorter-term glycemia levels rather than HbA1c. Additionally, fructosamine and GA are not influenced by anemia or hemoglobinopathies such as HbA1c is, and they can therefore be used in conditions where HbA1c is not reliable due to analytical or biological interferences^[62]. In conditions such as pregnancy^[76] and treatment modifications^[77] fructosamine and GA can detect changes in average blood glucose earlier than HbA1c and thus provide more timely information about the achievement of glycemetic control^[62,78,79].

Both fructosamine and GA are the markers of choice when glycemetic control needs to be assessed in patients with severe chronic kidney disease (CKD) (stages 4 and 5)^[80]. Additionally, in stage 5 CKD patients on hemodialysis, GA can be used as a predictor of overall survival and cardiovascular mortality^[81]. Due to the reduced production and lifespan of red blood cells and to erythropoietin treatment in CKD patients, HbA1c cannot be used as reliable marker, as it can significantly underestimate the true glycemetic status in these patients^[82].

The distribution of GA in healthy subjects has been described in diverse populations^[83,84]. The Large Atherosclerosis Risk in Communities (ARIC) study was conducted in a cohort of almost 12000 participants and proved a strong association of fructosamine and GA with the incidence of diabetes and microvascular complications (prevalent retinopathy and risk of CKD)^[85]. Together with fructosamine, GA was reported to be strongly associated with HbA1c and fasting glucose^[86]. Furthermore, a recent study by Bellia *et al*^[87] evaluated the potential clinical usefulness of GA for the diagnosis of diabetes in an asymptomatic Caucasian population (specifically in Europe) with an elevated risk of developing diabetes. At the GA cut-off of 13.5%, a high sensitivity (88.9%; 95%CI: 65.3-98.6) and a good specificity (60.4%; 95%CI: 54.8-65.9), was demonstrated for its possible screening use in similar subjects^[87].

It is important to note that fructosamine and GA measurements are not reliable in some physiological and pathological conditions. Every clinical condition that can affect protein and albumin metabolism (nephrotic syndrome, hyperthyroidism, glucocorticoid therapy, liver cirrhosis, *etc.*) may affect these results, where they would also require careful interpretation^[14,62]. Additionally, similar to HbA1c, fructosamine and GA are determined by genetic variants that are associated with both glycemetic and nonglycemetic components, both of which should be considered when putting the results in a clinical context^[84].

1,5-ANHYDROGLUCITOL

1,5-Anhydroglucitol (1,5-AG) is a monosaccharide that is structurally identical to D-glucose with the absence of the C-1 hydroxyl group. It is derived mainly through food intake and also absorbed by the intestine at a rate of approximately 4.4 mg/d. The main source of 1,5-AG is soy beans, but small amounts can be found in rice, pasta, fish, fruits, vegetables, tea, milk and cheese. The metabolic role of 1,5-AG is still quite unknown. It circulates in body in its free form and can be found in all organs and tissues (1,5-AG pool) with the total amount several times higher than that in plasma^[88]. A negligible amount is presumed to be synthesized *de novo*^[89]. 1,5-AG intake is regulated by its urinary excretion, and 99.9% of 1,5-AG is reabsorbed by the kidneys by the specific sodium glucose active cotransporter (SGLT4)^[88,90]. Reabsorption is competitively inhibited by glucose. When the plasma glucose level exceeds the renal threshold for glucosuria (approximately 10 mmol/L), 1,5-AG is excreted in the urine, which results in a rapid reduction of its serum levels^[91]. Thus, low values of 1,5-AG reflect both high circulating glucose levels and glucose fluctuation, or so-called hyperglycemic excursion^[92]. This biomarker may be useful to differentiate between diabetic patients with well-controlled HbA1c but with extensive glucose fluctuations^[93]. After normoglycemia is restored, the 1,5-AG concentration returns to its normal value at a rate of 0.3 µg/ml per day, and it can take up to 5 wk for this value to increase up to its normal level^[94]. Due to its half-life of approximately 1 to 2 wk, 1,5-AG can be used as a potential marker for short-term glycemia^[95]. Additionally, there is evidence that 1,5-AG reflects the 2-h postprandial glucose (PPG) values of the 2 preceding weeks in moderately controlled patients and is more sensitive and specific than HbA1c^[96]. PPG values are especially important for clinical decision-making concerning changes in the diet or in changes of the pharmacologic treatment of diabetes and overall glycemetic control^[97].

1,5-AG can be measured in serum, EDTA-plasma and urine samples. There are two commercially available enzymatic kits for its blood measurement: the Glyco-Mark™ (GlycoMark, Inc) kit that is used in United States and the Determiner-L (Kyowa Medex, Tokyo) kit that is used in Japan. Both of these methods can be applied to

automated chemistry analyzers. Recent data has shown a good between-method comparability despite slightly different results that were obtained in the same samples^[98]. Another method for the determination of 1,5-AG is chromatography, specifically gas chromatography-mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC). These methods are sensitive and precise but require sample preparation and are time-consuming and cumbersome^[99]. Urine, a sample with lower 1,5-AG levels, requires a more sensitive method such as liquid chromatography/mass spectrometry (LC/MS) or HPLC^[100].

Regarding its association with diabetes and microvascular complications, the ARIC study provided evidence that 1,5-AG levels were associated with prevalent retinopathy and incident CKD, particularly in patients who were diagnosed with diabetes. Despite the low association in nondiabetic subjects, there was a good risk prediction of incident diabetes in both groups^[86,101].

The results obtained from patients with certain conditions such as kidney disease or pregnancy must be carefully interpreted due to the changes in renal function during these conditions which influences the threshold for glucose excretion. Nevertheless, 1,5-AG can be reliable in subjects with mild to moderate renal insufficiency as a marker for glycemic control^[102]. Furthermore, 1,5-AG can be helpful in cases when frequent adjustments in therapy are required and glycemic control has to be maintained^[94].

Given the limitations of HbA1c and the recently collected evidence on the clinical utility of nontraditional markers of glycemia, their implementation in clinical practice is expected. The recently published reference intervals provide the most valuable tool in facilitating the translation of these biomarkers into routine clinical practice. In a healthy reference population of almost 1800 individuals, the reference ranges for fructosamine, GA and 1,5-AG were reported as 194.8-258.0 $\mu\text{mol/L}$, 10.7%-15.1% and 8.4-28.7 $\mu\text{g/mL}$, respectively^[103].

DIRECT MEASURES OF GLYCEMIA

Fasting and postprandial plasma glucose (FPG and PPG, respectively) are obvious measures of glycemia, providing “snapshot” glucose values for primary use in targeting treatment goals, which are currently set at ranges of 4.4-7.2 mmol/L for FPG and < 10.0 mmol/L for PPG^[1]. The contributions of these measures to HbA1c have been evaluated^[104], and significant association of PPG with cardiovascular risks was evidenced^[105]. Daily plasma glucose values are readily available to patients who perform SMBG as a part of their regular diabetes care but reviewing and interpreting the cumulative SMBG results may propose a significant challenge for healthcare professionals^[106].

Advances in both the analytical accuracy and software supporting SMBG, the development of continuous glucose monitoring sensors and, most recently, flash-glucose sensing technology, have prompted the development and validation of new, metrics-derived surrogate markers of glycemia which have improved our understanding of the complex glucose dynamics and have provided new tools for patients and healthcare providers in achieving optimal control of diabetes and reducing the frequency of acute and chronic complications of diabetes^[13,14].

Among the integrated SMBG-derived metrics, the glycemic risk assessment diabetes equation (GRADE) and average daily risk range (ADDR) were found to best correspond with the degrees of risk of hypo- and hyperglycemia that were associated with the glucose profile^[107], and they showed positive correlations with HbA1c and negative correlations with c-peptide levels^[108].

As opposed to the SMBG-derived profiles, which are based on a limited number of static plasma glucose measurements throughout the day, CGMS enable a continuous insight into daily glycemia, thus enabling an individualized approach and offering a powerful tool for patients in achieving their glycemic targets and mitigating glycemic excursion. CGMS has yielded previously unreachable measures of glycemia such as average glucose exposure, time in range, hypo- and hyperglycemia and glycemic variability (glucose excursions). The glycemic variability was considered to be a significant risk factor for developing complications that was not reflected by HbA1c levels^[13]. The advantages of using SMBG to improve patient outcomes have been amply evidenced in studies targeting various vulnerable populations of patients with diabetes such as children^[109], pregnant women^[110], the elderly^[111], and the patients suffering from diabetic kidney disease^[112] and from hypoglycemic episodes^[113]. However, the high costs, insurance-related limitations and patient- and healthcare provider-related attitudes still hinder a wider utilization of CGMS. The recently published International Consensus on Use of Continuous Glucose Monitoring is an

encouraging step forward and is aimed at providing technical and clinical recommendations on the use of CGMS in conjunction with HbA1c, and it provides a comprehensive insight into the state-of-the-art evidence supporting CGMS-derived metrics to improve patient care and clinical outcomes^[14].

CONCLUSION

Hyperglycemia is a key biochemical feature of diabetes that should be rigorously controlled and maintained in a range as close to normal as possible to mitigate the risk of diabetic complications. Both the level of and exposure to hyperglycemia, as well as glycemic variability, contribute to the pathogenesis of diabetic complications, with different patterns of disease pathogenesis in patients with type 1 or type 2 diabetes. Despite its analytical and biological limitations, HbA1c remains the key biomarker of long-term glycemic control. However, it has become apparent in recent years that other glycosylated proteins, 1,5-AG, and integrated measures from direct glucose testing by SMBG/CGMS may provide valuable data complementary to HbA1c, particularly in circumstances when the HbA1c results may be unreliable or insufficient to assess the risk of adverse outcomes (Table 3). Long-term associations of these alternative biomarkers of glycemia with the risk of diabetic complications need to be investigated to provide clinically relevant cut-off values and validate their utility in diverse populations of patients with diabetes.

Table 3 Characteristics of glycaemic biomarkers

Markers of hyperglycemia	Assessment period	Advantages	Limitations
HbA1c	2-3 mo	Fasting not necessary; low interindividual variability; screening tool for diabetes; association with diabetes complications; standardization	Surrogate biomarker analytical interferences; biological confounders; costs
Fructosamine Glycated albumin	2-3 wk	Fasting not necessary; inexpensive and easily automated; good correlation with HbA1c; association with diabetes complication; marker of choice in severe chronic kidney disease	Surrogate biomarker; higher interindividual variability; unreliable in conditions with altered protein and albumin metabolism (nephrotic disease, severe liver disease), thyroid dysfunction; not standardized
1,5-anhydroglucitol	1-2 wk	Fasting not necessary; glycemic excursion detection; good correlation with HbA1c; association with diabetes complications	Surrogate biomarker; unreliable in the setting of chronic kidney disease (stage 4 and 5), dialysis, pregnancy or other conditions with changes in renal threshold (sGLT inhibitors); not suitable for diabetes diagnosis
Fasting glucose	8-10 h	Current glycemic status; immediate availability for daily diabetes management SMBG/CGMS	Affected by acute illness and stress; SMBG and CGMS-accuracy
Postprandial glucose	2-4 h		
Indices of glycaemic variability	24-72 h	Short-term glucose dynamics; improves glycaemic control beyond HbA1c and patient's satisfaction/outcomes	CGMS mandatory; costs education; standardization

HbA1c: Hemoglobin A1c; SMBG: Self-monitoring of blood glucose; CGMS: Continuous glucose monitoring system.

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Effects of diabetic ketoacidosis in the respiratory system

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Abstract

Diabetes affects approximately 30 million persons in the United States. Diabetes ketoacidosis is one of the most serious and acute complications of diabetes. At the time of presentation and during treatment of diabetic ketoacidosis (DKA), several metabolic and electrolyte derangements can ultimately result in respiratory compromise. Most commonly, hypokalemia, hypomagnesemia and hypophosphatemia can eventually lead to respiratory muscles failure. Furthermore, tachypnea, hyperpnea and more severely, Kussmaul breathing pattern can develop. Also, hydrostatic and non-hydrostatic pulmonary edema can occur secondary to volume shifts into the extracellular space and secondary to increased permeability of the pulmonary capillaries. The presence of respiratory failure in patients with DKA is associated with higher morbidity and mortality. Being familiar with the causes of respiratory compromise in DKA, and how to treat them, may represent better outcomes for patients with DKA.

Key words: Diabetes ketoacidosis; Respiratory physiology; Mechanical ventilation; metabolic acidosis; Hyperventilation; Kussmaul breathing; Respiratory failure

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Core tip: Several electrolyte and metabolic derangements associated with diabetic ketoacidosis (DKA) and its treatment can affect the respiratory system. Since respiratory failure in DKA is associated with increased morbidity and mortality, the recognition and treatment of those derangements have the potential to improve outcomes in DKA.

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INTRODUCTION

Diabetes ketoacidosis (DKA) is one of the most serious and acute complications of diabetes. It is characterized by moderate hyperglycemia (blood glucose usually between 250 mg/dL and 800 mg/dL at presentation), metabolic acidosis, and presence of serum ketones with an elevated anion gap^[1]. It represents an extreme in the spectrum of hyperglycemia and presentation of complicated diabetes.

Diabetes affects approximately 30 million persons in the United States^[2]. Since 2009, there has been an increase of around 6% of hospitalizations due to DKA (from 19.5 to 30.2 per 1000 persons). However, the in-hospital mortality has declined at an annual average rate of 6.8% (from 1.1% to 0.4%)^[2].

The presence of DKA is accompanied by several electrolytes, metabolic and acid-base derangements that affect the respiratory system. Depletion of ions, such as potassium and phosphate, affect the respiratory muscles leading to acute respiratory failure^[3]. Reduction in colloid osmotic pressure increases lung water content, leading to noncardiogenic pulmonary edema and decrease in lung compliance^[4,5]. As a compensatory mechanism, the presence of metabolic acidosis will cause hyperventilation^[6].

Respiratory failure in DKA has been associated with increased morbidity and mortality^[3,7]. In this review, we analyze the common electrolytes, metabolic and acid-base abnormalities seen in DKA, their association with respiratory failure and its management.

ELECTROLYTE ABNORMALITIES

Potassium, magnesium and phosphorous are intracellular ions which serum concentrations decrease as a direct consequence of hyperglycemia and ketoacidosis (potassium), or as a consequence of the correction of acidosis with insulin (magnesium and phosphorous). A major goal in the treatment of DKA is to closely monitor these ions concentrations as DKA is corrected. Also, replace them on a timely fashion in order to prevent them from reaching critically low values. The clinical significance of their deficit is discussed below.

Potassium

Patients being admitted for DKA usually have a total body potassium deficit that averages 300 to 600 mEq^[8]. Osmotic diuresis is provoked by the hyperglycemia resulting from lack of insulin. In an attempt to maintain osmolality, the kidneys will retain sodium ions at the expense of potassium ions^[9]. Furthermore, when acidosis is present, hydrogen ions from the bicarbonate nucleus will be reabsorbed at the expenditure of potassium^[10].

The gastrointestinal tract is also responsible for potassium loss in DKA. The body will try to maintain osmotic pressure at the cost of tissue and serum electrolytes. An acute hyperkalemia will happen when potassium shifts into the extracellular fluid (ECF), causing gastric cells to preserve hydrogen ions concentration. Consequently, nausea, vomit and diarrhea will occur, promoting even more potassium loss^[11,12]. However, due to a shift of potassium from intracellular fluid into ECF caused by hyper osmolality and insulin deficiency, only 5% of patients with DKA will present with hypokalemia^[8,13].

When potassium levels fall below 2.5 mg/dL, severe ascending muscular weakness can occur^[14]. The muscular weakness can affect the respiratory muscles causing acute respiratory failure^[15], and requirement of mechanical ventilation^[16]. Aggressive potassium replacement should start once serum potassium concentration reaches a value of 3.3 mEq/L^[13].

Magnesium

At presentation of DKA, the levels of serum magnesium are usually normal. Excessive amounts of magnesium are excreted during acidosis, secondary to insulin deficiency^[17]. As the acidosis gets corrected, magnesium levels fall, reaching their nadir within the first 25 h of acidosis correction^[18,19].

Hypomagnesemia, defined as having a serum magnesium concentration below 1.6 mg/dL (0.66 mmol/L), usually doesn't lead to clinically significant symptoms until serum levels fall below 1.2 mg/dL (0.5 mmol/L)^[20].

Magnesium regulates intracellular calcium levels, influencing smooth muscle tone^[21]. Because of its role in regulating smooth muscle tone, magnesium deficiency has been associated with systemic hypertension, neuromuscular excitability, bronchoconstriction, coronary vasospasm and seizures^[22].

Muscular weakness and tetany associated with hypomagnesemia can affect the

respiratory muscles, impairing ventilation in patients who are spontaneously breathing and delaying extubation of mechanically ventilated patients^[22,23]. Empirical magnesium replacement has been associated with improvement of respiratory muscle power in patients with DKA^[23].

When treating patients with DKA, clinicians should aim to keep magnesium levels at normal range, since hypomagnesemia is associated with weakness of the respiratory muscles.

Phosphorous

Acidosis causes potassium shifts into the ECF and hyperglycemia causes phosphaturia by osmotic diuresis, which will ultimately lead to hypophosphatemia. However, DKA patients will present with normal phosphorous concentration due to the shift into the ECF associated with ECF volume concentration^[24]. The true state of phosphate equilibrium is revealed with volume expansion^[24,25].

Severe hypophosphatemia (< 1 mg/dL) is associated to the depletion of high-energy phosphate compounds in muscles, causing muscular weakness and rhabdomyolysis^[3,26].

Acute muscular weakness caused by hypophosphatemia in DKA has been associated with hypercapnic respiratory failure and prolonged mechanical ventilation in critically ill patients^[26-28].

Routine replacement of phosphorous in patients who presented with DKA is not beneficial and has been associated with worsening hypomagnesemia and causing hypocalcemia^[29,30]. However, if serum phosphate concentration falls below 1 mg/dL, or if hypophosphatemia is associated with cardiac dysfunction or respiratory depression, it should be replaced^[28,31,32].

HYPERVENTILATION

The presence of metabolic acidosis will normally generate a respiratory response. The reduction of serum bicarbonate and pH will result in hyperventilation and reduction in carbon dioxide (CO₂), partially preventing further fall in pH and bicarbonate concentration. Respiratory compensation for metabolic acidosis will cause the arterial CO₂ to decrease by 1.2 mmHg for each 1 meq/L fall in the serum bicarbonate^[33].

The respiratory response usually begins within 30 min of metabolic acidosis onset, and is generally complete within 12-24 h. However, a lag in respiratory compensation can occur when respiratory acidosis develops quickly; more than 4 meq/L of bicarbonate decrease in less than 6-12 h^[34,35].

There is a limit to the lungs' ability to compensate for metabolic acidosis. Even with serum bicarbonate concentrations below 6 meq/L, CO₂ levels cannot fall lower than 8-12 mmHg^[34]. Furthermore, the duration of the respiratory compensation is limited by respiratory muscle fatigue^[33,36].

Initially, patients will develop tachypnea, which is increased respiratory rate, leading to decrease in CO₂ concentration. With progression of acidosis, respiratory pattern evolves to hyperpnea, which is increased tidal volume, and ultimately, patients will develop a deep, fast and agonal pattern of breathing, named Kussmaul's respiration (Figure 1A-D)^[34,37].

Once patients with DKA develop Kussmaul's respiration, they are reaching the point of respiratory muscles fatigue, and mechanical ventilation should be considered^[34,38-40]. Furthermore, patients in DKA are severely "air hungry" prior to intubation, and are at higher risk to develop acute respiratory distress syndrome (ARDS)^[3,41] due to hyperpnea. Mechanical ventilation in these patients is particularly delicate, since a lung protective strategy, with low tidal volumes and controlled transpulmonary pressures, should be maintained, while attempting to increase minute-ventilation until metabolic acidosis is completely corrected^[42,43].

PULMONARY EDEMA

There are two types of pulmonary edema that have been described in patients with DKA: One associated with elevated pulmonary venous pressure and another associated with increased pulmonary capillary permeability. The diagnosis is made based on clinical findings of dyspnea, an A-a gradient on arterial blood gas and chest image showing bilateral pulmonary infiltrates.

Pulmonary edema due to elevated pulmonary venous pressure

Also known as hydrostatic pulmonary edema, it is usually existent at presentation of

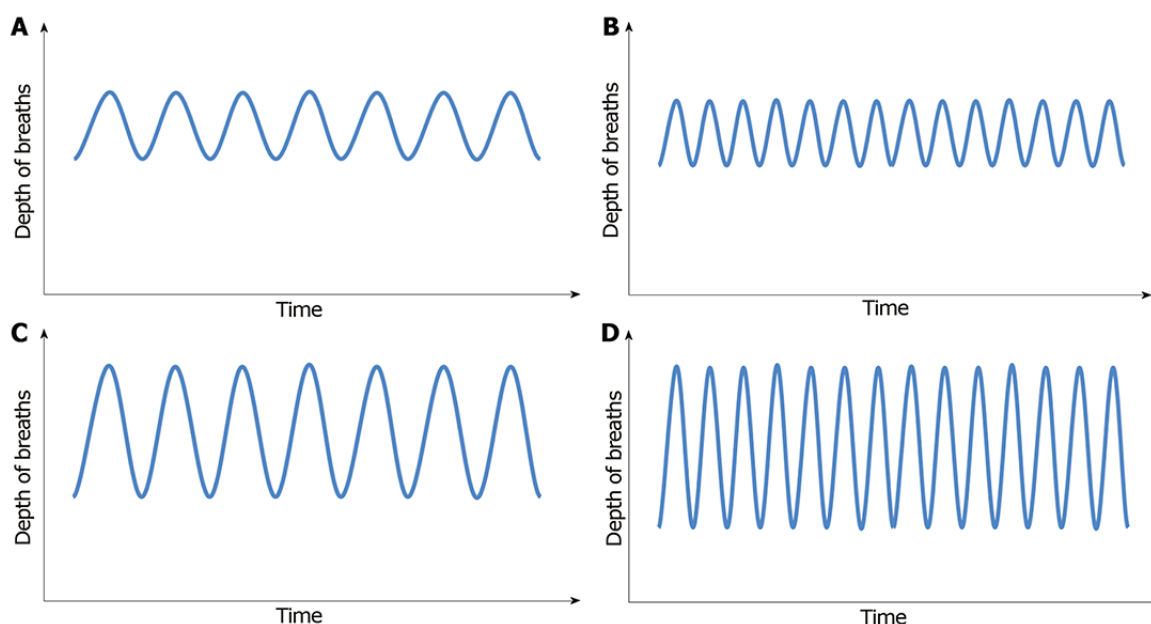


Figure 1 Depth of breaths. A: Normal (eupnea); B: Tachypnea - increased respiratory rate; C: Hyperpnea - normal rate, deep inspirations; D: Kussmaul's - tachypnea and hyperpnea.

DKA, is corrected during the treatment of DKA and is more common in patients with concomitant renal failure^[44-47]. The occurrence of circulatory overload and pulmonary edema with elevated pulmonary venous pressure is a result of the acute shift of an abundant volume of fluid into the extracellular compartment. This fluid shift happens as a consequence of solute accumulation in the extracellular compartment secondary to hyperglycemia^[44]. Therefore, correction of hyperglycemia shifts fluid back into cells, also correcting hydrostatic pulmonary edema. However, some patients might require hemodialysis and mechanical ventilation.

The degree of fluid shift and, consequently, the likelihood of developing hydrostatic pulmonary edema during a DKA episode are determined by the severity of hyperglycemia and by the volume status prior to the development of DKA^[47]. The amount of fluid transferred from the cells into the extracellular space is directly proportional to the changes in serum glucose concentration^[48]. The patients' volume status at the time of hyperglycemia onset is also a determinant of the volume that will shift into the extracellular space. Patients with baseline low extremity edema and/or anasarca have been shown to shift larger amounts of fluid and have a higher incidence of pulmonary edema, than those patients who are euvolemic when becoming hyperglycemic^[49].

Even though hydrostatic pulmonary edema has been described more commonly in patients with advanced renal disease, there are several cases reported in patients with DKA who developed pulmonary edema without having renal dysfunction. Several cases have been reported of takotsubo cardiomyopathy happening in the setting of DKA and causing pulmonary edema^[50,51]. There are also reports of myocardial dysfunction secondary to severe acidosis and electrolyte abnormalities^[52].

Pulmonary edema due to increased pulmonary capillary permeability

Also known as non-hydrostatic pulmonary edema, this type of pulmonary edema is caused by changes at the histological level of the alveolar epithelium. In diabetic patients, there is thickening of the alveolar epithelium and pulmonary capillary basal membrane, corroborating the presence of pulmonary microangiopathy^[53,54].

ARDS can develop during the course of DKA or during its treatment^[3], and it is more frequent and severe than hydrostatic pulmonary edema^[54,55]. The mechanism of ARDS in DKA is not completely understood. The most accepted explanation is activation of lymphocytes and release of cytokines, especially interleukin-1, which serum levels are much higher during treatment of DKA^[56-58].

The treatment of non-hydrostatic pulmonary edema in DKA is supportive. Focus should be on treating DKA and its exacerbating factor, early intubation and protective lung ventilation.

CONCLUSION

In DKA, respiratory failure is caused by several electrolytes, metabolic and cardiac and lung end-organ damage. Developing respiratory failure during DKA onset or treatment is associated with high mortality. Early recognition and treatment of the risk factors for the development of respiratory failure have the potential to decrease morbi-mortality of patients with DKA.

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Exploratory metabolomics of metabolic syndrome: A status report

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Abstract

Metabolic syndrome (MetS) is as a cluster of cardio-metabolic factors that greatly increase the risk of chronic diseases such as type II diabetes mellitus and atherosclerotic cardiovascular disease. In the United States, obesity, physical inactivity, aging, and genetics (to a minor extent) have arisen as risk factors for developing MetS. Although 35% of American adults suffer from MetS, its pathogenesis largely remains unknown. Worse, there is a lack of screening and optimum therapy for this disease. Researchers have consequently turned towards metabolomics to identify biomarkers to better understand MetS. The purpose of this review is to characterize various metabolites and their potential connections to MetS. Numerous studies have also characterized MetS as a disease of increased inflammation, and therefore this review also explores how metabolites play a role in various inflammatory pathways. Our review explores a broad range of metabolites including biogenic amines, branched chain amino acids, aromatic amines, phosphatidylcholines, as well as a variety of other molecules. We will explore their biochemical pathways and their potential role in serving as biomarkers.

Key words: Metabolic syndrome; Syndrome X, Metabolomics; Amino acids; Carnitine; Inflammation; Biomarkers; Diabetes

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Core tip: Metabolic syndrome (MetS) is a global epidemic that predisposes to type II diabetes mellitus, atherosclerotic cardiovascular disease and increased mortality. Whilst both insulin resistance and inflammation are advanced as pathogenic mechanisms, much work is needed to identify reliable biomarkers for this common cardio-metabolic disorder. In this mini-review, we provide a status report on the evolving field of metabolomics in MetS and it appears to offer some promising biomarkers such as

branched chain amino acids, lysine, carnitine, phosphatidylcholine (PC34:1) and PC34:2. However there is an urgent need to direct greater effort to the metabolome of MetS to unravel its pathophysiology and usher in much needed therapeutics.

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INTRODUCTION

Metabolic syndrome (MetS) describes a cluster of cardiometabolic risk factors that predisposes individuals to type II diabetes mellitus (T2DM) and atherosclerotic cardiovascular disease (ASCVD). MetS is defined by the Adult Treatment Panel (ATP) III criteria as having three of the five following features: increased triglycerides, reduced high-density lipoprotein (HDL)-cholesterol, plasma glucose of 100-125 mg/dL, increased waist circumference (WC), and hypertension. MetS affects approximately 35% of American adults and is increasing by drastic measures globally. Currently there is no optimal treatment for MetS, and consequently, there is a severe need to find new ways of approaching MetS in the hopes of finding better diagnostic and treatment modalities^[1]. Recently, studies assessing metabolomics have uncovered some insights into the pathology behind T2DM, CVD, and obesity^[2]. In previous studies, our lab demonstrated that MetS is a subclinical pro-inflammatory condition^[3]. We also have shown that this inflammation is present even in nascent MetS, which describes patients who meet diagnostic criteria for MetS without having confounding factors such as smoking, ASCVD, or T2DM. These findings suggest a causal role in MetS before the onset of serious sequelae. Various studies have suggested that metabolic diseases changes the levels of many amines, amino acids, and lipids^[4-6]. However, few studies have assessed the role of metabolites in MetS, and the biochemical alterations leading to metabolite changes are poorly understood. Therefore, in this review, we assess various metabolites and how they may be playing a role in the development, diagnosis, or management of MetS. We also evaluate if these metabolites may be related to inflammatory pathways, which could help elucidate their potential pathological role in MetS.

BIOGENIC AMINES: TMAO, CHOLINE, AND L-CARNITINE

Several recent studies have suggested that these biogenic amines have a role in the development of ASCVD and T2DM. It's been hypothesized that upon consumption of foods high in L-carnitine (LC) and choline, such as red meats, these amines are digested by gut microbes to produce trimethylamine. Ultimately this compound is converted to trimethylamine N-oxide (TMAO) in the liver^[7]. Some studies link TMAO with overall mortality in T2DM patients, predicting that higher circulating levels of TMAO are associated with a 2.1 to 2.7-fold increase in mortality, also seen after researchers adjusted for body mass index (BMI)^[8]. Others suggest that TMAO is linked with traits of obesity in mice receiving a high-fat diet, which suggests that the TMAO pathway is linked to obesity. For instance, Schugar *et al*^[9] illustrates a positive association between circulating levels of TMAO in mice fed a high fat and high sucrose diet, and body weight, fat mass, mesenteric adiposity, and subcutaneous adiposity. Moreover, a positive association between flavin-containing monooxygenase 3 gene, which encodes a TMAO-producing enzyme, and BMI and waist-to-hip ratio is established in these mice. Interestingly, this association in humans is not provided. Despite new insights into TMAO and its role in metabolic disease, the role of TMAO and its metabolites in the pathogenesis of the disease still remains elusive.

Choline

Choline is a quaternary ammonium compound commonly found in dairy and fish products. It is involved in the synthesis of phospholipids, lipoproteins, and neurotransmitters. Studies have found that choline consumption in healthy adults is related to inflammatory pathways, and subjects who consumed > 310 mg/d had 22%

lower C-reactive protein (CRP), 26% lower interleukin (IL)-6, and 6% lower tumor necrosis factor alpha (TNF α) levels^[10]. These results support a potential association between choline and the inflammatory process in healthy adults, but the exact role of choline in inflammatory pathways is unclear since some inflammatory markers were higher, while others were lower in this study. Other studies have shown that it has a role in CVD and is associated with key components of MetS including increased triglycerides, BMI, glucose, and WC. Furthermore, choline may also have some independent effects in metabolic disease. It's also been shown that betaine, formed by oxidized choline in the liver and kidney, is inversely associated with similar factors, suggesting a disruption of this pathway under conditions of mitochondrial dysfunction in MetS. This correlation between blood lipids and choline is in agreement with other studies showing that phosphatidylcholine (PC) supplementation in humans increases triglycerides without affecting cholesterol concentrations^[11]. Interestingly, studies show that when choline-deficient mice are fed a high-fat diet they have reduced glucose intolerance, whereas choline-replete mice fed the same diet show increased weight, triglycerides, hyperinsulinemia, and glucose intolerance^[12]. This study suggests that choline may have deleterious effects when coupled with fatty foods. Moreover, data from the Newfoundland CODING study illustrated a significant association between high human dietary consumption of choline and betaine and lowered insulin resistance. An inverse correlation was established between dietary choline and betaine intake and fasting glucose and insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and HOMA-B serum levels ($r = -0.08$ to -0.27 for choline, and $r = -0.06$ to -0.16 for betaine, $P < 0.05$). Conversely, increased choline and betaine dietary intakes positively correlated to quantitative insulin-sensitivity check index ($r = 0.16$ to 0.25 for choline, and $r = 0.11$ to 0.16 for betaine, $P < 0.01$). These associations were found in both genders after controlling for parameters such as age, physical activity, and daily caloric intake^[13]. Another study also demonstrated an association between high plasma concentrations of choline in human subjects and an adverse cardiometabolic risk-factor profile. More specifically, these high plasma choline concentrations were associated with low HDL-C levels, higher total homocysteine levels, higher BMI, and an greater odds of large-vessel cerebral vascular disease or history of cardiovascular disease^[14]. Though this provides further insight on the systemic effects of choline, further studies are needed to evaluate how choline is involved in metabolic disease, particularly MetS.

L-Carnitine

LC is also a quaternary ammonium compound found in meat products. The role of LC in MetS is largely understudied, but research following LC in other metabolic diseases may be predictive of its role in MetS. Interestingly, the deleterious role of LC in metabolic disease remains controversial. One study found that LC attenuates MetS in diet-induced obese rats by modulation of tissue fatty acids including inhibition of stearoyl-CoA desaturase-1 activity^[15]. Other studies suggest that LC supplementation at a dose of 1000 mg/d for 12 wk in humans with coronary artery disease resulted in reduced high sensitive CRP (hsCRP), IL-6, TNF α levels, and TNF α negatively correlated with LC levels ($r = -0.29$, $P = 0.02$) and antioxidant enzyme activities, superoxide dismutase ($r = -0.24$, -0.18 , and -0.19 ; $P = 0.03$, < 0.05 , and 0.05 for CRP, IL-6, and TNF α , respectively) and glutathione peroxidase ($r = -0.33$, -0.31 , and -0.19 ; $P < 0.01$, < 0.01 , and 0.06 for CRP, IL-6, and TNF α , respectively)^[16]. However, some have speculated that LC supplementation benefits may be dose dependent^[16,17]. While some studies report that LC supplementation reduced inflammatory factors, in the only paper published evaluating LC in a nascent form of MetS, we showed that LC had a 2.5-fold median increase ($P < 0.01$) and was positively correlated with soluble TNF receptor (sTNFR)-1 ($r = 0.51$, $P = 0.02$) and leptin ($r = 0.39$, $P = 0.02$), and inversely to the important anti-inflammatory adipokine, adiponectin ($r = -0.4$, $P = 0.02$)^[6].

Some studies also show LC may be involved in metabolic dysfunction. One study indicated that the carnitine palmitoyltransferase *1b166V* gene, coding for an enzyme involved in transferring long-chain fatty acids into the inner mitochondrial space, may have harmful effects in MetS such as increased fasting triglycerides, glucose, higher fatty liver index (FLI), and reduced insulin sensitivity^[18]. One of the few studies evaluating carnitine levels in humans showed that serum carnitine levels were increased in MetS patients with bipolar disorder and schizophrenia compared to the same subset of patients without MetS^[19]. Together these studies suggest that the role of LC in human metabolic disease may be potentially detrimental, possibly relating to inflammatory pathways. Because of the severe lack of data reporting LC in humans with MetS, future studies will be necessary to confirm the role of LC and its upstream and downstream products in MetS.

Recently we found that nascent MetS patients, without prior progression to CVD and T2DM, have higher levels of LC in urine samples. Since TMAO and choline were

not significantly increased in nascent MetS patients, our studies suggest that LC may play a larger role in MetS than previously believed^[6]. Furthermore, studies also show that lysine and methionine, two precursors of LC, are decreased in nascent MetS^[5], therefore increased LC may be driven by lysine and methionine depletion. Several studies show that the addition of LC in the diet of mice increased TMAO levels leading to worsened aortic lesions^[20], suggesting that LC may have a significant role in MetS and CVD. The precise role of LC in MetS remains largely unknown, and more research on this amine will be critical to evaluate if LC has a potential therapeutic or diagnostic role in MetS.

Trimethylamine N-oxide

Multiple studies report that TMAO and its precursors exacerbate glucose intolerance, inhibit hepatic insulin signaling, increase inflammation, and increase atheroma burden in mice and humans^[7,21]. It's also been shown that TMAO increases in MetS^[2]; however, these studies allowed for multiple confounding variables including smoking and diabetes. Furthermore, if these patients had renal impairment, this could have also skewed the results since TMAO increases as glomerular filtration rate decreases^[22]. Complicating the role of TMAO, some researchers have also found that TMAO levels are increased one year after patients undergo laparoscopic Roux-en-Y gastric bypass for morbid obesity, a therapy that reduces cardiovascular disease. Thus, the role of the TMAO and its metabolites remains unclear, especially in MetS^[23]. A recent study found that TMAO levels in adults stratified according to BMI had a positive association with adiposity and BMI, with highest TMAO levels in grade III obesity (BMI ≥ 40 kg/m²). Furthermore, FLI was tightly associated with TMAO levels. Specific cut-offs for circulating levels of TMAO to predict the presence of non-alcoholic fatty liver disease (NAFLD)-FLI and MetS were ≥ 8.02 μ M and ≥ 8.74 μ M, respectively. This finding suggests that TMAO may be an early biomarker of adipose dysfunction and NAFLD-FLI in circumstances where overt MetS is not present, but specific cut-offs may be needed to identify subjects at high risk for NAFLD-FLI^[24].

Studies have also explored the role of inflammation and these metabolites in nascent MetS. *In vivo* research has found that mice injected with TMAO showed an increase in inflammatory markers such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) and enhanced uptake of cholesterol in peritoneal macrophages, a critical step in atherosclerosis. The researchers proposed that TMAO promotes recruitment of active leukocytes to endothelial cells^[25]. Furthermore, numerous studies have suggested that inflammation is largely related to MetS. For example, in patient with MetS, there are increased levels of IL-1 β , IL-6, monocyte-NF κ B, and several macrophage immune receptors^[3]. One of the few studies assessing the role of TMAO and inflammation in MetS, found that TMAO significantly correlated with IL-6, endotoxin, and chemerin in patients with nascent MetS^[6], which further suggests that TMAO may have a role in ASCVD and metabolic disease *via* inflammatory mechanisms.

AMINO ACIDS

Alanine, Glutamate, and Glutamine

Alanine is a non-essential amino acid that can be synthesized by pyruvate and branched chain amino acids (BCAAs). In mammalian tissues and liver, alanine is vital in the glucose-alanine cycle. Amino acids are broken down to form glutamate by transamination. Through the actions of alanine aminotransferase (ALT), glutamate can then transfer its amino acid group to pyruvate, forming α -ketoglutarate and alanine, respectively. Alanine can then travel to the liver from the bloodstream^[26]. Numerous studies have shown that alterations to the alanine cycle, leading to increased levels of ALT may have implications in the development of T2DM and hyperglycemia. For instance, a study assessing if ALT is implicated in the development of MetS evaluated 1097 Caucasian men and women, and found that at follow-up, ALT was associated with fasting plasma glucose^[27]. Another study from Western Australian Health Department data linkage system found a strong association between ALT and MetS, independent of insulin resistance^[28]. There have been metabolomics studies that have found that alanine is linked to several traits associated with MetS including BMI, WC, triglycerides, hypertension, impaired glucose tolerance, and insulin resistance. The researchers proposed that glutamate likely stimulates glucagon release from pancreatic α cells and increases transamination of pyruvate to alanine, which strongly promotes gluconeogenesis in obesity^[26]. Furthermore, a study showed that alanine levels are increased in obesity and correlate with visceral adiposity in a Japanese population^[29]. Interestingly, BCAAs

seem to boost the conversion of pyruvate to alanine *via* short chain fatty acid production by gut microbiota^[30]. This may reflect an intricate role of various amino acids interconnected in metabolic dysfunction, and that liver metabolism likely plays a significant role in metabolic disease. Additionally, one study showed that serum ALT levels were significantly related to plasma CRP and lipid peroxides (LPO), regardless of the presence of underlying MetS, and that the presence of MetS and elevated ALT additively increased CRP and LPO. This study suggests that elevated serum ALT is a marker of active systemic inflammation and increased oxidative stress, independent of its relationship to MetS^[31].

Studies assessing metabolites and their relationship to metabolic risk factors found that an increased glutamate-glutamine ratio was associated with insulin resistance in individuals with metabolic risk factors and that glutamine-cycling pathways may have a prominent role in the development of metabolic risk. An increased glutamine-glutamate ratio was associated with lower risk of diabetes and the administration of glutamine in mice led to increased glucose tolerance and lower blood pressures. Additionally, glutamine-fed mice had the lowest plasma glucose levels compared to glutamate-fed and control-fed mice^[32]. An increased glutamine-glutamate ratio was also associated with decreased risk of future diabetes in a sample of 601 participants examined over 12 yr^[33]. Furthermore, a 2018 study of 563 Chinese adults identified a low glutamine-glutamate ratio as an independent risk factor for hyperglycemia^[34]. Studies have also identified glutamine as reducing pro-inflammatory cytokines, including IL-6, in human intestinal mucosa. Researchers also propose that glutamine could be helpful in modulating inflammatory conditions with imbalanced cytokine production^[35], which may prove valuable in treatment of MetS.

While the role of glutamine may be associated with metabolic wellness, glutamate may have an opposite effect. In a study of morbidly obese patients, those with pre-diabetes were found to have higher serum glutamate levels compared to non-diabetic controls. It was speculated that glutamate was elevated in morbidly obese patients due to an increased need for α -ketoglutarate in the tricarboxylic acid cycle (TCA) cycle to compensate for insulin resistance. This same study also found that morbidly obese non-pre-diabetic groups had increased levels of glutamate compared to non-obese and non-pre-diabetic groups, suggesting that obesity plays a role in glutamate metabolism^[36]. There have been other studies suggesting that glutamate levels are associated with insulin resistance^[37]. In a study of women with polycystic ovary syndrome, a disorder that shares features with MetS, investigators found that glutamate levels were down-regulated compared to controls (0.8-fold peak integral change in PCOS/controls). They proposed that glutamate is being used as an alternative energy source in patients with metabolic disorders, leading to its depletion^[38].

Additionally, new research suggests that glutamate levels identified by liquid chromatography/mass spectrometry in nascent MetS were significantly decreased compared to controls with median interquartile of 0.4 peak height ratio/creatinine peak height (range of 0.3-0.6) *vs* 2.3 (range of 1.1-3.6) respectively, and $P = 0.0001$. This study also found that gamma-aminobutyric acid (GABA) and D-pyroglutamic acid (PGA) were significantly increased in nascent MetS compared to controls with a 2.8-fold and 2.9-fold median increase and $P < 0.0001$ and $P = 0.004$ respectively. This study also identified a novel metabolite of gut microbiota tryptophan interaction, N-acetyl-D-tryptophan (NAT), was decreased by 90% in nascent MetS patients compared to controls ($P < 0.001$). The authors propose that this decrease in glutamate in nascent MetS could be due to its conversion to both GABA and PGA, which are both increased in this patient population. Researchers also found that GABA correlated significantly with WC, systolic blood pressure (SBP), chemerin, leptin, fetuin A, and endotoxin. PGA correlated positively with IL-6, leptin, fetuin A, and nitrotyrosine. NAT was inversely correlated with WC, SBP, BMI, triglycerides, hsCRP, Toll-like receptor 4 (TLR-4), IL-6 blood glucose, chemerin, and retinol binding protein 4. While GABA and PGA positively correlated with biomediators of inflammation and cardiometabolic risk factors of MetS, the NAT was inversely correlated. This study suggests that GABA and PGA may be contributing to the pro-inflammatory state on MetS while NAT could mitigate the inflammatory response^[39]. This new finding could explain decreased glutamate levels in nascent metabolic disease and suggests possible therapeutic or diagnostic opportunity for early stages of MetS. Still, alanine and glutamine pathways offer a complex prospective in metabolic disease. Research in MetS is limited, and studies need to identify if this pathway can be targeted for diagnostic and treatment purposes.

Aspartate and asparagine

Asparagine is required for the development and function of the human brain. It is also known to play a critical role in the synthesis of ammonia. In the human body,

oxaloacetate is converted to aspartate using transaminases. An amino group is transferred from glutamate to oxaloacetate making α -ketoglutarate and aspartate. Aspartate can accept an amine group from glutamine to form asparagine. Asparagine and aspartate are associated with numerous medical conditions, but they may also have a role in metabolic dysfunction. While some studies have shown that it is elevated in obesity^[29], another study found that asparagine, but not aspartate, is inversely related to numerous metabolic traits including BMI, WC, insulin, HOMA-IR, triglycerides, systolic and diastolic blood pressure (DBP), while directly relating to HDL. Interestingly, in this same study, aspartate but not asparagine, was inversely related to glucose in human subjects^[32]. This suggests that aspartate and asparagine levels may both be involved in metabolic disease; however, their exact roles may not necessarily overlap. Moreover, a study evaluating amino acids in a male Mediterranean population with MetS found asparagine to be inversely associated with MetS^[2]. Therefore, the current evidence suggests that asparagine and aspartate may have protective roles in MetS or may be depleted as a consequence of disease progression.

Arginine

Arginine is another amino acid that may play a role in metabolic disease. It is most well-known for being the precursor for biosynthesis of nitric oxide and therefore aiding vasodilation, but it also has a role in cell division, wound healing, excretion of ammonia, immune function, and hormone release. The research related to arginine and metabolic disease is limited, though several studies report that it is dramatically increased in obese versus lean individuals^[30]. However, there have been other studies suggesting it has a protective role since consumption of low sugar and protein biscuits that were enriched with L-arginine enhanced endothelial function, improved metabolism, insulin sensitivity, and insulin secretion in MetS subjects^[40]. Supplementing 4.5 g per day of arginine for four weeks in overweight adults has also been shown to decrease postprandial vasospasm when baseline arginine levels were low^[41]. To complicate the role of arginine further, another study in diabetic rats found that supplementation of L-arginine did not improve insulin resistance, but did improve lipid metabolism where plasma triglyceride levels decreased after oral lipid administration ($P < 0.05$)^[42]. Additionally a study assessing metabolite profiling to identify metabolic risks in humans found that arginine significantly correlated with triglyceride levels^[32], which further suggests that arginine may play a role in dyslipidemia related to metabolic disease. Research has also shown that in a renal mass reduction (RMR) model of chronic renal failure, a 12-wk treatment of 1.25 g/L of L-arginine in the drinking water of rats improves kidney function by significantly reduced serum creatinine (2.3 to 1.3 mg/dL), serum urea (128.3 to 72.2 mg/dL), urine protein (104.8 to 49.2 ml/24 hr), as well as increased creatinine clearance (0.77 to 1.8 mL/min) ($P < 0.05$ for all factors). After 12 wk of L-arginine treatment in RMR mice, there was improved the systolic blood pressure (from 207.0 to 169.1 mm Hg, $P < 0.05$) and decreased pro-inflammatory cytokines including IL-1 α (69.4 to 47.9 pg/mL), IL-1 β (86.7 to 51.5 pg/mL), IL-6 (89.3 to 45.8 pg/mL), and TNF α (26.4 to 18.0 pg/mL) ($P < 0.05$ for all cytokines)^[43]. Therefore, arginine may play a role in reducing pro inflammatory cytokines and kidney function, both of which may have implications in the development of MetS. Accordingly, there is a potential role of supplemental arginine for the purpose of reducing inflammation. Human studies have identified that L-arginine treatment can ameliorate endothelial dysfunction, inflammation, oxidative stress, adipokine release, and insulin sensitivity in T2DM and coronary artery disease patients^[44,45]. Interestingly, a study assessing the relationship between plasma asymmetrical dimethyl L arginine (ADMA) and inflammation found that ADMA was directly correlated with inflammation and soluble adhesion markers in pre-diabetic subjects^[46]. Ganz *et al*^[47] also recently measured serum levels of arginine and ADMA in 105 persons with T2DM compared to controls and found that arginine was decreased in diabetics (64 ± 28 vs 75 ± 31 μ mol/L) while ADMA was unchanged. Additionally, low arginine and high ADMA were associated with diabetic microvascular complications. There was no significant difference in BMI between the non-diabetic and T2DM groups (30.5 vs 30.6), suggesting that while arginine is increased in obesity, it has a tendency to decrease in an insulin resistant state. These studies suggest a complex interaction between arginine, ADMA and inflammation including mechanisms involved in endothelial dysfunction in a pre-diabetic and diabetic state and further research is needed to determine how arginine is related to metabolic disease, and to evaluate the circumstance under which its effects are beneficial or harmful.

Histidine

Histidine is a semi-essential amino acid with anti-inflammatory functions that it is

decreased in T2DM^[48], liver injury^[49], CVD^[50], and chronic kidney disease^[51]. Studies have shown that histidine levels are higher in obese patients after bariatric surgery including sleeve gastrectomy, proximal Roux-en Y gastric bypass, and distal Roux-en Y gastric bypass^[52]. Another randomized control study in obese women found that histidine supplementation of 4 g/d for 12 wk decreased inflammatory cytokines TNF- α (-28.3%), IL-6 (-29.3%) and improved oxidative stress by measurement of antioxidants superoxide dismutase (16.1%) and glutathione peroxidase (9.0%) in obese women with MetS. Histidine supplementation in this group also significantly decreased the HOMA-IR, BMI, WC, fat mass and non-esterified fatty acids by 18.9%, 2.9%, 3.7%, 6.0% and 18.1% after histidine supplementation, respectively^[53]. Recent studies assessing the role of histidine on metabolic changes found that histidine supplementation may alter serum and urine metabolomic and amino acid profiles of obese women. Histidine supplementation of 4 g/d for 12 wk significantly decreased lipids and glucose, thus supporting a practical application of histidine in preventing and treating chronic metabolic diseases, such as MetS. Interestingly, this same study found that histidine supplementation resulted in increased choline, betaine, and TMAO levels, suggesting that these amines may have an interconnected role in metabolic dysfunction^[48]. In studies profiling metabolites and how they are associated with metabolic risk factors, histidine was only associated with triglycerides and DBP, but not glucose, BMI, or insulin, which are key features of MetS^[32]. This suggests that histidine may play a more complicated role in MetS and may be indirectly associated with the disease pathology.

Methionine/cysteine

Methionine is an essential sulfur-containing amino acid that contributes to both anabolic metabolism and the reduction of free radicals. One study recently observed that methionine levels were elevated in diabetic obese rats with leptin missense mutations^[54], which is in line with the model of metabolic dysregulation proposed by Adams in an insulin resistant and obese state. In this model, he proposed that reduction in branched-chain α -keto acid dehydrogenase (BCKD) activity affected metabolism of α -ketobutyrate into propionyl-CoA. Since α -ketobutyrate is a downstream product of methionine, it was theorized that buildup of α -ketobutyrate led to upstream buildup of methionine. This excess of methionine is also thought to increase cysteine and cystine, consequentially leading to a buildup of tyrosine^[55]. Interestingly, Reddy *et al*^[5] observed that methionine is decreased in nascent MetS despite increases in tyrosine and isoleucine, which are also BCKD substrates. This suggests a fundamental difference in pathway directionality between nascent MetS patients and those with fulminant obesity and diabetes. Reddy *et al*^[5] also observed that methionine inversely correlated with LC, which is formed by trimethylation of lysine *via* S-adenosylmethionine. Furthermore, LC was increased in nascent MetS^[6], while medium chain acylcarnitine levels were not significantly increased in the same patient population^[4]. These findings suggest that LC may be increased as a result of depleting methionine without expected changes in acylcarnitines, and, as a result, this may indicate a dysregulation of fatty acid metabolism in nascent MetS. Additionally, adiponectin, a regulator of fatty acid oxidation was also decreased in this population. This is also supported by Bene *et al*^[56] who observed that a group of 38 MetS patients had elevated total carnitine, comparable free carnitine, and increased C3 and C4 acylcarnitine levels compared to controls, while medium and long chain levels were reduced.

Another possible explanation for the decrease in methionine is the proinflammatory state associated with nascent MetS. Accumulation of visceral fat leads to increased inflammatory cytokines and subsequent generation of intracellular reactive oxygen species^[57]. This oxidative stress leads to increased need for the reducing agent glutathione, which is formed by glutamate and cysteine. Since cysteine is a formed from methionine, increased oxidative stress could upregulate this pathway. However, Reddy *et al*^[5] observed no correlations between methionine and the following markers of oxidative stress: Oxidized low-density lipoprotein (oxLDL), monocyte superoxide, and nitrotyrosine. Given their exclusion of patients with liver disease, this may indicate that anti-oxidative pathways become more prevalent in more established MetS populations, especially those with NAFLD/Non-alcoholic steatohepatitis. Mohorko *et al*^[58] found that cysteine was significantly higher in a group with a single selection criterion for MetS compared to controls and even further increased with two components, but without any increases in methionine or homocysteine. Interestingly, cysteine did not correlate with CRP or TNF- α . In another study with 984 insulin resistant Hispanic children, researchers found no association between cysteine and IL-6, MCP-1, and CRP^[59]. A possible explanation for the increases in cysteine could be due to increased dietary intake of methionine causing upregulation of its transsulfuration pathway, rather than a response to oxidative

stress^[60]. Current evidence suggests that while methionine and cysteine are indeed dysregulated in obesity, T2DM, and nascent MetS, the mechanisms of dysregulation may differ significantly with regards to both inflammatory and metabolic profiles.

Lysine

Lysine is an essential amino acid with basic properties that is synthesized *via* the diaminopimelate and α -aminoadipate pathways. In nascent MetS, Reddy *et al*^[5] also observed a substantial decrease (92%) in the basic amino acid lysine, which inversely correlated with LC, like methionine. While HOMA-IR was elevated in their MetS population, this suggests insulin-induced BCKD inhibition leads to rerouting of lysine and methionine to fatty acid oxidation instead of a buildup in nascent MetS. Iida *et al*^[61] similarly observed increased levels of α -aminoadipate, a product of lysine degradation, suggesting some catabolic process in MetS. Reddy *et al*^[5] also observed that lysine inversely correlated with numerous markers of inflammation including endotoxin, TLR-4, and IL-6. Moreover, acetylation of lysine is seen in states of insulin resistance and is also thought to play a role in immunomodulation^[61,62]. This inverse correlation may indicate an attempt to blunt the inflammatory response, leading to a depletion in lysine. Furthermore, diets rich in grain legumes, which are abundant in lysine, are protective against T2DM and salient features of MetS including CVD and increased LDL^[63], thus further suggesting that lysine may have potential protective effects, especially in metabolic diseases. Reddy *et al*^[5] also support this finding as lysine inversely correlated with WC, SBP, DBP, glucose, while positively correlating with HDL-cholesterol. This data offers promising research for dietary lysine supplementation in mitigating features of MetS.

BRANCHED CHAIN AMINO ACIDS

The branch-chain amino acids include isoleucine, leucine, and valine, all of which are metabolized by BCKD. A 2018 meta-analysis of four cohorts of patients with T2DM showed that all three BCAAs are elevated by approximately 40% in the setting of poor glycemic control^[64]. Similarly, Reddy's study saw increases in isoleucine levels in a nascent MetS population compared to controls^[5]. These findings are all consistent with insulin-induced impairment of BCKD activity^[55]. However, in a study of rats being fed BCAA, the connection between insulin resistance and isoleucine was only observed in the presence of a high fat diet^[37]. It was proposed that BCAA buildup was secondary to increased fatty acid oxidation, which increases the NADH/NAD⁺ ratio. This leads to impairment of BCKD activity, glycolysis, and the TCA cycle. Consistent with this proposition, isoleucine more strongly correlated with markers of adiposity such as leptin, WC, and BMI than HOMA-IR ($P = 0.09$) in Reddy's study. Isoleucine also inversely correlated with HDL-cholesterol and directly correlated with systolic and DBP^[5]. Therefore, isoleucine holds some promise as an early predictor of MetS because it correlates with every risk factor included in the ATP III criteria. Isoleucine has further use as a marker of underlying inflammation, as it positively correlates with IL-6, endotoxin, and oxLDL^[65]. Though increased isoleucine seems to be a long-downstream byproduct of insulin and fatty acid oxidation induced metabolic dysregulation, it provides valuable information related to many aspects of the inflammatory and metabolic profile.

Leucine may also provide comparable utility as an inflammatory and metabolic marker, correlating with TNF- α and HOMA-IR, and negatively associating with adiponectin and HDL-cholesterol^[48]. TNF- α is thought to further increase serum BCAA levels by inhibiting its uptake in adipose tissue^[66,67]. Valine is perhaps the least well-studied BCAA in the setting of MetS, but is increased in the setting of insulin resistance and adiposity. This was observed in Fiehn's study of obese diabetic African American women and is suggestive of findings in a MetS population^[68]. Increases in these BCAAs have similarly been observed in other MetS patient groups. In a population of middle-aged Mediterranean males with MetS, Ntzouvani *et al*^[2] observed that isoleucine, valine, and leucine were all significantly increased even after correction for T2DM and liver function. A 2018 study of 563 Chinese adults again showed increased BCAAs in the setting of hyperglycemia and correlations with elevated serum LDL, triglycerides, and decreased HDL^[34]. Similarly, other studies have also shown that increases in BCAA correlate significantly with MetS in Chinese, African American and Caucasian population^[33,69].

C5 acylcarnitine is formed from breakdown of isoleucine and leucine prior to interaction with BCKD, while C3 acylcarnitine is formed from valine and isoleucine after metabolism by BCKD^[70]. Therefore, C5 and C3 acylcarnitine levels may provide additional information about the activity of BCKD in MetS. If BCKD is indeed

impaired in MetS, one would expect C5 serum levels to be elevated due to pathway rerouting and possible reduction in C3 levels from upstream inhibition. However, a study recently compared acylcarnitine levels in four groups divided by obesity and metabolic wellness, and the group that most closely aligned with the ATP III criteria showed an increased ratio of C3 and C5 to total acylcarnitines as well as increased levels of C3 carnitine. This indicates that acylcarnitine formation occurs both upstream and downstream of BCKD^[71]. Bene *et al*^[56] observed similar increases in serum C3 and C5 acylcarnitine^[56]. While the findings for C5 acylcarnitine are consistent with BCKD inhibition, C3 acylcarnitine levels are not. C3 acylcarnitine is additionally formed from non BCAAs, including odd-chain fatty acids and threonine, providing us with little reductive information in this regard. Though the BCAAs are universally increased in MetS and evidence suggests ties to both inflammation and fatty acid oxidation, the other pathways leading to C3 acylcarnitine formation must also be explored in order to better assess BCKD activity.

AROMATIC AMINES

Phenylalanine

Phenylalanine is an essential amino acid that has been implicated in the onset of insulin resistance and T2DM. Studies have reported increased serum concentrations of phenylalanine in obese, insulin-resistant, and diabetic subjects^[55]. More specifically, Wang *et al*^[33] showed that phenylalanine significantly correlated with fasting insulin, HOMA-IR, HOMA-B, and oral glucose tolerance test levels. In addition to BCAAs, aromatic amino acids such as phenylalanine have been shown to be predictors of insulin resistance at 6 year follow-up in normoglycemic young adults. This predictive value is especially pronounced in men. It is theorized that altered aromatic amino acid metabolism precedes insulin resistance in early adulthood before the onset of impaired fasting glucose^[72]. Wijekoon *et al*^[73] illustrated that phenylalanine levels were 55% higher in young insulin-resistant rats compared to non-obese rats. Despite phenylalanine's significant role in promoting insulin resistance, little is known of how this mechanism occurs. Future studies of the pathogenesis of phenylalanine dysregulation with regards to insulin resistance and its clinical utility of predicting diabetes should be conducted.

Tyrosine

Tyrosine is an aromatic amino formed from the essential amino acid phenylalanine *via* the enzyme phenylalanine hydroxylase. As discussed earlier, some researchers propose that BCKD inhibition leads to buildup of methionine, which is then shunted to cysteine/cystine formation when confronted by states of oxidative stress. Cystine then inhibits tyrosine aminotransferase, leading to a buildup of tyrosine and its precursor, phenylalanine^[55]. Additionally, the Framingham Heart Study found that tyrosine was associated with future risk for diabetes^[33]. Reddy *et al*^[5] and Mohorko *et al*^[58] both saw increases in tyrosine in MetS populations without T2DM and CVD. However, Mohorko *et al*^[58] saw associations between tyrosine and TNF- α , CRP, HOMA-IR and adiponectin, while Reddy *et al*^[5] observed no associations between tyrosine and any of the salient features of MetS. Reddy *et al*^[5] proposed that tyrosine may be a bystander in the disease process, but since this study did not record study participant's diet, definitive conclusions are difficult to ascertain. Mohorko's patients had significantly increased protein intake with increasing features of MetS, suggesting that serum tyrosine levels may be partially explained by diet rather than altered metabolism^[5,58].

Tryptophan

Tryptophan is an aromatic essential amino acid that must be obtained from dietary sources. Chen *et al*^[74] conducted a metabolic profiling study that found circulating tryptophan levels increased in obese subjects compared to healthy lean subjects. These tryptophan levels were lowered after appropriate dietary modifications. Moreover, he found that tryptophan serum levels were independently and positively associated with T2DM risk. In contrast to phenylalanine and tyrosine, more is known about the biochemical pathway of how tryptophan mediates insulin resistance. Significantly increased activity of the rate limiting enzyme 2,3-dioxygenase was seen in patients with T2DM. Downstream metabolites such as kynurenine and xanthurenic acid were subsequently elevated in patients with T2DM^[75]. These metabolites have been shown to play an important role in regulating insulin resistance, pancreatic beta-cell function, and glucose homeostasis. For instance, xanthurenic acid is associated with higher insulin resistance and higher odds of diabetes^[76]. Additionally, metabolism of

kynurenine is intimately linked to inflammation and immune response. Higher levels of kynurenine metabolites are found in peripheral tissue for inflammatory disorders such as cancer and T2DM^[77]. In contrast, some researchers have also failed to establish a link between T2DM and tryptophan levels^[55]. Because of its relatively elucidated biochemical pathway and the ability of patients to control intake through diet, tryptophan poses as a potentially powerful clinical marker that could be used to detect and lower risk for T2DM^[75].

Phospholipids

Phosphatidylcholines (PC) are major phospholipid components of plasma lipoprotein classes and the only phospholipids known to be required for lipoprotein assembly and secretion. Moreover, PC play a critical role in regulating the quantities of circulating lipoproteins such as very low-density lipoproteins (VLDLs) and HDLs. Studies have shown that increased levels of these PCs in the blood serum of subjects correlated positively with obesity and insulin resistance^[65]. Weinberg^[78] illustrated significant associations between WC and PC concentrations and a positive association between lysophosphatidylcholine [LPC (14:0)] and diacylphosphatidylcholine [DPC (32:3)]. Another study identifying global lipidomics characterized LPC, PC (32:1), PC (34:2), and PC (34:6) as having significant odds ratios for progression to T2DM^[79]. The ADVANCE study also identified PCs, such as PC (34:1), that were associated with future cardiovascular incidents in male T2DM patients^[80]. We further investigated the role of PCs in patients with nascent MetS and found that PC34:2 correlated with various features of MetS, including fasting glucose, triglycerides, and WC. This biomarker also correlated with pro-inflammatory markers including IL-1 β , IL-8, and hsCRP and identified with features of adipose tissue dysfunction through its positive correlation with leptin and inverse correlation with adiponectin. In contrast to the ADVANCE study, our study did not find significant increases of PC34:1 in patients with MetS^[4]. Given their correlation with inflammatory biomarkers, adipose dysregulation, and progression to chronic disease such as T2DM, PCs should be characterized and explored further.

DISCUSSION AND CONCLUSION

Despite the high incidence of MetS and connection to a variety of chronic diseases, there remains limited knowledge about its pathogenesis, treatment, and prevention. Numerous studies have characterized MetS as a pro-inflammatory disease. Accordingly, changes to a variety of metabolite levels have been observed. Analysis of these particular metabolites may help to better characterize MetS and its pathogenesis.

Biogenic amines such as choline, LC, and TMAO are found in red meats. Increased quantities of these amines have been found to induce inflammatory pathways and increase the risk of metabolic diseases. For instance, increased dietary consumption of choline was found to be associated with an adverse cardiometabolic profile and insulin resistance. Additionally, TMAO was observed to be associated with a variety of inflammatory markers such as IL-6, endotoxin, and chemerin in nascent MetS. Other amino acids have been shown to be both protective and risk factors for MetS. For instance, BCAA and alanine have been linked to insulin resistance, while histidine and lysine were observed to decrease inflammation and oxidative stress. Branched chain and aromatic amino acids have also been associated with the pathogenesis of insulin resistance and serve as promising biomarkers for predicting the onset of insulin resistance in normo-glycemic patients. PCs have also emerged as biomarkers that correlated with features of MetS, as well as adipose tissue dysfunction and inflammation.

Although the pathogenesis of MetS remains elusive, metabolomics research offers a promising bridge to understanding the disease from a different perspective. Characterization of many biomarkers gives different avenues through which further research can be conducted. The role of systemic metabolomics for prediction of diseases such as MetS is expanding. For instance, Pujos-Guillot *et al.*^[81] utilized a combination of untargeted metabolomics and parameters which included clinical, socioeconomic, and dietary subject characteristics to reveal phenotypic changes five years before the onset of MetS. Significant differences between 50 metabolites were found in subjects who would later develop MetS versus control subjects. This integrative approach of systemic metabolomics to characterize MetS on the sub-phenotypic level represents the types of future studies that can be potentially performed in the future in the field of metabolomics^[81]. However, the role of many biomarkers, especially tyrosine and phenylalanine, in the pathogenesis of MetS needs

to be further clarified. Further research should also be conducted in fields such as lipidomics so that a wider array of biomarkers, such as PC34:2, can be identified. Despite ongoing advances in the field of metabolomics, our review of metabolomics in MetS identifies a critical gap in the current understanding of how metabolites relate to the specific pathogenesis of metabolic disease. More importantly, continued implementation of these biomarkers as predictive or therapeutic tools for MetS should be aggressively pursued.

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Case Control Study

Diabetes in the Kokan region of India

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Abstract

BACKGROUND

BKL Walawalkar Hospital is situated near the village of Dervan in the Kokan region of the state of Maharashtra in India. A survey of 2200 surrounding villages showed 51.8% adults had body mass index (BMI) below $<18.5 \text{ kg/m}^2$ and only 4.5% were overweight. A survey of 11521 adolescent girls from rural schools showed 64% prevalence of thinness. In the same region, government survey reported the prevalence of diabetes around 7%, and 70% prevalence of leanness. This reinforced the fact that the overall population of Kokan is lean. Hence, we decided to investigate body composition of diabetic people from our hospital clinic by carrying out a clinic-based case control study.

AIM

To study body composition of diabetics in a rural clinic of Kokan.

METHODS

In a case-control study, 168 type 2 diabetic patients (102 men) attending the outpatient department at a rural hospital and 144 non-diabetic controls (68 men) in the Chiplun area of the Kokan region were recruited. History of diabetes (age of onset, duration), anthropometric measurements (height, weight, waist and hip circumference) were recorded. Body composition was measured by bioimpedance using the TANITA analyzer.

RESULTS

More than 45% of diabetic subjects had a 1st degree family history of diabetes, and more than 50% had macrovascular complications. The average BMI in diabetic subjects was 24.3 kg/m^2 . According to World Health Organization standards, prevalence of underweight was 8% and that of normal BMI was around 50%. Underweight and normal diabetic subjects (men as well as women) had

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significantly lower body fat percentage, higher muscle mass percentage, lower visceral fat and lower basal metabolic rate when compared to their overweight counterparts.

CONCLUSION

The diabetic population in Kokan has near normal body composition, and BMI has considerable limitations in assessing body composition and it also lacks sensitivity for assessing risk for diabetes in this population. High prevalence of family history of diabetes may point towards genetic predisposition. Leanness is an inherent characteristic of this population and its metabolic significance needs further investigations with a larger sample size.

Key words: Body composition; Diabetes; Metabolism; Malnutrition; Kokan

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Core tip: As per government survey, leanness is widespread in Kokan but diabetes is also on the rise. We studied lean body mass in diabetics in our clinic. Lean individuals had lower body mass index but better percent muscle mass compared to overweight. This could be metabolic response to less caloric intake despite heavy physical activity. This mechanism needs to be clarified. The diabetic population in Kokan has near normal body composition and body mass index has considerable limitations. Therefore, the physiological process producing these deviations in body composition and its metabolic significance need further investigations on larger scale.

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INTRODUCTION

Recent years have seen a considerable increase in the burden of chronic non-communicable diseases (diabetes, hypertension and coronary heart disease) in clinical practice in urban India. Recent studies in urban populations have shown an unexpectedly high prevalence of diabetes, and the prevalence is rising rapidly^[1]. Type 2 diabetes mellitus (T2DM), previously called non-insulin dependent diabetes mellitus (or NIDDM) is a familial disease, on the verge of becoming a pandemic in developing countries like India^[2]. T2DM is the most prevalent form of diabetes seen in India and constitutes more than 95% of the diabetic population^[3]. Migrant Indians in Canada, Britain, the Netherlands, United States and Singapore also have a higher prevalence of diabetes compared to the native populations^[4-8].

In contrast, the prevalence of diabetes and coronary heart disease in rural India has been shown to be low. However, there are very few prevalence studies in rural India^[9-11] due to remoteness of the villages and lack of health infrastructure for epidemiological studies. Rapid socio-economic developments over the last 2 decades have made the rural population particularly vulnerable to non-communicable diseases. Therefore, there is a need to obtain reliable data on non-communicable diseases in rural India, study the risk factors, and plan effective preventive measures.

In Europe and America, the majority of patients with T2DM are obese. In 1965, Tripathy and Kar^[12] highlighted leanness among elderly diabetics in India. Other studies in India have reported a prevalence of low body weight/lean T2DM ranging from 1.6% to 26%^[13,14]. A review by Dulloo *et al.*^[15] highlighted limitations of body mass index (BMI) in assessment of body composition and lack of sensitivity for assessing disease risk, particularly in those who have normal or slightly elevated body weight.

BKL Walawalkar Hospital, situated near the village of Dervan in Ratnagiri district of the state of Maharashtra, has actively promoted rural health care through a variety of education and holistic outreach programs for more than 22 years. The hospital serves the poor socio-economic class populations of the adjoining villages. In 2003-2010 our hospital carried out house-to-house surveys of 2200 villages in the area. In this survey, 51.8% of the subject had BMI < 18.5 kg/m² and only 4.5% were

overweight, with BMI > 25 kg/m². A survey of 11521 adolescent girls from rural schools conducted between 2011 and 2017 showed that 64% of the girls were in grade 1 to 3 of thinness, based upon International Obesity Task Force standards. Stunting was seen in 22% to 28% of the girls. Thus, the overall population of Kozkan is lean in their body stature.

The National Family Health Survey^[9] conducted by the government of India reported on the prevalence of diabetes in the same region based on random blood sugar as 9% among men and 5.8% among women. The same survey also reported more than 70% prevalence of leanness based on BMI among men as well as women. This again reinforced the leanness of the population of Kozkan. Hence, we decided to investigate more about body composition of diabetic people from our hospital clinic by carrying out a clinic-based case control study. The health infrastructure in our hospital provided us the opportunity to study the profile of diabetic as well as non-diabetic subjects.

MATERIALS AND METHODS

This study was carried out in the outpatient department of BKL Walawalkar Hospital and Rural Medical College in the Ratnagiri district.

Diabetic patients from the outpatient department were enrolled in the study. Non-diabetic control subjects were mostly spouses of the patients in the hospital or were from the hospital staff.

The following data was recorded from the clinical history for diabetic subjects: age at diagnosis of diabetes; family history of diabetes; and history of macrovascular complications (hypertension, ischemic heart disease, cardiovascular disease and cerebral vascular disease) and microvascular complications (nephropathy, neuropathy and retinopathy).

The following anthropometric parameters were measured on diabetic as well as non-diabetic subjects: height; weight; waist and hip circumferences. BMI and waist-to-hip ratio (commonly known as WHR) were calculated. Subjects were classified as underweight, normal and overweight using World Health Organization standards for BMI^[16]. Those with BMI < 25 kg/m², that is those who were underweight or normal, were classified as lean. We used International Diabetes Federation criteria^[17] for the waist circumference to classify the subjects as centrally obese.

Body composition was assessed on all the subjects using the Tanita BC 420-MA analyzer (Tanita Corporation, Tokyo, Japan). It measured bioelectrical impedance by passing alternating current through the subject to measure the water content. Body composition measurements (fat mass, lean mass, total body water) were obtained as mass as well as percentage. In addition, we also obtained visceral fat, fat free mass, total body water (TBW) as mass as well as percentage, and basal metabolic rate (BMR).

In total, 201 diabetic subjects reported to the outpatient department of medicine. Those with diabetes with pregnancy, severe chronic illness, pancreatic disease and type-1 diabetes were excluded. After these, 168 diabetic subjects (102 men) were left to form the sample of diabetic subjects. We recruited 144 non-diabetic control subjects (68 men).

Statistical methods

Data is presented as mean ± SD for continuous and as percent for categorical variables. Analysis of variance and χ^2 test was used to compare continuous and categorical variables for differences in groups. Comparison of anthropometric parameters between diabetic and non-diabetic subjects was adjusted for current age. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, United States).

Ethics

Informed and written consent was obtained from all the subjects for use of their data. Ethical approval from the institute's ethics committee was also obtained for the data analysis.

Our institute's ethics committee is registered with the government of India. Earlier, its registration number was EC/755/INST/MH/2015. The registration expired in August 2018 and was subsequently renewed, with the new registration number as EC/755/INST/MH/2015/RR-18.

The study was conducted from January 2018 to June 2018.

RESULTS

Subject characteristics

Table 1 shows clinical characteristics (age, anthropometry and body composition measurements) of subjects. Non-diabetic subjects (men as well as women) were significantly younger ($P < 0.001$). In diabetic subjects the mean age of diagnosis of diabetes was similar for men and women. Height was similar in both sexes, when comparing diabetic and non-diabetic. Diabetic women were heavier and had higher BMI. Diabetic women also had higher hip circumference. Both diabetic men and women had significantly higher WHR. Body fat percentage as well as mass was similar in diabetic and non-diabetic men but it was significantly higher in diabetic women. In both sexes the muscle mass percentage was similar between diabetics and non-diabetics but diabetic men had higher muscle mass. Diabetic women had higher visceral fat. Diabetic and non-diabetic subjects (men as well as women) had similar TBW and TBW% but BMR was higher in diabetic women compared to non-diabetic women.

Table 2 shows the categorical data. In diabetic subjects the proportion of those with 1st and 2nd degree family history of diabetes was similar in both sexes. No control subject had family history of diabetes. A substantial number (around 70%) of diabetic subjects reported macrovascular complications. Diabetics (men as well as women) had higher proportion of those overweight and centrally obese compared to their non-diabetic counterparts.

Body composition and BMI

In diabetic subjects of both sexes, those lean had significantly lower body fat (percentage as well as mass), lower muscle mass but high muscle mass percentage, lower visceral fat, lower fat free mass, higher total TBW%, but lower TBW and BMR compared to their overweight counterparts. Similar differences were observed in non-diabetic subjects between those lean and overweight of both sexes, except for TBW% which was similar in lean and overweight non-diabetic women (Tables 3 and 4).

DISCUSSION

There are many reports on the profile of diabetic subjects in rural regions of India^[18-28] but this is the first report from the Ratnagiri district of the Kozkan area. Although from a small sample size, our data demonstrated that more than 50% of the men as well as the women subjects with diabetes were lean with a BMI well below the normal BMI cutoff of 25 kg/m². Our study is clinic-based and not community-based. The National Family Health Survey^[9] conducted by the government of India determined the prevalence of diabetes in the same region based on random blood sugar, being 9% among men and 5.8% among women. The same survey also found more than 70% prevalence of leanness based on BMI among men as well as women. This could be because the population in this region is constitutionally small.

Body composition depends on genetic makeup, dietary habits, physical activities, and susceptibility to chronic illness. Lean diabetes has been described in many populations across the world^[29,30], and there have been extensive reports on lean diabetes in India. A prospective study across nine centers in India found about one-fourth of T2DM patients to be lean or with BMI below 19 kg/m². Prevalence of type 2 lean DM across the centers varied from 11% to 25%^[13]. A recent report^[31] showed significantly higher prevalence of T2DM without overweight and obesity in Indians compared to white Caucasians in the United States. Diabetes in lean patients has been described before^[32,33]. There are reports on lean diabetes from other regions of South Asia, India, and Africa^[29,34,35]. Populations described in these reports were lean, had a history of childhood malnutrition, and had poor socioeconomic status.

Another notable observation in our subjects was the increased muscle mass percentage but low BMR in lean subjects compared to those overweight subjects. Usually, BMR is directly proportional to muscle mass and as a metabolic response to starvation, the primary concern is to supply energy to the brain^[36]. In our study, the BMR was less in spite of better muscle mass. This could be because of less caloric intake despite heavy physical activity. Adverse intrauterine or early postnatal environment with insufficient nutrients has been suggested as a mechanism of beta cell failure in lean diabetics^[29]. Another Indian study^[37] found that type 2 diabetics had an unfavorable body fat distribution, with an increase in visceral fat compared to that in non-diabetics. However, there are differing opinions on the causality of this association^[38,39].

Visceral fat is more important than total body fat, as excess visceral fat is a risk factor for both pre-diabetes and diabetes, being more so in Indians compared to other Asian populations. In our study, the diabetic subjects had higher visceral fat, and

Table 1 Anthropometry and body composition

	Diabetic		Non-diabetic		P for diabetic vs non-diabetic	
	Men, n = 102	Women, n = 66	Men, n = 68	Women, n = 76	Men	Women
Age						
Current age	55.2 (13.1)	57.9 (9.5)	41.7 (12.0)	38.2 (10.2)	0.000	0.000
Age at diagnosis	48.7 (12.1)	50.9 (10.5)				
Anthropometry						
Height, cm	164.2 (6.4)	151.1 (6.3)	162.7 (8.4)	152.1 (7.7)	0.17	0.13
Weight, kg	64.7 (11.7)	56.6 (12.1)	59.9 (13.5)	50.4 (11.9)	0.27	0.001
BMI, kg/m ²	24.0 (3.9)	24.7 (4.9)	22.5 (4.3)	21.7 (4.4)	0.45	0.002
Waist circumference, cm	92.0 (9.9)	87.5 (11.4)	81.3 (12.6)	76.0 (9.8)	0.000	0.000
Hip circumference, cm	94.2 (9.2)	97.3 (11.9)	89.8 (10.3)	89.4 (10.5)	0.22	0.000
WHR	0.98 (0.08)	0.90 (0.09)	0.90 (0.09)	0.85 (0.07)	0.000	0.045
Body composition by bio-impedance						
Body fat, %	22.6 (5.7)	35.6 (6.8)	21.3 (6.8)	30.5 (7.2)	0.87	0.026
Body fat, kg	15.2 (6.2)	21.2 (8.4)	14.1 (8.7)	15.7 (6.3)	0.89	0.000
Muscle mass, %	73.2 (5.4)	60.7 (6.4)	72.9 (8.7)	64.4 (10.5)	0.13	0.38
Muscle mass, kg	46.7 (5.9)	33.6 (4.4)	43.4 (8.1)	33.4 (7.1)	0.03	0.19
Visceral fat level	12.1 (4.4)	7.2 (2.6)	8.6 (5.3)	4.6 (2.9)	0.23	0.019
Fat free mass, kg	49.3 (6.3)	35.6 (4.8)	46.1 (8.2)	34.8 (6.3)	0.068	0.057
TBW, %	54.0 (3.6)	46.7 (3.6)	53.5 (3.5)	48.3 (5.7)	0.69	0.077
TBW, kg	34.7 (5.4)	25.6 (4.0)	31.8 (6.0)	24.5 (4.6)	0.12	0.056
BMR, calories/time	1350.2 (188.9)	1059.8 (168.9)	1291.0(231.4)	1043 (185.6)	0.30	0.004

Data are mean (standard deviation). P is adjusted for current age for anthropometry; body composition parameters are already age adjusted. BMI: Body mass index; WHR: Waist-to-hip ratio; TBW: Total body water; BMR: Basal metabolic rate.

within the diabetics those who are lean had significantly lower visceral fat. Significantly higher muscle mass percentage in the low and normal BMI group of diabetics than in those overweight shows that BMI has limitations pertaining to detailing of body composition. This striking peculiarity in type 2 diabetics in our subjects is bound to influence the natural history of diabetes and it needs further study.

The Kokan region is characterized by mountainous terrain with poor soil quality, hot humid weather, poverty and deep-rooted superstitions which have led to widespread malnutrition amongst people^[40]. Our hospital is located in a remote, rural area, and our study population is from a tribal region. A study from our center on 1290 school-going rural adolescents found underweight prevalence of 72%^[41]. In a pilot study on adolescent girls from Kokan, a high prevalence of micronutrient deficiencies was also found. More than 65% were deficient in calcium, zinc and folic acid, and were malnourished^[42]. In our hospital, 41.9% of the babies delivered were low birth weight (birth weight < 2500 g)^[43]. The Tata Memorial Rural Out Reach Program (known as TMCROP) was implemented by our hospital in all 2200 villages in Kokan, and all villagers were screened for cancer by household survey. In that survey, 51.7% population had a BMI less than 18.5 kg/m² and only 4.5% could be classified as obese. These findings highlighted the leanness of the population of Kokan.

In our current study, more than 40% had family history for diabetes, which may suggest genetic predisposition; although, we do not have any genetic data on our subjects. Inadequacy of BMI in distinguishing leanness has suggested future studies should investigate the role of body composition in the development of lean diabetes^[44].

Our study has some notable strengths. It has yielded the first report from the Kokan region, where malnutrition is very much prevalent. Unlike many other reports on diabetic subjects from other regions of India^[18-28], we have collected the data on body composition. There are, also, many limitations to our study. The sample size is small and it used cross-sectional data from a rural diabetic clinic. No sample size calculations were done. We were only able to recruit a smaller number of controls, making the study prone to bias. We were constrained by the remoteness of the area

Table 2 Family history, diabetic complications, and anthropometric morbidity

	Diabetic		Non-diabetic		P for diabetic vs non-diabetic	
	Men, n = 102	Women, n = 66	Men, n = 68	Women, n = 76	Men	Women
Diabetes history						
Family history						
1 st degree	45 (44)	34 (51)				
2 nd degree	7 (7)	9 (14)				
Complications						
Macrovascular	72 (69.9)	47 (71.2)				
Microvascular	13 (12.1)	1 (1.5)				
Underweight	8 (7.8)	7 (10.6)	13 (19.1)	20 (26.3)		
Normal	54 (52.9)	27 (40.9)	35 (51.5)	41 (53.9)	0.07	0.001
Overweight	40 (39.2)	32 (48.5)	20 (29.4)	15 (41.9)		
Centrally obese	59 (58)	49 (74)	19 (28.4)	29 (39.7)	0.000	0.000

Data are n (%).

where the hospital is situated. We could not use Dual-Energy X-Ray Absorptiometry (commonly known as DEXA; the current gold standard for body composition) because of the high equipment costs. We measured the body composition by bioelectrical impedance, using the TANITA body composition analyzer, which is a low cost, convenient and noninvasive technology, but concerns have been raised in the past about the validity of the analyzer^[45,46] and there is an urgent need to develop an ethnicity-specific equation for the Asian Indian population. We could not report on other cardiovascular risk markers (lipids, blood pressure) nor on the socioeconomic status of the participants as very little data were available as a part of patient history. We are aware of the fact that these subjects were diagnosed with diabetes at much earlier age. Recruitment of controls from the hospital setting has induced inherent selection bias. Thus, there is a need for a large community-based prospective study investigating the role of lean mass in development of diabetes in this region.

To conclude, we attempted to investigate the role of lean body mass in development of diabetes in the predominantly underweight diabetic population of Kokan. The underlying mechanism of lean diabetes has not yet been fully explored and more studies are needed. The diabetic population in Kokan has near-normal body composition, and BMI has considerable limitations. Therefore, the physiological process producing these deviations in body composition and its metabolic significance need further investigations using larger sample sizes.

Table 3 Body mass index and body composition in diabetics

	Diabetic, <i>n</i> = 168				<i>P</i> -value for lean vs overweight	
	Men, <i>n</i> = 102		Women, <i>n</i> = 66		Men	Women
	Lean, <i>n</i> = 62	Overweight, <i>n</i> = 40	Lean, <i>n</i> = 34	Overweight, <i>n</i> = 32		
Body fat, %	19.6 (4.4)	27.5 (4.0)	30.6 (4.7)	40.9 (4.1)	0.000	0.000
Body fat, kg	11.5 (3.5)	21.0 (5.1)	15.3 (5.4)	27.2 (6.7)	0.000	0.000
Muscle mass, %	76.2 (4.1)	68.5 (3.8)	65.4 (4.4)	55.7 (3.9)	0.000	0.000
Muscle mass, kg	43.8 (4.7)	51.3 (4.7)	31.1 (2.9)	36.3 (4.1)	0.000	0.000
Visceral fat level	10.0 (3.9)	15.3 (3.1)	5.2 (1.6)	9.3 (1.7)	0.000	0.000
Fat free mass, kg	46.2 (4.9)	54.1 (4.9)	32.8 (3.2)	38.5 (4.4)	0.000	0.000
TBW, %	55.3 (3.6)	52.0 (2.5)	48.6 (3.5)	44.5 (2.4)	0.000	0.000
TBW, kg	31.9 (4.1)	39.0 (4.4)	23.1 (2.3)	28.6 (3.6)	0.000	0.000
BMR, calories/time	1251.6 (139.1)	1502.3 (152.5)	953.7 (100.8)	1172.7 (153.3)	0.000	0.000

Data are mean (standard deviation). TBW: Total body water; BMR: Basal metabolic rate.

Table 4 Body mass index and body composition in non-diabetics

	Non-diabetic, <i>n</i> = 144				<i>P</i> -value for lean vs overweight	
	Men, <i>n</i> = 68		Women, <i>n</i> = 76		Men	Women
	Lean, <i>n</i> = 48	Overweight, <i>n</i> = 20	Lean, <i>n</i> = 61	Overweight, <i>n</i> = 15		
Body fat, %	18.8 (6.3)	27.6 (2.6)	28.4 (5.5)	39.1 (6.9)	0.000	0.000
Body fat, kg	11.0 (6.6)	21.5 (9.1)	13.6 (4.5)	24.7 (5.4)	0.000	0.000
Muscle mass, %	75.5 (8.1)	68.7 (7.1)	65.9 (10.8)	58.1 (6.4)	0.000	0.000
Muscle mass, kg	40.7 (6.0)	50.1 (8.6)	32.0 (6.2)	38.9 (7.8)	0.000	0.000
Visceral fat level	6.2 (4.3)	14.3 (1.9)	3.6 (1.9)	8.8 (2.2)	0.000	0.000
Bone mass, kg	2.2 (0.4)	2.8 (0.3)	1.8 (0.4)	2.3 (0.5)	0.000	0.000
Fat free mass, kg	42.7 (6.6)	54.1 (5.7)	33.3 (4.7)	41.0 (8.5)	0.000	0.000
TBW, %	54.6 (3.5)	51.0 (1.9)	48.8 (6.1)	46.1 (3.1)	0.000	0.075
TBW, kg	29.0 (4.3)	38.5 (4.1)	23.3 (3.3)	29.4 (5.8)	0.000	0.000
BMR, calories/time	1196.2 (185.2)	1521.5 (159.6)	1001.2 (148.4)	1228.7 (221.8)	0.000	0.000

Data are mean (standard deviation). TBW: Total body water; BMR: Basal metabolic rate.

ARTICLE HIGHLIGHTS

Research background

Recent years have seen a considerable increase in the burden of diabetes, hypertension and coronary heart disease in clinical practice in urban India. Recent studies in urban populations have shown an unexpectedly high prevalence of diabetes, and the prevalence is rising rapidly.

Research motivation

BKL Walawalkar Hospital carried out house-to-house surveys of 2200 villages in 2003-2010. In that survey, 51.8% of the subjects had body mass index (BMI) < 18.5 kg/m² and only 4.5% were overweight, with BMI > 25 kg/m². Another survey of 11521 adolescent girls from rural schools that was conducted in 2011-2017 showed that 64% of the girls had grade 1 to 3 thinness, based on the International Obesity Task Force standards, and stunting was seen in 22% to 28% of the girls. Thus, the overall population of Kozkan is lean in their body stature. The same survey also found a more than 70% prevalence of leanness based on BMI among men as well as women. This, again, reinforced the leanness of the population of Kozkan.

Research objectives

In order to investigate body composition of diabetic people from the BKL Walawalkar Hospital Clinic, a clinic-based case control study was carried out.

Research methods

One hundred sixty-eight type 2 diabetic patients (102 men) attending the outpatient department at a rural hospital and 144 non-diabetic controls (68 men) in the Chiplun area of the Kokan region were recruited. History of diabetes and anthropometric measurements were recorded, and body composition was measured by bioimpedance using the TANITA analyzer. All analyses were performed using SPSS 16.0 statistical software.

Research results

In this study, more than 45% of diabetic subjects had a 1st degree family history of diabetes, and more than 50% had macrovascular complications. The average BMI in the diabetic subjects was 24.3 kg/m². Underweight and normal diabetic subjects (men as well as women) had significantly lower body fat percentage, higher muscle mass percentage, lower visceral fat and lower basal metabolic rate compared to their overweight counterparts. Our data pave the way for a new theory of undernutrition as a risk factor in predisposing the Kokan population to diabetes.

Research conclusions

Undernutrition should also be considered as a risk factor for diabetes in lean patients. The molecular basis and physiological adaptations to undernutrition need to be explored.

Research perspectives

Lean diabetics had significantly lower body fat percentage, higher muscle mass percentage, lower visceral fat and lower basal metabolic rate compared to overweight diabetics. This could indicate a metabolic response to less caloric intake despite heavy physical activity, and this mechanism needs to be investigated. The diabetic population in Kokan has near-normal body composition and BMI has considerable limitations. Therefore, the physiological process producing these deviations in body composition and its metabolic significance need further investigations on a larger scale.

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Case Control Study

Relationship between sonographically measured median nerve cross-sectional area and presence of peripheral neuropathy in diabetic subjects

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statement: The study was approved by the Ethical committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife, Osun State, Nigeria, and a copy is uploaded with the submission.

Informed consent statement:

Written informed consent was obtained from all study participants before their inclusion

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Abstract**BACKGROUND**

Neuropathy is a common complication of diabetes mellitus resulting from direct damage by hyperglycemia to the nerves and/or ischemia by microvascular injury to the endoneurial vessels which supply the nerves. Median nerve is one of the peripheral nerves commonly affected in diabetic neuropathy. The median nerve size has been studied in non-Nigerian diabetic populations. In attempt to contribute to existing literature, a study in a Nigerian population is needed.

AIM

To evaluate the cross-sectional area (CSA) of the median nerve using B-mode ultrasonography (USS) and the presence of peripheral neuropathy (PN) in a cohort of adult diabetic Nigerians.

METHODS

Demographic and anthropometric data of 85 adult diabetes mellitus (DM) and 85 age- and sex-matched apparently healthy control (HC) subjects were taken. A complete physical examination was performed on all study subjects to determine the presence of PN and modified Michigan Neuropathy Screening Instrument

into the study.

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(MNSI) was used to grade its severity. Venous blood was taken from the study subjects for fasting lipid profile (FLP), fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) while their MN CSA was evaluated at a point 5 cm proximal to (5cmCATL) and at the carpal tunnel (CATL) by high-resolution B-mode USS. Data was analysed using SPSS version 22.

RESULTS

The mean MN CSA was significantly thicker in DM subjects compared to the HC at 5cmCATL ($P < 0.01$) and at the CATL ($P < 0.01$) on both sides. The presence of diabetic peripheral neuropathy (DPN) further increased the MN CSA at the CATL ($P < 0.05$) but not at 5cmCATL ($P > 0.05$). However, the severity of DPN had no additional effect on MN CSA 5 cm proximal to and at the CATL. There was no significant association between MN CSA and duration of DM and glycemic control.

CONCLUSION

Thickening of the MN CSA at 5cmCATL and CATL is seen in DM. Presence of DPN is associated with worse thickening of the MN CSA at the CATL but not at 5cmCATL. Severity of DPN, duration of DM, and glycemic control had no additional effect on the MN CSA.

Key words: Median nerve; Cross-sectional area; Sonography; Diabetics; Peripheral neuropathy

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Core tip: We report median nerve cross-sectional area findings in diabetics of Nigerian origin. This study demonstrates that the median nerve is thicker at the carpal tunnel and 5 cm proximal to the carpal tunnel in diabetic subjects than age- and sex-matched healthy controls. Further thickening in the median nerve size is seen in the presence of diabetic peripheral neuropathy at the carpal tunnel but not at a point 5 cm proximal to it. Median nerve size has no significant relationship with age, gender, severity of diabetic peripheral neuropathy, duration of diabetes mellitus or glycemic control in diabetic subjects.

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INTRODUCTION

Diabetes mellitus (DM) is used to describe several diseases where there is a persistent increase in blood sugar level due to deficiency in the production and/or action of insulin^[1]. Broadly, diabetes mellitus can be classified into two major forms, type 1 or insulin-dependent and type 2 or non-insulin dependent DM according to insulin secretion or action respectively^[2]. The prevalence of DM is on the increase worldwide in both developed and developing countries^[1,2]. Diabetic peripheral neuropathy (DPN) is the most common complication of DM and is seen in patients with types 1 and 2 DM. DPN and peripheral nerve dysfunction have common signs and symptoms in people with diabetes when other aetiological factors of the defect are not considered^[3].

After a long-term persistent hyperglycemia, DPN usually becomes symptomatic in type 1 DM while it is obvious in type 2 DM at detection or after a period of insufficient blood sugar level control^[4].

Organ impairment or failure is related with prolonged hyperglycemia of diabetes, and some of the organs that can be affected include the eyes, kidneys, nerves, heart, and blood vessels^[5]. Damage to peripheral nerves can occur directly from elevated blood sugar level or indirectly from reduced blood flow to nerves^[1].

Diabetic neuropathy is responsible for substantial morbidity, increased mortality

and impaired quality of life of diabetic patients^[6]. Therefore, early detection of nerve dysfunction is important to appropriately care for patients with diabetic neuropathy^[7].

The characteristic signs and symptoms of diabetic neuropathy basically suggest its diagnosis and confirmatory neurophysiological tests are required^[8].

Although electroneuromyography and nerve conduction studies (NCS) are the major electro-neurophysiological methods for diagnosing pathology associated with the median nerve and other peripheral nerves, they only allow assessment of peripheral nerve function, but fail to provide any data on their morphology or the possible visible pathomorphology of the surrounding structures and tissues^[9,10].

Magnetic resonance imaging (MRI) of the median nerve provides excellent morphological details of the nerve. Magnetic resonance imaging of peripheral nerves is known as Magnetic Resonance Neurography (MRN)^[11,12]. It is used to assess peripheral nerve entrapments and impingements as well as localization and grading of nerve injuries and lesions^[11,12]. Magnetic Resonance Neurography could be morphological or functional MRN^[11]. The morphological MRN is based on 3D MRI sequences with or without fat suppression while the functional MRN is based on Diffusion-weighted imaging (DWI). DWI, an MR Neurographic technique used to measure the limited random movement of water molecules within tissues depends on the Brownian motion of water^[13,14]. Diffusion within nerve fibres or white matter of the brain is usually high and tends to be directed towards the path of minimum opposition to the moving molecules and this is used to generate the final image in DWI. Diffusion tensor imaging (DTI) is an extension of DWI that enables measurement of Brownian movement of water molecules in nerves^[11-15]. However, MRI is not widely available, patient selective, expensive, and involves the use of time-consuming techniques.

Ultrasonography (USS) is preferred to MRI as a diagnostic method for a variety of reasons such as noninvasiveness, low cost, accessibility, approach, *etc*^[15]. The assessment of extremely small peripheral nerves *via* ultrasound has been made possible by employing Diagnostic high-resolution USS^[6]. The median nerve can be examined as it courses from the arm to the hand using high-resolution USS. Ultrasound can be used to evaluate the shape, size, and echo-texture of the MN. The major disadvantage of ultrasound is that it is operator dependent as it requires trained experienced hands with appropriate high resolution equipment^[15].

This study aimed to compare the MN cross-sectional area (CSA) measured on USS between DM subjects and age- and sex-matched apparently healthy controls (HC), and evaluate the relationship between MN CSA and presence and/ or severity of DPN.

MATERIALS AND METHODS

Subjects and study area

The study was approved by the Ethics and Research Committee of our hospital. Eighty-five consenting DM patients aged between 18 and 80 years and an equal number of age- and sex-matched HC subjects were randomly recruited from the Endocrinology Unit of the Department of Medicine of our hospital. Hypertensives, current smokers and alcohol consumers, subjects with thyroid disease, liver disease, previous history of carpal tunnel operation, inflammation, malignancy, and elevated total cholesterol after serum fasting lipid profile (FLP) were excluded from the study. Our hospital is a tertiary hospital and one of the major referral centers for diabetes care in the Southwestern zone of Nigeria. It serves a catchment area of about 170000 people.

Clinical parameters

Physical examination was carried out on all prospective study subjects by the managing endocrinologist for the presence and severity of peripheral neuropathy. The modified clinical history part of Michigan Neuropathy Screening Instrument (MNSI) questionnaire^[11] was administered to all study participants and scored over 15 to determine and grade peripheral neuropathy (PN). A total "Yes" score of 1-5 represented mild PN, a score of 6-10 moderate and 11-15 represented severe PN as done previously by Moghtaderi *et al*^[16].

Phalen test^[12] was performed to rule out carpal tunnel syndrome.

In the erect position with the acantho-meatal line set parallel to the floor, subjects had their weight in kilogram (Kg) and height in meters (M) measured without their shoes on. Their body weight and height were measured to the nearest 0.1 kg and 0.1 meter respectively using a mechanical physician weighing scale attached with a height gauge (model ZT-160, China).

Body mass index (BMI) was determined for all study participants using the formula: $BMI = \text{Weight}/\text{height}^2$

Laboratory parameters

Venipuncture of the antecubital veins in the left arm was done under sterile conditions for all study participants after an overnight fast of at least 8-12 h. 5 mL of venous blood was taken from each study subject for the assessment of fasting lipid profile (FLP) and glycated hemoglobin (Hb1Ac). The sample was emptied into an EDTA (Ethylene diamine tetra acetic acid) bottle and sent to chemical pathology laboratory for FLP using bioassay systems EnzyChrom cholesterol assay kit (E2CH-100) and Hb1Ac by chromatography using Siemens Hb1Ac machine (model SEMDIA-10311134, United States). Chromatography was either done immediately or sample stored in the refrigerator at a temperature of 2-8 °C and test done within 7 d.

Fasting Blood Glucose (FBG) was determined from finger prick specimen using a glucometer (Accu-check, Roche 365702101104) based on the glucose oxidase method.

Sonographic technique for median nerve assessment

All sonographic examinations were performed using the MINDRAY Real-time ultrasound machine (Model DC-7) equipped with a linear array probe, with a transducer frequency of 6MHz-12MHz. Each participant was seated on examination couch with a pillow on his/her lap. The forearm was placed supine on the pillow with elbow and fingers semi-flexed during the examination of the median nerve. The patient was instructed not to move the fingers during the examination period.

Following adequate positioning, coupling gel was applied to the anterior part of the wrist joint, over the carpal tunnel (CATL) and 5 cm proximal to it (5cmCATL). The volar wrist crease and pisiform bone were used as external reference points and landmarks during scanning. The transducer was positioned at right angles to the distal wrist crease and longitudinal axis of the forearm within the carpal tunnel inlet. The MN was identified and its major and minor axes were taken (Figure 1). Intra-observer variability was minimized by taking three measurements and recording the mean value. The CSA was calculated by the indirect method using the formula: $CSA = \text{major axis} \times \text{minor axis} \times \pi \times 1/4 \text{ (mm}^2\text{)}$ ^[13]; where π is a mathematical constant and is equal to 3.142.

Statistical analysis

All data were entered into the computer spreadsheet using Statistical Package for Scientific Solutions (SPSS) version 22.0 for Windows (SPSS, Chicago, IL, United States). Quantitative variables were indicated as mean \pm SD, while qualitative variables were indicated as frequencies and percentages.

Independent Sample *t*-test was used to compare MN CSA between DM and apparently HC subjects. A subgroup analysis amongst the DM subjects was done between those with and those without PN using independent *t*-test. Median nerve CSA was further compared between DM without PN, with mild PN and moderate/severe PN using Analysis of Variance (ANOVA).

Pearson Correlation was done to determine the relationship between MN CSA, clinical and laboratory parameters of the DM and HC subjects.

A level of $P \leq 0.05$ was considered as statistically significant for all tests.

The statistical review of this study was done by a biomedical statistician^[17-20].

RESULTS

There were significant differences in mean weight ($P = 0.003$), BMI ($P = 0.010$), DBP ($P = 0.001$), MAP ($P = 0.023$) and FBG ($P = 0.001$) between the diabetic and control groups while mean age ($P = 0.602$), total cholesterol ($P = 0.622$), height ($P = 0.473$) and SBP ($P = 0.557$) were similar in them. Both groups were well matched for Gender (Table 1).

Diabetic subjects had significantly higher median nerve CSA than their age- and sex-matched apparently healthy controls at the CATL level ($12.5 \pm 2.5 \text{ mm}^2$ vs $8.8 \pm 1.7 \text{ mm}^2$ ($P < 0.01$) on the right and $12.3 \pm 2.5 \text{ mm}^2$ vs $8.6 \pm 1.7 \text{ mm}^2$ ($P < 0.01$) on the left) and at 5cmCATL ($8.0 \pm 2.0 \text{ mm}^2$ vs $5.3 \pm 1.2 \text{ mm}^2$ ($P < 0.01$) on the right and $7.9 \pm 1.9 \text{ mm}^2$ vs $5.4 \pm 1.4 \text{ mm}^2$ ($P < 0.01$) on the left) (Table 2).

The median nerve CSA was significantly higher in diabetics with PN compared to diabetics without PN ($12.9 \pm 2.5 \text{ mm}^2$ vs $11.8 \pm 2.4 \text{ mm}^2$, $P = 0.049$) at the level of the CATL but not at 5cmCATL ($8.0 \pm 2.2 \text{ mm}^2$ vs $8.0 \pm 2.0 \text{ mm}^2$, $P = 0.856$) (Table 3).

There was no association between median nerve CSA and severity of PN as median nerve CSA did not significantly differ between the absent, mild, and moderate/severe PN categories of diabetic subjects at the CATL ($P = 0.062$) and at 5cmCATL ($P = 0.145$).

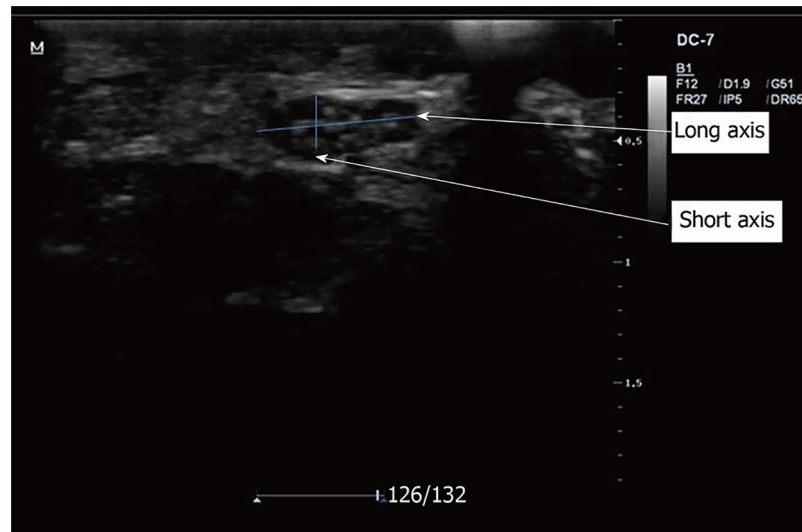


Figure 1 Transverse B-mode sonogram of the median nerve showing its long and short axes.

even after post-hoc Scheffe analysis for intergroup differences (Table 4).

Median nerve CSA at 5cmCATL and at the CATL did not show significant association with age greater than 60 years, duration of DM and glycemic control in both HC and DM subjects. However, MN CSA was significantly more thickened in males compared to females among the HC at both points of measurement. The association of MN CSA with gender was not found among DM subjects even after subgroup analysis of only those with DPN (Table 5).

Duration of DM, FBG and, HbA1c did not show any significant correlation with MN CSA in the diabetic subjects (Table 6).

DISCUSSION

Diabetic neuropathy is a relatively early and common complication affecting approximately 30% of DM patients^[14]. The prevalence of DPN from clinical assessment using the MNSI questionnaire was 60% in this study. This is at best an estimate since the gold standard for assessing PN, the nerve conduction test was not employed in our study.

We found higher MN CSA in the diabetic cohort which consisted of both types 1 and 2 DM subjects compared to non-diabetic HC at the 2 points of measurement. The finding of increased MN CSA in the diabetics relative to HC in our study agrees with those of 2 other hospital-based studies by Watanabe *et al*^[9] in 30 type 2 DM subjects aged 36 to 83 years with mean age of 59.8 ± 10.2 years and 32 healthy volunteers aged 24-72 years with mean age of 53.7 ± 13.9 years in Japan, and Agirman *et al*^[21] in 63 Type 2 DM subjects and 14 controls with mean age 47.6 ± 13.1 years in Turkey even though they were conducted in type 2 DM subjects only in racially and geographically different settings.

We also observed a significant additional increase in MN CSA in diabetics in the presence of DPN at the CATL similar to the findings of Watanabe *et al*^[8] and Zaidman *et al*^[22] which suggests that DPN can cause an increase in MN CSA apart from the hyperglycemic effect of DM. This statistical difference was however low ($P = 0.49$). The low statistical difference of MN CSA between subjects with and without PN in our study may be due to the fact that we did not diagnose PN using electrophysiological tests like NCS and as such, we may have misclassified some of the subjects with PN as those without PN. The statistically low significant difference between DM subjects with and without PN seen at the CATL was however not significant at 5cmCATL^[23].

Furthermore, this study failed to establish a significant association between MN CSA and severity of DPN. It can, therefore, be inferred from the index study that the presence of DPN probably increases MN CSA to a given threshold beyond which no further increase is possible. Therefore, our study shows that the presence and not severity of DPN can give an additional thickening of the MN CSA at the CATL.

Long-term hyperglycemic state has been implicated in the occurrence of DPN^[14,18,19]. Both FBG and HbA1c are short-term and long-term monitors of glycemic control respectively. The long-term monitor (HbA1c) gives a good estimate of glycemic

Table 1 Demographic, anthropometric and clinical characteristics of the study subjects *n* (%)

Variables	DM, <i>n</i> = 85	HC, <i>n</i> = 85	<i>P</i> -value
Age (yr)			
mean \pm SD ¹	61.7 \pm 11.1	60.9 \pm 10.3	0.620
< 50 yr	11 (12.9)	11 (12.9)	0.965
50-59 yr	25 (29.4)	27 (31.8)	
60-69 yr	22 (25.9)	23 (27.1)	
\geq 70 yr	27 (31.8)	24 (28.2)	
Gender			
Male	44 (51.8)	44 (51.8)	1.000
Female	41 (48.2)	41 (48.2)	
Height (m) ¹	1.66 \pm 0.08	1.65 \pm 0.08	0.473
Weight (Kg) ¹	71.3 \pm 13.6	65.4 \pm 11.5	0.003
BMI (Kg/m ²) ¹			
mean \pm SD ¹	26.0 \pm 5.3	24.1 \pm 4.3	0.010
Underweight	3 (3.5)	4 (4.7)	0.072
Normal BMI	35 (41.2)	51 (60.0)	
Overweight	31 (36.5)	21 (24.7)	
Obese	16 (18.8)	9 (10.6)	
Systolic BP (mmHg) ¹	129.8 \pm 20.7	131.9 \pm 24.3	0.557
Diastolic BP (mmHg) ¹	84.2 \pm 11.5	82.8 \pm 13.1	0.404
Total cholesterol (mmHg) ¹	4.90	4.78	0.622
FBG (mmol/L) ¹	7.00 \pm 2.16	5.15 \pm 0.06	0.001
HbA1c in % (Mean \pm SD)	8.69 \pm 2.46	-	-
Duration of DM in months median (range)	48.0 (0.3-312)	-	-
Peripheral neuropathy	51 (60)	-	-

¹*P* values < 0.05 are significant. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DM: Diabetes mellitus; FBG: Fasting blood glucose; HbA1c: Glycated haemoglobin.

control over a period of 3 months duration. Diabetic subjects were dichotomised into those with good and poor glycemic control based on their HbA1c levels in this study. Glycemic control did not show significant association with the MN CSA at the 2 points of measurements among the DM subjects, even after a subgroup analysis of only those with DPN. There was also no significant correlation between HbA1c levels and MN CSA at both points of measurement (5cmCATL: $r = -0.012$, $P = 0.916$ and CATL: $r = 0.034$, $P = 0.758$). This observation contrasts the findings of Watanabe *et al*^[8] in 32 DM subjects who reported significant correlation between MN CSA and HbA1c levels despite fewer sample size in their study relative to ours. Genetic and racial differences may have contributed to this.

Since long-term hyperglycaemic state has been implicated in the occurrence of DPN^[14,18,19], and MN CSA further thickened in the presence of PN in diabetics in this study, it would have been expected that a poor glycaemic state would be associated with a further thickening of the MN CSA in the diabetics with PN and show positive correlation with HbA1c. Factors that were not explored in this study such as impaired insulin signalling, insulin growth factor and C-peptide that mediate DPN as suggested by Dobretsov *et al*^[24] and /or genetic factors may have contributed to the contrasting findings in our study.

Duration of DM of more than 5 years had no additional effect on the MN CSA at the 2 points of measurement. The duration of diabetes was estimated from the time of diagnosis in a hospital in this study. This obviously is a conservative estimation as patients would have had the disease before presenting to the hospital. This may be responsible for the insignificant association between MN CSA and duration of DM seen in our study even among DM subjects with PN.

In this study, we used a modified clinical history part of Michigan Neuropathy Screening Instrument (MNSI) questionnaire to identify subjects with peripheral neuropathy. Peripheral neuropathy could be large fiber mono-neuropathy/polyneuropathy or isolated small fiber neuropathy. We could only have been sure of MN neuropathy in our subjects if we had performed neurophysiological study of the MN. Also, we only assessed MN in this study on ultrasound. It is

Table 2 Comparison of median nerve cross-sectional area between diabetics and healthy controls

Median nerve CSA (mm ²)		DM, n = 85	HC, n = 85	P-value
Right	Carpal tunnel	12.5 ± 2.5	8.8 ± 1.7	< 0.01
	5 cm proximal	8.0 ± 2.0	5.3 ± 1.2	< 0.01
Left	Carpal tunnel	12.3 ± 2.5	8.6 ± 1.7	< 0.01
	5 cm proximal	7.9 ± 1.9	5.4 ± 1.4	< 0.001

CSA: Cross sectional area; DM: Diabetic subjects; HC: Healthy control subjects. *P* values < 0.05 are significant.

possible that our study participants may have had mono-neuropathy not affecting the MN or have isolated small fibre neuropathy. This could have accounted for the lack of correlation between MN CSA and disease duration, FBG, HBA1c levels in the index study.

DPN symptoms are induced by factors such as total hyperglycemic exposure, high lipid levels, blood pressure, increased height, exposure to high concentrations of ethanol. Also, hereditary factors are considered. In addition to the matching of our DM and HC subjects for age and sex, the results of their anthropometric and laboratory parameters showed that there was no significant differences in their height, BP and TC. Confounders like hypertension, smoking, and alcohol consumption were also eliminated by excluding subjects with a history of these risk factors from the two study groups.

Evidence from this study may have been limited by the selection bias of our hospital-based setting as subjects enrolled were only those that presented in the teaching hospital. We, however, minimised this by recruiting consecutive consenting subjects into the study. Another limitation is the fact that we did not confirm neuropathy using neurophysiological tests like NCS which uses supramaximal stimuli and recruits non-selectively all available fibres (both large and small fibres) and involves the proximal and distal parts of the nerve trunks. NCS would have picked abnormality in the function of the nerves even in the absence of clinical symptoms and as such we would have been able to diagnose subclinical peripheral neuropathy in our study subjects.

However, our findings are unique in that we report findings from diabetics of Nigerian origin. To the best of our knowledge, MN CSA measured on ultrasound in diabetic neuropathy has not been reported in Nigeria prior to this study.

We conclude from our study that DM subjects had thicker MN CSA at 5cmCATL and at CATL compared to their age- and sex-matched HC, Diabetics with PN had thicker MN CSA at the CATL but not at 5cmCATL compared with those without PN and MN CSA had no significant relationship with age, gender, severity of DPN, duration of DM or glycemic control in diabetics.

We recommend the CATL over 5cmCATL as the point of measurement for MN CSA when evaluating the MN in diabetics as both the increase in the MN CSA secondary to DM and additional thickening in presence of DPN seen in this study occurred at the CATL while only the increase secondary to DM occurred at 5cmCATL. Evaluation of the MN CSA in DM subjects is only recommended before DPN sets in as no additional thickening of the MN CSA with a worsening grade of DPN was seen in this study.

Table 3 Comparison of median nerve cross-sectional area between diabetics with and diabetics without diabetic peripheral neuropathy

Median nerve CSA (mm ²)	Diabetic peripheral neuropathy		P-value
	Absent <i>n</i> = 34	Present <i>n</i> = 51	
Carpal tunnel	11.8 ± 2.4	12.9 ± 2.5	0.049
5cm Proximal	8.0 ± 2.2	8.0 ± 2.0	0.856

CSA: Cross sectional area. *P* values < 0.05 are significant.

Table 4 Comparison of median nerve cross-sectional area between diabetics without diabetic peripheral neuropathy, diabetics with mild diabetic peripheral neuropathy and diabetics with moderate/severe diabetic peripheral neuropathy

Median nerve CSA (mm ²)	Peripheral neuropathy			P-value
	Absent (<i>n</i> = 34)	Mild (<i>n</i> = 32)	Moderate/severe (<i>n</i> = 19)	
Carpal tunnel	11.8 ± 2.4 ^a	13.2 ± 2.6 ^a	12.3 ± 2.1 ^a	0.062
5 cm Proximal	7.9 ± 2.2 ^b	8.3 ± 2.2 ^b	7.6 ± 1.4 ^b	0.145

CSA: Cross sectional area; DPN: Diabetic peripheral neuropathy; NB: The same alphabets in each row indicate insignificant differences using scheffe post hoc test to evaluate intergroup differences.

Table 5 Comparison of median nerve cross-sectional area between categories of age, gender, duration of diabetes mellitus, and glycemic control at carpal tunnel and 5 cm proximal to the carpal tunnel

		Median nerve CSA (mm ²) at CATL					
		DM_nonDPN	P-value	DM_DPN	P-value	HC	P-value
Duration of DM	≤ 5 yr	11.4 ± 1.8	0.65	12.9 ± 2.4	0.99	-	-
	> 5 yr	11.8 ± 3.3		12.9 ± 2.7		-	
FBG	≤ 7 mmol/L	11.6 ± 2.3	0.79	13.2 ± 2.9	0.34	-	-
	> 7 mmol/L	11.4 ± 2.0		12.5 ± 1.8		-	
HBA1c	≤ 7%	11.0 ± 1.8	0.45	12.3 ± 2.4	0.20	-	-
	> 7%	11.7 ± 2.3		13.2 ± 2.6		-	
Age	≤ 60 yr	11.1 ± 1.9	0.25	12.9 ± 2.3	1.00	8.7 ± 1.5	0.37
	> 60 yr	12.0 ± 2.4		12.9 ± 2.7		8.4 ± 1.9	
Sex	Female	11.3 ± 1.9	0.62	13.2 ± 2.6	0.42	8.1 ± 1.2	0.02
	Male	11.7 ± 2.4		12.6 ± 2.4		9.0 ± 2.0	
		Median nerve CSA (mm ²) at 5cmCATL					
		DM_nonDPN	P-value	DM_DPN	P-value	HC	P-value
Duration of DM	≤ 5 yr	7.6 ± 1.9	0.87	7.7 ± 1.7	0.13	-	-
	> 5 yr	7.5 ± 1.5		8.5 ± 2.2		-	
FBG	≤ 7 mmol/L	7.5 ± 1.3	0.74	8.2 ± 1.9	0.57	-	-
	> 7 mmol/L	7.7 ± 2.5		7.9 ± 2.0		-	
HBA1c	≤ 7%	7.5 ± 1.4	0.86	7.7 ± 2.2	0.29	-	-
	> 7%	7.6 ± 1.9		8.3 ± 1.8		-	
Age	≤ 60 yr	7.3 ± 1.5	0.32	8.4 ± 2.1	0.25	5.5 ± 1.2	0.79
	> 60 yr	7.9 ± 2.2		7.8 ± 1.8		5.4 ± 1.5	
Sex	Female	7.0 ± 1.4	0.10	7.8 ± 1.7	0.37	4.9 ± 0.7	0.00
	Male	8.0 ± 2.0		8.3 ± 2.2		5.9 ± 1.6	

CATL: Carpal tunnel; 5cmCATL: 5 cm proximal to the carpal tunnel; DM_nonDPN: Diabetics without diabetic peripheral neuropathy; DM_DPN: Diabetics without diabetic peripheral neuropathy; HC : Healthy controls. *P* values < 0.05 are significant.

Table 6 Relationship between median nerve cross-sectional area on ultrasound and clinical/laboratory parameters of the diabetic

subjects

Variables	CATL		5cmCATL	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Age	-0.041	0.898	-0.050	0.650
Sex	-0.058 ¹	0.601	-0.050 ¹	0.651
Weight	0.094	0.390	-0.041	0.708
Height	0.042	0.703	0.025	0.817
BMI	0.091	0.407	-0.056	0.611
SBP	-0.126	0.251	0.050	0.648
DBP	0.043	0.693	0.050	0.648
TC	0.092	0.402	0.092	0.056
Duration of DM	0.110 ²	0.318	0.151 ²	0.167
FBG	-0.059	0.591	0.026	0.811
HbA _{1c}	-0.012	0.916	0.034	0.758

¹Point-biserial correlation coefficient;

²Spearman correlation coefficient. CATL: Carpal tunnel; 5cmCATL: 5 cm proximal to the carpal tunnel; *r*: Pearson correlation coefficient; CSA: Cross sectional area; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DM: Diabetes mellitus; FBG: Fasting blood glucose; HbA_{1c}: Glycated haemoglobin *P* values < 0.05 are significant.

ARTICLE HIGHLIGHTS

Research background

Peripheral neuropathy (PN) is a common complication of diabetes mellitus. High-resolution ultrasonography gives good morphological detail in the peripheral nerves.

Research motivation

Sonographic measurement of the Median nerve cross-sectional area may be a valuable tool in addition to clinical examination in identifying subjects with peripheral neuropathy in regions where standard electrophysiological studies like nerve conduction test are not available.

Research objectives

We evaluated the relationship between median nerve cross-sectional area (CSA) and the presence of PN in a cohort of adult diabetic Nigerians.

Research methods

A one-year cross-sectional study carried out in diabetic subjects recruited in the endocrinology unit of a Nigerian tertiary hospital and age- and sex-matched controls.

Research results

This study demonstrates that the median nerve is thicker in CSA at the carpal tunnel (CATL) and 5 cm proximal to the carpal tunnel (5cmCATL) in diabetic subjects than in age- and sex-matched healthy controls. Further thickening in the median nerve CSA is seen in the presence of diabetic peripheral neuropathy at the carpal tunnel but not at a point 5 cm proximal to it. Median nerve size has no significant relationship with age, gender, severity of DPN, duration of DM or glycemic control in our diabetic subjects.

Research conclusions

This study done in Diabetics of Nigerian origin adds to the current literature that Diabetic subjects have thicker MN CSA compared to their age- and sex-matched controls. Median nerve CSA was also thicker at the CATL in Diabetics with PN than in those without PN.

Research perspectives

We suggest the need for longitudinal studies in diabetic subjects who have median nerve neuropathy confirmed with nerve conduction test to elucidate the progressive effect of diabetic peripheral neuropathy on MN CSA.

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Retrospective Cohort Study

Early vs late oral nutrition in patients with diabetic ketoacidosis admitted to a medical intensive care unit

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Abstract**BACKGROUND**

Diabetic ketoacidosis (DKA) has an associated mortality of 1% to 5%. Upon admission, patients require insulin infusion and close monitoring of electrolyte and blood sugar levels with subsequent transitioning to subcutaneous insulin and oral nutrition. No recommendations exist regarding the appropriate timing for initiation of oral nutrition.

AIM

To assess short-term outcomes of oral nutrition initiated within 24 h of patients being admitted to a medical intensive care unit (MICU) for DKA.

METHODS

A retrospective observational cohort study was conducted at a single academic medical center. The patient population consisted of adults admitted to the MICU with the diagnosis of DKA. Baseline characteristics and outcomes were compared between patients receiving oral nutrition within (early nutrition group) and after (late nutrition group) the first 24 h of admission. The primary outcome was 28-d mortality. Secondary outcomes included 90-d mortality, MICU and hospital lengths of stay (LOS), and time to resolution of DKA.

RESULTS

There were 128 unique admissions to the MICU for DKA with 67 patients

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receiving early nutrition and 61 receiving late nutrition. The APACHE (Acute Physiology and Chronic Health Evaluation) IV mortality and LOS scores and DKA severity were similar between the groups. No difference in 28- or 90-d mortality was found. Early nutrition was associated with decreased hospital and MICU LOS but not with prolonged DKA resolution, anion gap closure, or greater rate of DKA complications.

CONCLUSION

In patients with DKA, early nutrition was associated with a shorter MICU and hospital LOS without increasing the rate of DKA complications.

Key words: Diabetes mellitus; Diabetic ketoacidosis; Diabetic complications; Acidosis; Ketosis; Critical care

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Core tip: Considering variability of timing in reinstitution of oral diet in patients with diabetic ketoacidosis and lack of guideline recommendations, we investigated whether early oral nutrition is safe. We found that oral feeding instituted in the first 24 h appeared safe and resulted in shorter intensive care unit and hospital lengths of stay.

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INTRODUCTION

Diabetic ketoacidosis (DKA), a potentially dangerous complication of diabetes, has an associated mortality of 1% to 5%^[1]. It causes severe insulin deficiency, electrolyte abnormalities, and dehydration, and often requires admission to an intensive care unit (ICU). Upon admission, patients require insulin infusion and close monitoring of electrolyte and blood sugar levels with subsequent transitioning to subcutaneous insulin and oral nutrition. No recommendations exist regarding the appropriate timing for initiation of oral nutrition. Potential disadvantages of oral nutrition administered within the first 24 h of admission to an ICU (early nutrition) include difficulty in blood sugar monitoring and insulin dosing, altered mental status predisposing to aspiration, and worsening of nausea, vomiting, and abdominal pain.

Due to these concerns and the lack of definitive recommendations, many institutions have varying protocols regarding the initiation of oral nutrition. Our study investigates the safety of early nutrition in adult DKA patients admitted to a medical ICU (MICU).

MATERIALS AND METHODS

A retrospective observational cohort study was conducted at a single academic institution (Baylor Scott and White Heath, Temple, TX, United States) from December 2015 to January 2017. The study was approved by the local institutional review board and granted a waiver of informed consent. The study participants included all patients admitted to the MICU with the diagnosis of DKA. Only the first admission during the specified time frame for each patient was included. Exclusion criteria were age less than 18 years old, pregnancy, admission with DKA to a general ward or other type of ICU and leaving the hospital against medical advice. Data was collected by review of the electronic medical records. The time of first oral intake was labeled as the initiation of oral nutrition. The resolution of DKA was defined as achieving a serum glucose < 200 mg/dL and satisfying two of the following three criteria: pH ≥ 7.3, serum bicarbonate ≥ 15 meq/L, and anion gap ≤ 12. The anion gap was corrected using the value of the closest serum albumin measurement^[1]. The severity of DKA was defined by arterial pH, serum bicarbonate, anion gap, and presence of altered

mentation according to the American Diabetes Association consensus statement^[1]. Early nutrition was defined as the initiation of nutrition within the first 24 h of admission. Late nutrition was defined as the initiation of nutrition after the first 24 h of admission.

Statistical analysis

Characteristics of the study sample were assessed using descriptive statistics. Frequencies and percentages were reported for categorical variables and means and standard deviations (or medians and ranges, if appropriate) were reported for continuous variables. Wilcoxon-Mann-Whitney tests were used to compare non-normally distributed continuous variables between groups. Chi-square and Fisher exact tests were used to compare categorical variables between groups. SAS version 9.4 and StatXact version 11 software was used to perform the statistical analysis. Statistical significance is expressed as ^a $P < 0.05$, ^b $P < 0.01$.

RESULTS

There were 330 admissions to the MICU for a diagnosis of DKA. After excluding repeated hospitalizations and those satisfying exclusion criteria, the final cohort consisted of 128 unique patient admissions. Of those patients, 67 received early nutrition and 61 received late nutrition.

Baseline characteristics are described in Table 1. The patient population had a mean age of 47.3 (SD = 17.7) years, 50.8% were female and race was predominately white (65%). The severity of illness scores, Acute Physiology and Chronic Health Evaluation (APACHE) IV mortality and APACHE IV length of stay (LOS) scores, were 9.9 (SD = 18.5) and 4.6 (SD = 1.75), respectively. Comparing the early and late nutrition groups found no statistically significant difference between the groups in age, race, severity of illness based on APACHE IV mortality and LOS indices and DKA severity. A statistically significant difference between the early and late nutrition groups existed in terms of sex (37% *vs* 62% female, $P = 0.0047$).

Outcomes are described in Table 2. The overall 28-d mortality was 3.1 % (4 patients) and 90-d mortality was 3.9% (5 patients). Mean hospital and MICU LOS were 6.16 (SD = 6.54) and 2.21 (SD = 3.37) days respectively. There were no differences in the early and late nutrition groups in terms of mortality at 28 d (2.34% *vs* 0.78%, $P = 0.62$) and at 90 d (2.36% *vs* 1.57%, $P = 1.00$). Early nutrition group was not associated with longer mean time to anion gap closure ($P = 0.1642$) or DKA resolution ($P = 0.1410$). There was a significant decrease in the ICU LOS (1.38 *vs* 3.12, $P = 0.0002$) and overall hospital LOS (4.16 *vs* 8.35 $P = 0.0001$) in the early versus the late nutrition group.

Additionally, no significant difference in mean number of episodes of hyperkalemia (0.56 *vs* 0.43, $P = 0.37$), hypoglycemia (0.97 *vs* 1.54, $P = 0.18$), or severe acidosis (0.04 *vs* 0.20, $P = 0.18$) existed between the early and late nutrition groups. However, fewer episodes of hypokalemia (1.18 *vs* 2.21, $P = 0.0022$) and hypophosphatemia (0.73 *vs* 1.67, $P = 0.0052$) occurred in the early nutrition group.

DISCUSSION

We found that initiating oral nutrition in patients with DKA within the first 24 h of admission to the MICU was safe and decreases hospital and MICU LOS in our cohort of patients. Our 90-d mortality rate is consistent with prior studies^[2]. The overall low mortality rate made the comparison between the early and late nutrition groups unlikely to reach statistical significance. Our analysis also demonstrated no difference in secondary outcomes, including time to normalization of the anion gap and resolution of DKA, and mean instances of hypoglycemia, hyperkalemia, and severe acidosis. However, a significant decrease in instances of hypokalemia and hypophosphatemia occurred. Finally, ICU and overall hospital LOS was significantly shorter for the early nutrition group.

DKA results in over 100000 admissions per year in the United States and has significant medical costs^[1]. Mortality rates remain low between 1%-2.4%, with the cause of death in DKA patients often stemming from concurrent acute medical conditions and comorbidities^[2,3]. The most appropriate location of care delivery for these patients is dictated by local practices, and recent studies report favorable outcomes with management on general hospital wards^[4].

The role of nutrition in critical care cannot be overemphasized. The stress of critical illness places an enormous metabolic demand on the body^[5]. Adequate nutrition has multiple advantages that include replenishing energy stores and protecting against

Table 1 Baseline characteristics

	Entire cohort	Early nutrition	Late nutrition	P value
<i>n</i>	128	67	61	
Age, mean (yr)	47.3 (SD = 17.7)	45.7 (SD = 18.4)	49.1 (SD = 16.9)	0.1970
Race (<i>n</i>)				
African American	25% (32)	23.9% (16)	26.2% (16)	0.1950
Caucasian	65% (83)	67.2% (45)	62.3% (38)	
Other	10% (13)	8.9% (7)	11.5% (7)	
Female sex (<i>n</i>)	50.8% (65)	37.3% (25)	62% (38)	0.0047
DKA severity				
Mild	51	33	28	0.8997
Moderate	36	19	17	
Severe	31	15	16	
Mean APACHE IV Mortality	9.9 (SD = 18.5)	6.0 (SD = 12.7)	14.1 (SD = 22.5)	0.1170
Mean APACHE IV LOS	4.6 (SD = 1.8)	4.2 (SD = 1.5)	4.8 (SD = 2.0)	0.8400

APACHE: Acute Physiology and Chronic Health Evaluation; LOS: Length of stay; SD: Standard deviation.

ICU- and hospital-acquired complications^[5]. However, the optimal nutritional components in the ICU remain controversial, and new evidence challenges the intuitive tendency to supplement critically ill patients with high-calorie nutrition^[6].

Increasing evidence suggests that ketone bodies play a role in hunger control through a yet an unknown process^[7]. This facilitated introduction of the ketogenic diet as an effective modality of weight loss. Additionally, elevated free fatty acid (FFA) levels, which are often observed in starvation states, have been shown to reduce food intake by acting on specific hypothalamic neurons^[7]. As it pertains specifically to DKA, a higher degree of ketonemia and elevated circulating FFA could suppress hunger and potentially explains the delay in oral intake when initiated upon the patient's demand. In our study, beta hydroxybutyrate (BHB) and FFA levels were not measured. Varying degrees of ketonemia in the study groups may have contributed to the difference in LOS and time to resolution of DKA. However, we found no statistical difference between the groups in either the level of severity of DKA in both groups.

The potential for certain types of food to exacerbate ketosis may lead many physicians to withhold oral nutrition during DKA. Although reducing patients' initial oral intake of a low-carbohydrate diet might promote ketogenesis, the magnitude of its effect is low compared with the ketosis caused by uncontrolled diabetes. The maximum level of ketonemia achieved by a physiologic ketosis due to diet is 7-8 mmol/L as compared with > 25 mmol/L found in DKA^[7]. The dietary augmentation of ketosis likely becomes even less significant with the initiation of insulin treatment and carbohydrate delivery to the cells.

In our institution, every patient diagnosed with DKA is admitted to the MICU as a result of level of clinical care related to a continuous insulin infusion. This practice provided the opportunity to assess the safety of early nutrition in all DKA patients. Despite the widespread use of DKA severity for the purposes of deciding the appropriate level of care, the direct link between estimated severity and outcomes has not been established. Nevertheless, individual components of severity assessment, such as mental status and pH, have been associated with worsened outcomes. Altered mental status in particular could be a manifestation of a more severe underlying condition preventing patients from early nutrition and disproportionately worsening outcomes in the late nutrition group. The DKA severity based on available measurements of initial bicarbonate concentration, pH, and GCS did not differ between the groups in our study. Additionally, there was no statistically significant differences between the groups in the severity of illness represented by APACHE IV mortality and LOS scores.

Patients with DKA often have abdominal pain, nausea, and vomiting, ultimately leading to oral-intake intolerance. Consensus guidelines associate patients' readiness to eat with resolution of ketoacidosis^[1]. However, it is possible that when oral nutrition was administered on demand in our study, patients having more severe DKA and worse symptoms on presentation would end up in the late oral nutrition group. This may have implications in further studies investigating any benefit of mandatory early oral nutrition in DKA where randomization would be a key to

Table 2 Outcomes

	Early nutrition	Late nutrition	P value
Mean time to AG normalization (h)	11.7 (SD = 15.6)	20.0 (SD = 40.7)	0.1642
Mean time to DKA resolution (h)	15.4 (SD = 18.8)	19.6 (SD = 32.6)	0.1410
Mortality at 28 d (n)	2.34% (3)	0.78 (1)	0.6300
Mortality at 90 d (n)	2.34% (3)	1.57% (2)	1.0000
Hospital LOS (d)	4.16 (SD = 2.63)	8.35 (SD = 8.85)	0.0001
ICU LOS (d)	1.38 (SD = 1.17)	3.12 (SD = 4.58)	0.0002
Mean number of complication occurrences:			
Hypoglycemia	0.97 (SD = 1.49)	1.54 (SD = 2.47)	0.1804
Hypokalemia	1.18 (SD = 1.4)	2.21 (SD = 2.1)	0.0022
Hyperkalemia	0.43 (SD = 0.72)	0.56 (SD = 0.89)	0.3706
Hypophosphatemia	0.73 (SD = 0.9)	1.67 (SD = 2.4)	0.0052
Severe acidosis	0.04 (SD = 0.21)	0.20 (SD = 0.73)	0.1356

DKA: Diabetic ketoacidosis; ICU: Intensive care unit; LOS: Length of stay; SD: Standard deviation.

ensure similar severity of ketoacidosis in the investigation groups.

To control for possible delay in meeting the strict DKA resolution criteria, we separately analyzed the time to normalization of anion gap as this likely represents cessation of ketosis with no change in outcomes. Both of these results were consistent with prior studies^[8]. While patients starting oral nutrition after the first 24 h of admission had longer time to DKA resolution and anion gap normalization, neither was statistically significant. Notably, both the time to AG closure and to resolution of acidosis in the late nutrition group were less than 24 h. It is possible that the delay in oral diet resumption may have contributed to delayed transfer of these patients out of the MICU.

Our study confirmed the existing variability among physicians regarding the optimal timing of initiating oral nutrition in patients DKA. Although the study population size was likely too small to demonstrate a significant difference in the mortality, oral nutrition provided to DKA patients on demand appears to be safe. Early reinstitution of oral nutrition did not result in worsening of DKA complications and was associated with improvement in hypokalemia and hypophosphatemia. Finally, on-demand oral nutrition reinitiated within the first 24 h of admission has the potential to shorten ICU and overall hospital LOS.

ARTICLE HIGHLIGHTS

Research background

Diabetic ketoacidosis (DKA) is a common reason for hospitalization in patients with diabetes. It results in significant morbidity, mortality, and financial burden. Research and quality improvement efforts have been put forth to investigate the triggers and risk factors associated with ketoacidosis to prevent initial episode of DKA and minimize recurrence. In the meantime, the standard of care in management of DKA has been more clearly defined attention to serum glucose levels, electrolytes, acidosis and diligent evaluation for and treatment of the underlying etiology. Together, these advances resulted in significant reduction of mortality associated with DKA over the years. Nevertheless, many aspects of care for DKA patients remains unanswered, including severity stratification and appropriate level of care. Many institutions continue to accept patients with DKA to the intensive care unit (ICU) due to frequent electrolyte and glucose monitoring and meticulous insulin titration. Minimizing financial burden and hospital acquired complications associated with frequent and prolonged ICU stay is the subject of current and future investigations.

Research motivation

Tolerance of oral diet is regarded as a marker for resolution of ketoacidosis in DKA patients. Its administration is often postponed until biochemical confirmation of the resolution of ketoacidosis due to fear of unpredictable glucose and electrolyte changes. We hypothesized that allowance of on demand oral nutrition in DKA patients is safe and has a potential to decrease the length of hospitalization.

Research objectives

We aim to compare the mortality, rate of complications, and length of stay between DKA patients receiving oral nutrition before and after the first 24 h of ICU admission.

Research methods

Retrospective data collection was conducted establishing the demographics, initial biochemical characteristics, and outcomes of patients admitted to our single academic medical center. Outcomes included common complications of DKA, 28- and 90-d mortality, and length of ICU and hospital stay. Bivariate analysis was then performed comparing these variables between the two subgroups defined by the timing of their first oral intake.

Research results

The timing of oral nutrition in DKA patients was heterogeneous between different care teams with 52.3% of patients restarting oral intake in the first day of admission. This did not result in increased mortality (2.34% *vs* 0.78%, $P = 0.62$) or rate of complications such as hyperkalemia (0.56 *vs* 0.43, $P = 0.37$), hypoglycemia (0.97 *vs* 1.54, $P = 0.18$), or severe acidosis (0.04 *vs* 0.20, $P = 0.18$). Despite having similar overall illness severity and severity of DKA itself, the DKA patients who received oral nutrition in the first 24 h of their admission had a shorter ICU (1.38 *vs* 3.12, $P = 0.0002$) and (4.16 *vs* 8.35 $P = 0.0001$) hospital stay.

Research conclusions

Early oral nutrition (defined as oral intake in the first 24 h) administered on demand in patients admitted to ICU with DKA has a potential to safely reduce the length of stay.

Research perspectives

The study introduces the possibility of early oral nutrition in DKA to improve the length of stay. Further prospective randomized investigation is necessary to validate this finding.

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Insulin resistance is associated with subclinical vascular disease in humans

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Abstract

Insulin resistance is associated with subclinical vascular disease that is not justified by conventional cardiovascular risk factors, such as smoking or hypercholesterolemia. Vascular injury associated to insulin resistance involves functional and structural damage to the arterial wall that includes impaired vasodilation in response to chemical mediators, reduced distensibility of the arterial wall (arterial stiffness), vascular calcification, and increased thickness of the arterial wall. Vascular dysfunction associated to insulin resistance is present in asymptomatic subjects and predisposes to cardiovascular diseases, such as heart failure, ischemic heart disease, stroke, and peripheral vascular disease. Structural and functional vascular disease associated to insulin resistance is highly predictive of cardiovascular morbidity and mortality. Its pathogenic mechanisms remain undefined. Prospective studies have demonstrated that animal protein consumption increases the risk of developing cardiovascular disease and predisposes to type 2 diabetes (T2D) whereas vegetable protein intake has the opposite effect. Vascular disease linked to insulin resistance begins to occur early in life. Children and adolescents with insulin resistance show an injured arterial system compared with youth free of insulin resistance, suggesting that insulin resistance plays a crucial role in the development of initial vascular damage. Prevention of the vascular dysfunction related to insulin resistance should begin early in life. Before the clinical onset of T2D, asymptomatic subjects endure a long period of time characterized by insulin resistance. Latent vascular dysfunction begins to develop during this phase, so that patients with T2D are at increased cardiovascular risk long before the diagnosis of the disease.

Key words: Diabetes; Cardiovascular risk; Arterial stiffness; Arterial elasticity; Intima-media thickness; Vascular calcification; Insulin resistance; Animal protein; Vegetable protein

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Core tip: Vascular injury associated to insulin resistance includes impaired vasodilation in response to chemical mediators, reduced distensibility of the arterial wall (arterial stiffness), vascular calcification, and increased thickness of the arterial wall. Vascular dysfunction associated to insulin resistance is present in asymptomatic subjects and predisposes to cardiovascular diseases, such as heart failure, ischemic heart disease, stroke, and peripheral vascular disease. Structural and functional vascular disease associated to insulin resistance is highly predictive of cardiovascular morbidity and mortality.

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INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality particularly in patients with diabetes. Cardiovascular risk in this population group begins decades prior the clinical diagnosis of the disease and is not fully explained by traditional risk factors such as hypercholesterolemia and smoking. Multiple investigations provide compelling evidence of an association between insulin resistance by itself and cardiovascular risk in the general population and patients with diabetes. More insulin-resistant subjects endure higher cardiovascular risk compared to those who are more insulin-sensitive^[1]. A causative link between insulin resistance by itself and vascular disease is very likely to exist, but the pathogenic mechanisms that explain the vascular dysfunction related to insulin resistance remain elusive. There is conclusive evidence that dietary habits that include animal protein increase the risk of type 2 diabetes (T2D) and cardiovascular disease whereas dietary patterns with elevated content of vegetable protein reduce the risk of both disorders^[2]. Population groups that change their dietary routine to augment animal protein intake experience a dramatic increase in the rate of T2D and cardiovascular events^[3]. Animal protein consumption activates glucagon secretion. Glucagon is the primary hormone that opposes insulin action. Animal protein ingestion may predispose to T2D and cardiovascular events by intensifying insulin resistance via glucagon secretion (Figure 1)^[4].

Asymptomatic individuals with insulin resistance experience striking vascular damage that is not justified by traditional cardiovascular risk factors, such as hypercholesterolemia or smoking. Vascular injury related to insulin resistance develops progressively in asymptomatic subjects during a period of time that may begin during childhood. A long phase of insulin resistance and latent vascular injury precedes the clinical onset of T2D increasing cardiovascular risk before the diagnosis of the disease^[5-7]. Accordingly, subclinical vascular dysfunction is evident in patients with screen-detected T2D^[8]. Vascular damage associated with insulin resistance includes functional and structural vascular injury, such as impaired vasodilation, loss of elasticity of the arterial wall (arterial stiffness), increased intima-media thickness of the arterial wall, and vascular calcification. (Figure 2) The presence of subclinical vascular disease associated with insulin resistance is highly predictive of future cardiovascular events^[9-12].

INSULIN RESISTANCE IS INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL IMPAIRMENT OF VASCULAR REACTIVITY

Vascular smooth muscle cells normally undergo contraction or relaxation to regulate the magnitude of the blood flow according to physiological conditions. Normal endothelial cells generate vasoactive substances that modulate the reactivity of vascular smooth muscle cells. Among them, nitric oxide is a short-lived gas that

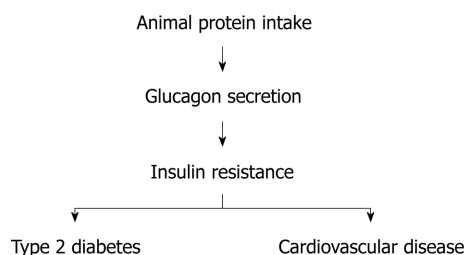


Figure 1 A simplified proposed mechanism underlying vascular disease associated with insulin resistance.

induces vasodilation. Acetylcholine is an endogenous transmitter that activates endothelial nitric oxide production by acting on muscarinic receptors. Acetylcholine induces endothelium-dependent vasodilation while exogenous sources of nitric oxide (such as nitroglycerin and sodium nitroprusside) induce endothelium-independent vasodilation. In response to increased blood flow, vascular smooth muscle cells normally relax to produce vasodilation and accommodate the elevated blood flow. Flow-mediated vasodilation is attributed to nitric oxide release by endothelial cells. The degree of flow-mediated vasodilation is considered a measure of endothelium-dependent vasodilation and can be determined by ultrasonography performed at the brachial artery^[13-15].

A number of investigations show that insulin resistance is independently associated with blunted flow-mediated arterial vasodilation in asymptomatic healthy individuals compared to control subjects^[5,16,17].

Similarly, insulin resistance is associated with limited vasodilation in response to metacholine chloride, a muscarinic agent. The increment in blood flow in response to metacholine is lower in insulin-resistant subjects compared to insulin-sensitive controls^[18].

Likewise, arterial response to exogenous sources of nitric oxide, such as nitroglycerin, sodium nitroprusside, and nitrates is impaired in subjects with insulin resistance compared to control subjects^[10,16,18].

Similarly to healthy subjects, flow-mediated vasodilation is defective in nondiabetic patients with coronary heart disease, compared to control subjects. On multivariate analysis, the extent of flow-mediated vasodilation is correlated with serum high-density lipoprotein (HDL)-c, but not with low-density lipoprotein (LDL)-c or total cholesterol levels^[10].

Impairment of flow-mediated vasodilation associated with insulin resistance is already apparent in childhood. Obese children show impaired arterial vasodilation compared to control children. Further, regular exercise over 6 mo restores abnormal vascular dysfunction in obese children. The improvement in flow-mediated vasodilation after 6-mo exercise program correlates with enhanced insulin sensitivity, reflected by reduced body mass index (BMI), waist-to-hip ratio, systolic blood pressure, fasting insulin, triglycerides, and LDL/HDL ratio^[19].

In normal weight and overweight adolescents, there is a gradual deterioration of flow-mediated vasodilation with worsening of insulin resistance evaluated by the euglycemic hyperinsulinemic clamp^[20].

INSULIN RESISTANCE IS INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL ARTERIAL STIFFNESS

Loss of distensibility of the arterial wall (arterial stiffness) leads to elevated systolic blood pressure and consequently increases cardiac afterload resulting in left ventricular hypertrophy that contributes to the development of congestive heart failure. In addition, arterial stiffness leads to reduced diastolic blood pressure, which may deteriorate diastolic coronary blood flow contributing to ischemic heart disease^[21,22] (Figure 3). Arterial stiffness is associated with wide pulse pressure (systolic blood pressure minus diastolic blood pressure)^[7,23].

Parameters that estimate arterial stiffness include blood pressure, pulse pressure, pulse-wave velocity, augmentation index, coefficients of distensibility and compliance, and the Young's elastic modulus, which includes intima-media thickness and estimates arterial stiffness controlling for arterial wall thickness^[6]. Pulse-wave velocity is the speed of the pressure wave generated by left ventricular contraction. Arterial stiffness impairs the ability of the arterial wall to cushion the pressure wave and increases pulse-wave velocity^[21]. Augmentation is the pressure difference

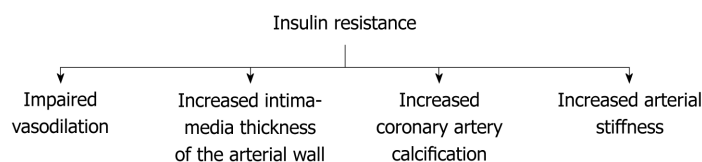


Figure 2 Pathophysiological changes associated with insulin resistance-mediated vascular disease.

between the second and first systolic peaks of the central arterial pressure waveform. Increased augmentation reflects arterial stiffness^[24,25]. The augmentation index has been defined as augmentation divided by pulse pressure, being a measure of peripheral wave reflection. A higher augmentation index reflects increased arterial stiffness^[26,27].

Age is consistently associated with arterial stiffness, but the loss of arterial elasticity related with age is not justified by conventional cardiovascular risk factors. Insulin resistance becomes deeper with age and may be a major pathophysiological determinant of arterial stiffness in the elderly population^[12,28,29].

Numerous investigations document an association between insulin resistance and subclinical arterial stiffness in nondiabetic individuals across all ages. Arterial stiffness related to insulin resistance begins early in life and progresses in asymptomatic subjects during a latent period of time before the diagnosis of cardiovascular disease. Subclinical arterial stiffness associated with insulin resistance strongly predicts future cardiovascular events. Conventional cardiovascular risk factors do not explain the loss of arterial elasticity related to insulin resistance^[7,22].

Arterial stiffness is apparent in asymptomatic subjects with insulin resistance ascertained either by its clinical expression, the metabolic syndrome, or by estimates of insulin sensitivity.

Estimates of insulin resistance are associated with subclinical arterial stiffness

In a variety of population groups, insulin resistance identified by different estimates is consistently associated with measures of arterial stiffness independently of classic cardiovascular risk factors (Table 1).

The Atherosclerosis Risk in Communities study is a prospective population-based trial with African American and Caucasian participants. A cross-sectional analysis showed an independent association between insulin resistance (assessed by glucose tolerance tests) and arterial stiffness. Subjects with insulin resistance had stiffer arteries compared to those with normal glucose tolerance after adjustment for confounding factors^[6].

Similarly, insulin resistance (glucose tolerance tests) in individuals from the general population was independently associated with arterial stiffness estimated by distensibility and compliance of the carotid, femoral and brachial arteries, compared to normal glucose tolerance. Arterial stiffness worsened with deteriorating glucose tolerance^[22].

Comparable findings were obtained in healthy Chinese subjects. Insulin resistance (impaired glucose tolerance) was independently associated with arterial stiffness (estimated by brachial-ankle pulse-wave velocity) compared to normal glucose tolerance. Normoglycemic subjects with altered glucose metabolism have increased arterial stiffness^[30].

Likewise, arterial stiffness (brachial artery pulse-wave velocity) is positively correlated with postprandial glucose and negatively correlated with plasma adiponectin level, suggesting that arterial stiffness is greater in patients with insulin resistance compared to those with normal glucose tolerance^[17].

Assessment of insulin resistance with the euglycemic hyperinsulinemic clamp is also independently associated with subclinical arterial stiffness of the common carotid and femoral arteries evaluated by pulse-wave velocity in asymptomatic healthy adults^[21]. In patients with hypertension, insulin resistance (glucose tolerance tests) is independently associated with arterial stiffness (carotid-femoral pulse-wave velocity and pulse pressure) as well^[31,32].

Several studies document an association between insulin resistance evaluated by the homeostasis model assessment (HOMA) index and arterial stiffness in asymptomatic individuals from different population groups. In healthy subjects and in Korean post-menopausal women, insulin resistance is independently associated with increased arterial stiffness (evaluated by brachial-ankle, aortic and peripheral pulse-wave velocity). Arterial stiffness increases sequentially with the degree of insulin resistance^[33,34]. Analogous findings are observed in normotensive normoglycemic first-degree relatives of patients with diabetes. Arterial stiffness

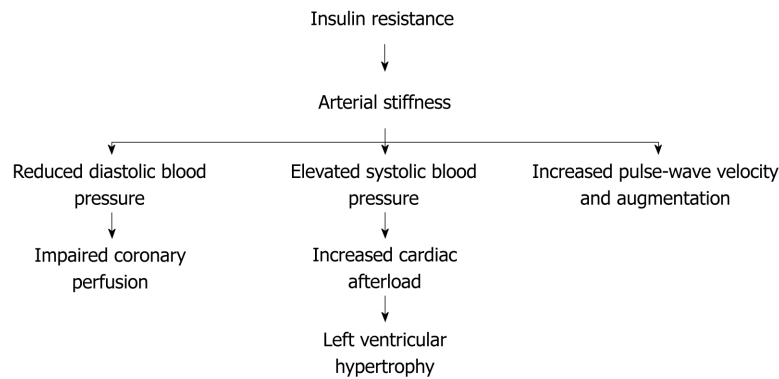


Figure 3 Cardiovascular disease associated to arterial stiffness.

(carotid-femoral pulse-wave velocity) is increased in the relatives with insulin resistance compared to those more insulin-sensitive^[35]. Insulin resistance and arterial stiffness (augmentation index and pulse-wave velocity) were compared in Indigenous Australians (a population group with elevated rate of T2D) and European Australians. The Indigenous population group had higher HOMA-IR values and increased arterial stiffness compared to their European counterparts, suggesting that intensified insulin resistance among Indigenous participants contributes to explain increased arterial stiffness in this group^[4].

Subclinical arterial stiffness is already present in children and adolescents with insulin resistance, compared to insulin-sensitive control subjects. In healthy children and adolescents from the general population of different countries, insulin resistance (HOMA-IR values) is independently associated with increased arterial stiffness evaluated by carotid-femoral pulse-wave velocity or brachial artery distensibility compared to control subjects^[11,36,37]. In obese children and adolescents, a profound independent effect of insulin resistance on vascular compliance has been observed. Insulin-resistant subjects (HOMA-IR) experience increased vascular stiffness (aortic pulse-wave velocity) compared to control individuals^[38-40]. In normal weight and overweight adolescents, insulin resistance assessed by euglycemic hyperinsulinemic clamp is associated with higher augmentation index, indicating that insulin resistance in adolescents is related to increased arterial stiffness^[20].

Clinical manifestations of insulin resistance are associated with subclinical arterial stiffness

The metabolic syndrome is a cluster of clinical features that reflects insulin resistance, including obesity, systolic hypertension, dyslipemia (hypertriglyceridemia and low HDL-c), and hyperinsulinemia. The metabolic syndrome and its individual components have been independently associated with arterial stiffness. Patients with any clinical expression of insulin resistance experience subclinical arterial stiffness that is not explained by conventional cardiovascular risk factors. Arterial stiffness has been considered a further clinical manifestation of insulin resistance^[7] (Table 2).

The metabolic syndrome is associated with arterial stiffness: The longitudinal association between the metabolic syndrome and arterial stiffness was investigated in the Cardiovascular Health Study. Metabolic syndrome at baseline (obesity, systolic hypertension, hyperinsulinemia and hypertriglyceridemia) independently predicted increased arterial stiffness (aortic pulse-wave velocity) at follow-up^[28].

In the Atherosclerosis Risk in Communities study, the joint effect of elevated glucose, hyperinsulinemia and hypertriglyceridemia (reflecting insulin resistance) is independently associated with arterial stiffness in subjects from the general population^[6]. Similarly, the clustering of at least three components of the metabolic syndrome is related with increased carotid artery stiffness among healthy participants across all age groups in the Baltimore Longitudinal Study on Aging independently of other cardiovascular risk factors^[41].

Likewise, the metabolic syndrome is strongly and independently associated with reduced distensibility of the common carotid artery in healthy women from the general population^[9]. In 12517 subjects with no history of cardiovascular disease, systolic hypertension, obesity, hypertriglyceridemia, and hyperuricemia are independent determinants for arterial stiffness (brachial-ankle pulse-wave velocity) on multiple regression analysis^[29]. Arterial stiffness (augmentation index and pulse-wave velocity) was compared in Indigenous and European Australians. Factor

Table 1 Studies that find an independent association between insulin resistance and subclinical arterial stiffness unexplained by classic cardiovascular risk factors

Ref.	Population group	Insulin resistance	Arterial stiffness
Salomaa <i>et al</i> ^[6]	African American and Caucasian	IGT	Arterial compliance, Young's elastic modulus
Henry <i>et al</i> ^[22]	General population	IGT	Arterial compliance
Shin <i>et al</i> ^[30]	Healthy Chinese subjects	IGT	Brachial-ankle PWV
Liye <i>et al</i> ^[17]	IGT versus normal glucose tolerance	IGT, serum adiponectin levels	Brachial artery PWV
Giltay <i>et al</i> ^[21]	Healthy subjects	Hyperinsulinemic euglycemic clamp	Carotid-femoral PWV
Vyssoulis <i>et al</i> ^[32]	Patients with hypertension	IGT	Carotid-femoral PWV
Sengstock <i>et al</i> ^[31]	Patients with hypertension	Frequently sampled IV tolerance test	Aortic PWV, pulse pressure
Kasayama <i>et al</i> ^[33]	Healthy adults	HOMA	Brachial-ankle PWV
Park <i>et al</i> ^[34]	Postmenopausal women	HOMA-IR	Aortic and peripheral PWV
Maple-Brown <i>et al</i> ^[4]	Indigenous Australians	HOMA-IR	Augmentation index
Scuteri <i>et al</i> ^[35]	Family history of diabetes	HOMA-IR	Carotid-femoral PWV
Sakuragi <i>et al</i> ^[36]	Prepubescent children	HOMA-IR	Carotid-femoral PWV
Whincup <i>et al</i> ^[11]	British children	HOMA-IR	Brachial artery distensibility
Gungor <i>et al</i> ^[38]	Children and adolescents	HOMA-IR	Aortic PWV
Iannuzzi <i>et al</i> ^[39]	Children and adolescents	HOMA-IR	Aortic PWV
Tomsa <i>et al</i> ^[20]	Adolescents	Hyperinsulinemic euglycemic clamp	Augmentation index

IGT: Impaired glucose tolerance; PWV: Pulse-wave velocity; HOMA: Homeostasis model assessment; HOMA-IR: Homeostasis model assessment-insulin resistance.

analysis revealed that metabolic syndrome components clustered with Indigenous Australian participants. Arterial stiffness was more pronounced among Indigenous compared to European Australians^[4].

Subclinical arterial stiffness is already present in children and adolescents with the metabolic syndrome, suggesting that insulin resistance plays an important role in the early pathogenesis of vascular disease. British and Chinese children and adolescents with the metabolic syndrome have increased arterial stiffness compared to control children after adjustment for covariates. There is a strong graded inverse relationship between the number of metabolic syndrome components and brachial artery distensibility^[11,37]. In obese children, common carotid artery stiffness is more prominent in the group with the metabolic syndrome compared to the control group^[42]. Normoglycemic young adults (mean age 20 years) with a positive family history of T2D have higher BMI and fasting insulin and increased arterial stiffness (aortic pulse-wave velocity) than their counterparts without T2D relatives^[43]. The longitudinal relationship between the metabolic syndrome identified in childhood and arterial elasticity assessed in adulthood was investigated in a prospective population-based cohort study with 21 years of follow-up, the Cardiovascular Risk in Young Finns Study. Childhood metabolic syndrome (obesity, systolic hypertension, hypertriglyceridemia and hyperinsulinemia) predicts independently carotid artery stiffness in adulthood^[44].

Obesity is associated with arterial stiffness: Longitudinal and cross-sectional studies consistently show that measures of adiposity (BMI, waist circumference, waist-to-hip ratio, body fat, and abdominal fat) are independently associated with estimates of arterial stiffness in diverse population groups. This association is already apparent during childhood and cannot be explained by traditional cardiovascular risk factors. In a population-based setting, adulthood obesity (BMI and waist-to-hip ratio) is associated with increased stiffness of carotid, femoral, and brachial arteries after adjusting for cardiovascular risk factors. Arterial distensibility consistently decreased with higher BMI^[9,45]. Similarly, obesity (BMI and waist circumference) is independently related to increased arterial stiffness (augmentation index) in Indigenous Australians free of T2D compared to European Australians^[46]. In female twins, abdominal adiposity is a determinant of arterial stiffness (augmentation index) independent of genetic effects and other confounding factors^[47].

The association between adiposity parameters and increased arterial stiffness begins during childhood. In obese children, there is a marked effect of insulin resistance associated with obesity on vascular compliance. Obese children are more insulin-resistant and have stiffer arteries compared with lean controls^[39,40]. In a population-based setting, childhood obesity (BMI and waist circumference) is

Table 2 Studies that find an independent association between the clinical expression of insulin resistance and subclinical arterial stiffness unexplained by classic cardiovascular risk factors

Ref.	Population group	Insulin resistance	Arterial stiffness
Mackey <i>et al</i> ^[28]	Elderly	Metabolic syndrome	Aortic pulse-wave velocity
Salomaa <i>et al</i> ^[6]	General population	Metabolic syndrome Hyperinsulinemia	Arterial compliance, Young's elastic modulus
Scuteri <i>et al</i> ^[41]	Healthy subjects	Metabolic syndrome	Carotid artery stiffness
Van-Popele <i>et al</i> ^[9]	Women	Metabolic syndrome Obesity Dyslipemia	Carotid artery stiffness
Tomiyama <i>et al</i> ^[29]	Healthy subjects	Metabolic syndrome Systolic hypertension	Brachial-ankle pulse-wave velocity
Maple-Brown <i>et al</i> ^[4]	Indigenous versus European Australians	Metabolic syndrome	Augmentation index, pulse-wave velocity
Whincup <i>et al</i> ^[11]	British children	Metabolic syndrome Obesity Hyperinsulinemia	Brachial artery distensibility
Xi <i>et al</i> ^[37]	Chinese children	Metabolic syndrome	Brachial artery distensibility
Iannuzzi <i>et al</i> ^[42]	Obese children	Metabolic syndrome	Carotid artery stiffness
Hopkins <i>et al</i> ^[43]	Relatives of patients with type 2 diabetes	Metabolic syndrome	Aortic pulse-wave velocity
Juonala <i>et al</i> ^[44]	Children	Metabolic syndrome Hyperinsulinemia	Carotid artery stiffness
Zebekakis <i>et al</i> ^[45]	General population	Obesity	Carotid, femoral, and brachial arteries stiffness
Maple-Brown <i>et al</i> ^[46]	Indigenous versus European Australians	Obesity	Augmentation index
Greenfield <i>et al</i> ^[47]	Female twins	Abdominal obesity	Augmentation index
Sakuragi <i>et al</i> ^[35]	Children	Obesity Dyslipemia Hyperinsulinemia	Brachial artery distensibility
Gungor <i>et al</i> ^[38]	Adolescents and young adults	Obesity Dyslipemia	Aortic pulse-wave velocity
Jourdan <i>et al</i> ^[47]			
Urbina <i>et al</i> ^[49]			
Kappus <i>et al</i> ^[50]			
Wildman <i>et al</i> ^[51]	Young and older adults	Obesity	Aortic pulse-wave velocity
Iannuzzi <i>et al</i> ^[39]		Systolic hypertension	Aortic pulse-wave velocity
Kasayama <i>et al</i> ^[33]		Dyslipemia	
Cecejia <i>et al</i> ^[12]		Hyperinsulinemia	
Urbina <i>et al</i> ^[52]		Triglyceride/HDL-c	Aortic pulse-wave velocity

associated with increased arterial stiffness after adjustment for confounding factors. There is a strong graded inverse relationship between BMI and brachial artery distensibility. This association is apparent even at BMI levels below those considered to represent obesity^[11,36]. Similar results are observed in adolescents and young adults. Obesity is associated with subclinical arterial stiffness independently of cardiovascular risk factors^[38,48-50].

The association between obesity and arterial stiffness (aortic pulse-wave velocity) was evaluated in young adults (20 to 40 years, 50% African American) and older adults (41 to 70 years, 33% African American). Obesity parameters (BMI, waist circumference, hip circumference, and waist-to-hip ratio) were strongly correlated with higher aortic pulse-wave velocity, independently of risk factors. Obesity is an independent and strong predictor of aortic stiffness for both races and age groups^[51].

Systolic hypertension, dyslipemia, and hyperinsulinemia are associated with arterial stiffness: Other clinical manifestations of insulin resistance, including systolic hypertension^[12,29,39,40], dyslipemia^[9,33,36,38-40,52], and hyperinsulinemia^[6,11,36,40,44] are also consistently associated with different measures of arterial stiffness independently of other cardiovascular risk factors, in diverse population groups, across all ages. Longitudinal studies such as the Atherosclerosis Risk in Communities study and the Multi-Ethnic Study of Atherosclerosis have shown that arterial stiffness predicts the development of systolic hypertension^[53,54]. In healthy subjects 10 to 26 years old,

triglyceride-to-HDL-c ratio is an independent predictor of arterial stiffness after adjustment for cardiovascular risk factors, particularly in the obese. Arterial stiffness rose progressively across tertiles of triglyceride-to-HDL-c ratio^[52].

INSULIN RESISTANCE IS INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL STRUCTURAL CHANGES OF THE ARTERIAL WALL

Similarly to arterial stiffness, a gradual increase in carotid intima-media thickness occurs with age. A systematic review documents a strong association between age and carotid intima-media thickness in healthy subjects and individuals with cardiovascular disease. This relationship is not affected by cardiovascular risk factors. Ageing is associated with magnification of insulin resistance that may explain the increase in intima-media thickness^[55].

Insulin resistance either ascertained by estimates or by its clinical expression is associated with increased intima-media thickness and increased calcification of the arterial wall in asymptomatic subjects. This association is not mediated by classic cardiovascular risk factors, suggesting that insulin resistance plays a crucial role in the development of initial vascular damage (Table 3).

Estimates of insulin resistance are associated with increased thickness of the arterial wall and increased coronary calcification

Increased thickness of the arterial wall: In healthy subjects from the Kuopio Ischemic Heart Disease Risk Factor study, insulin resistance was determined by the euglycemic hyperinsulinemic clamp technique and the presence of subclinical vascular disease in the femoral and carotid arteries was evaluated by ultrasonography. Subjects with asymptomatic vascular disease were more insulin-resistant compared to control subjects^[56].

The association between insulin resistance and subclinical vascular disease was confirmed in healthy Swedish men. Insulin resistance was determined by the hyperinsulinemic euglycemic clamp in subjects with high cardiovascular risk (hypercholesterolemia, smoking) and subjects with no cardiovascular risk factors. Asymptomatic vascular disease was evaluated by B-mode ultrasound of the common carotid artery. A negative correlation between insulin sensitivity and carotid intima-media thickness was observed in both population groups (high and low cardiovascular risk). Participants with insulin resistance had greater carotid wall thickness compared to insulin-sensitive subjects^[57].

A similar association between insulin resistance and subclinical vascular disease (increased intima-media thickness of the arterial wall) was observed in healthy Caucasian participants of the Insulin Resistance Atherosclerosis Study. Insulin sensitivity was evaluated by the frequently sampled intravenous glucose tolerance test with analysis by the minimal model of Bergman. Asymptomatic vascular disease was assessed by the measurement of intima-media thickness of the carotid artery by B-mode ultrasonography. In Caucasian men, insulin resistance is associated with a subclinical increase in carotid intima-media thickness, after adjustment for traditional cardiovascular risk factors^[58].

The independent association between insulin resistance (HOMA-IR) and subclinical vascular disease (increased carotid intima-media thickness) has been confirmed in healthy subjects of four ethnic groups (non-Hispanic Whites, African-Americans, Hispanic Americans, and Chinese Americans) from the Multi-Ethnic Study of Atherosclerosis^[59].

In asymptomatic patients with impaired glucose tolerance, insulin resistance (calculated by the insulin sensitivity check index) is strongly associated with severe carotid atherosclerosis (assessed by ultrasonography) on multiple regression analysis after adjustment for confounders. Carotid intima-media thickness correlated inversely with insulin sensitivity^[60].

The association between insulin resistance and asymptomatic increased intima-media thickness is apparent in childhood. In healthy children, insulin resistance measured with the euglycemic hyperinsulinemic clamp is associated with higher carotid intima-media thickness^[61]. Likewise, obese children aged 6-14 years with higher HOMA-IR had increased carotid intima-media thickness compared to control children^[39].

Increased coronary artery calcification: Insulin resistance is also associated with subclinical coronary artery calcification. Asymptomatic subjects with insulin resistance (HOMA-IR) have increased coronary calcification score (derived from

Table 3 Studies that find an independent association between insulin resistance and subclinical vascular calcification or increased intima-media thickness of the arterial wall unexplained by traditional cardiovascular risk factors

Ref.	Population group	Insulin resistance	Vascular disease
Laakso <i>et al</i> ^[56]	Healthy subjects	Euglycemic hyperinsulinemic clamp	Increased carotid IMT
Agewall <i>et al</i> ^[57]	Healthy men	Euglycemic hyperinsulinemic clamp	Increased carotid wall thickness
Howard <i>et al</i> ^[58]	Healthy Caucasians	Frequently sampled IV glucose tolerance test	Increased carotid IMT
Bertoni <i>et al</i> ^[59]	Multiethnic healthy subjects	HOMA-IR	Increased carotid IMT, elevated coronary calcium
Rajala <i>et al</i> ^[60]	Healthy subjects	Insulin sensitivity check index	Increased carotid IMT
Iannuzzi <i>et al</i> ^[39]	Obese children	HOMA-IR	Increased carotid IMT
Ryder <i>et al</i> ^[61]	Healthy children	Euglycemic hyperinsulinemic clamp	Increased carotid IMT
Arad <i>et al</i> ^[62]	Healthy subjects	HOMA-IR	Elevated coronary calcium score
Ong <i>et al</i> ^[63]	Healthy subjects	HOMA-IR	Elevated coronary calcium score
Meigs <i>et al</i> ^[64]	Healthy subjects	Glucose tolerance tests	Coronary artery calcification
Dabelea <i>et al</i> ^[65]	Healthy and type 1 diabetes children	Glucose disposal rate	Coronary artery calcification
Reilly <i>et al</i> ^[66]	Family history of cardiovascular disease	HOMA-IR	Coronary artery calcification
Qasim <i>et al</i> ^[67]	Family history of cardiovascular disease	HOMA-IR	Coronary artery calcification
Young <i>et al</i> ^[68]	Patients with coronary artery disease	Glucose tolerance test	Coronary artery calcification
Shinozaki <i>et al</i> ^[69]	Family history of cardiovascular disease	Glucose tolerance test	Coronary artery calcification

HOMA-IR: Homeostasis model assessment-insulin resistance; IMT: Intima-media thickness.

electron-beam computed tomography) that is not explained by traditional cardiovascular risk factors^[59,62,63].

In the Framingham Offspring Study, there is a graded increase in subclinical coronary artery calcification with worsening insulin resistance (impaired glucose tolerance) among asymptomatic subjects^[64].

The association between insulin resistance (estimated glucose disposal rate) and coronary artery calcification was examined among patients with type 1 diabetes and healthy subjects in the Coronary Artery Calcification in Type 1 Diabetes study. Insulin resistance was independently associated with coronary artery calcification (electron-beam computed tomography) in both population groups^[65].

In the Study of Inherited Risk of Coronary Atherosclerosis, insulin resistance (HOMA-IR) is associated with coronary artery calcification after adjustment for confounding factors in asymptomatic subjects with a family history of premature cardiovascular disease. The HOMA-IR index predicts coronary artery calcification scores beyond other cardiovascular risk factors in this population group^[66,67].

In normoglycemic patients with coronary artery disease, insulin resistance (glucose tolerance tests) is associated with severity of the coronary disease documented by coronary arteriography compared to control subjects. Nondiabetic patients with coronary artery disease are insulin-resistant compared to control subjects^[68,69].

Clinical manifestations of insulin resistance are associated with subclinical structural damage to the arterial wall

The metabolic syndrome and its individual components are associated with subclinical structural vascular disease that is not explained by conventional cardiovascular risk factors.

The metabolic syndrome: In healthy participants of several studies, including the Atherosclerosis Risk in Communities study, the Baltimore Longitudinal Study on Aging study, and the Multi-Ethnic Study of Atherosclerosis, the metabolic syndrome is independently associated with asymptomatic increased carotid intima-media thickness across all age groups and ethnicities^[6,41,59,70]. Likewise, the metabolic syndrome is associated with coronary artery calcification independently of other cardiovascular risk factors in asymptomatic subjects with a family history of premature cardiovascular disease participants of the Study of Inherited Risk of Coronary Atherosclerosis^[66].

Subclinical vascular damage is detectable at young age in the presence of metabolic

syndrome. Asymptomatic carotid intima-media thickness is increased in children with metabolic syndrome as compared with healthy control children, after adjustment for confounders^[71]. Regular exercise over 6 mo improves the metabolic syndrome and reduces carotid intima-media thickness in obese children compared to control subjects^[19].

In analyses from four cohort studies (Cardiovascular Risk in Young Finns study, Bogalusa Heart study, Princeton Lipid Research study, Insulin study) with a mean follow-up of 22.3 years, the presence of the metabolic syndrome during childhood is associated with higher carotid intima-media thickness in adulthood^[72].

In the Bogalusa Heart study, postmortem examinations performed in children and adolescents from a biracial (African American and Caucasian) community showed that the antemortem presence of the metabolic syndrome (obesity, dyslipemia, and hypertension) strongly predicted the extent of vascular disease in the aorta and coronary arteries^[73].

In the Pathobiological Determinants of Atherosclerosis in Youth study, arteries collected from autopsies aged 15-34 years whose deaths were accidental showed that vascular disease in the aorta and right coronary artery is associated with the presence of impaired glucose tolerance, obesity, hypertension, and low HDL-c level. This association is not explained by hypercholesterolemia or smoking^[74].

Obesity: In healthy asymptomatic adults, greater BMI and waist-to-hip ratio are independently associated with increased carotid intima-media thickness^[70,75]. Increased diameter of the arterial wall associated with obesity is present in several areas of the arterial system, including carotid, femoral and brachial arteries. Across a wide age range, intima-media thickness of several arteries increased with higher BMI in a population-based sample of participants^[45].

The independent relationship between obesity and subclinical increased intima-media thickness of carotid and femoral arteries is present in children and adolescents. Obese children have increased carotid and femoral intima-media thickness compared to control children^[39,48,50]. In a prospective cohort of children and adolescents, BMI assessed at 11, 15, and 18 years was associated with higher carotid intima-media thickness after controlling for confounders. Overweight/obese subjects had higher carotid intima-media thickness compared to subjects with normal BMI^[76].

In analyses from four cohort studies (Cardiovascular Risk in Young Finns study, Bogalusa Heart study, Princeton Lipid Research study, Insulin study) with a mean follow-up of 22.3 years, childhood BMI was associated with higher carotid intima-media thickness in adulthood^[72].

Systolic hypertension, dyslipemia, and hyperinsulinemia: Fasting hyperinsulinemia is independently associated with greater carotid intima-media thickness and coronary artery calcification in asymptomatic healthy subjects^[6,62,64,70]. The association between hyperinsulinemia and increased carotid intima-media thickness is similar in African American and Caucasian subjects^[6,70]. The Mexico City Diabetes study investigated the longitudinal relationship between systolic hypertension and vascular damage in a population-based prospective trial. In normotensive subjects who progress to hypertension (prehypertensive subjects), baseline carotid intima-media thickness increased in comparison with subjects who remained normotensive. After adjusting for multiple cardiovascular risk factors, converter status was independently associated with a higher carotid intima-media thickness^[77].

Autopsy examinations from the Pathobiological Determinants of Atherosclerosis in Youth study show that systolic hypertension is associated with greater vascular injury in both the aorta and right coronary artery (particularly fibrous plaques) in subjects throughout the 15-34 year age span. The association of hypertension with vascular damage remained after adjusting for BMI and glycohemoglobin^[74].

Longitudinal autopsy studies conducted in children and adults show that low HDL-c is independently associated with vascular disease. The degree of vascular lesions in both the aorta and right coronary artery is negatively associated with serum HDL-c on multiple regression analysis^[73,74,78,79].

SUBCLINICAL VASCULAR DISEASE ASSOCIATED WITH INSULIN RESISTANCE PREDICTS CARDIOVASCULAR DISEASE

Subclinical structural and functional vascular dysfunction associated with insulin resistance in otherwise healthy subjects is highly predictive of future cardiovascular events. Reduced vasodilation, loss of arterial distensibility, and increased arterial

intima-media thickness in asymptomatic subjects are all associated with future cardiovascular disease.

In a systematic review and meta-analysis of prospective studies, impaired brachial flow-mediated vasodilatation was associated with future cardiovascular events both in asymptomatic and diseased population groups^[15]. Impaired nitroglycerin-mediated vasodilatation of the brachial artery has been independently associated with coronary artery calcification in a population-based study^[80]. In a prospective study, impaired coronary vasoreactivity was independently associated with a higher incidence of cardiovascular events. Baseline coronary vasoreactivity in response to several stimuli (acetylcholine, sympathetic activation, increased blood flow, and nitroglycerin) predicted incident cardiovascular events at follow-up, after adjustment for traditional cardiovascular risk factors^[81].

The ability of arterial stiffness to predict cardiovascular events independently of other cardiovascular risk factors has been documented in cross-sectional and prospective studies, systemic reviews and meta-analyses.

Prospective studies show that increased arterial stiffness (estimated by wide pulse pressure, carotid-femoral pulse-wave velocity, and common carotid distensibility) is a powerful predictor of incident cardiovascular events in asymptomatic individuals from the general population, patients with hypertension, subjects with impaired glucose tolerance, and patients with T2D beyond classic cardiovascular risk factors^[82-84]. A systematic review of cross-sectional studies concludes that arterial stiffness is highly predictive of cardiovascular events^[12]. A systematic review and meta-analysis of longitudinal studies that followed-up 15877 subjects for a mean of 7.7 years concludes that aortic stiffness (expressed as aortic pulse-wave velocity) is a strong predictor of future cardiovascular events, cardiovascular mortality, and all-cause mortality, independently of classic cardiovascular risk factors. The predictive value of increased arterial stiffness is larger in patients with higher baseline cardiovascular risk states, such as renal disease, coronary artery disease, or hypertension compared with low-risk subjects (general population)^[85].

The prospective association between arterial stiffness and postmortem vascular damage was investigated among elderly subjects. There was a weak correlation between baseline arterial stiffness (pulse-wave velocity) and the degree of vascular damage observed at autopsy^[86].

A large cross-sectional study with 10828 participants investigated the ability of brachial-ankle pulse-wave velocity for screening cardiovascular risk in the general population. On multivariate analysis, brachial-ankle pulse-wave velocity was associated with cardiovascular risk independently from conventional risk factors^[87].

In a population-based cohort study in the elderly (Rotterdam study), arterial stiffness had a strong positive association with structural vascular disease. Aortic and carotid stiffness (assessed by carotid-femoral pulse-wave velocity and common carotid distensibility) was associated with carotid intima-media thickness after adjustment for cardiovascular risk factors^[88].

Subclinical carotid intima-media thickness predicts cardiovascular events in healthy subjects and patients with coronary artery disease. A systematic review and meta-analysis concluded that carotid intima-media thickness is a strong independent predictor of future vascular events, although data for younger individuals are limited^[89]. A prospective cohort study of women shows that increased carotid intima-media thickness predicts cardiovascular events during 7-year follow-up regardless of glucose tolerance and other cardiovascular risk factors^[90]. In a systematic review, population groups with cardiovascular disease had a higher carotid intima-media thickness compared to population groups free of cardiovascular disease^[55].

CONCLUSION

Numerous studies provide compelling evidence of an association between insulin resistance and subclinical cardiovascular disease that is not explained by traditional cardiovascular risk factors, such as hypercholesterolemia or smoking. Pathogenic mechanisms underlying vascular damage linked to insulin resistance are undefined. Vascular injury associated with insulin resistance begins early in life and includes impaired vasodilation, loss of arterial distensibility, increased intima-media thickness of the arterial wall and increased arterial calcification. Subclinical vascular dysfunction associated with insulin resistance in otherwise healthy subjects is highly predictive of future cardiovascular events. Reduced vasodilation, loss of arterial distensibility, increased arterial intima-media thickness and vascular calcification in asymptomatic subjects are associated with future cardiovascular disease.

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Retrospective Cohort Study

New results on the safety of laparoscopic sleeve gastrectomy bariatric procedure for type 2 diabetes patients

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Abstract

BACKGROUND

It has been established that bariatric surgery, including laparoscopic sleeve gastrectomy (LSG), has a positive impact on type 2 diabetes mellitus (T2DM). However, less frequently T2DM is reported as a risk factor for complications with this type of surgery.

AIM

To evaluate the safety of LSG in T2DM.

METHODS

A retrospective cohort study was conducted over patients admitted for LSG from January 2008 to May 2015. Data was collected through digitized records. Any deviation from normal postoperative care within the first 60 d was defined as an early complication, and further categorized into mild or severe.

RESULTS

Nine hundred eighty-four patients underwent LSG, among these 143 (14.5%) were diagnosed with T2DM. There were 19 complications in the T2DM group (13.3%) compared to 59 cases in the non-T2DM (7.0%). Out of 19 complications in the T2DM group, 12 were mild (8.4%) and 7 were severe (4.9%). Compared to the non-T2DM group, patients had a higher risk for mild complications (Odds-ratio 2.316, CI: 1.163-4.611, $P = 0.017$), but not for severe ones ($P = 0.615$). An increase of 1% in hemoglobin A1c levels was associated with a 40.7% increased risk for severe complications ($P = 0.013$, CI: 1.074-1.843) but not for mild ones.

CONCLUSION

Our data suggest that LSG is relatively safe for patients with T2DM. Whether pre-operative control of hemoglobin A1c level will lower the complications rate has to be prospectively studied.

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Core tip: Laparoscopic sleeve gastrectomy is the most popular bariatric procedure worldwide today. Its impact among diabetic patients has been beneficial regarding diabetes control. This study is the first to examine the safety of the procedure in this subgroup of the population. We found that the diagnosis of diabetes mellitus is associated with an increased rate of mild postoperative complications but not with severe ones. Elevated hemoglobin A1c is a good predictor for the risk of severe complications.

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INTRODUCTION

Bariatric surgery is the standard of care for obesity and related morbidity. In the past few years, the most popular procedure worldwide has become laparoscopic sleeve gastrectomy (LSG) and accounting for more than 50% of bariatric procedures in the United States since 2014^[1,2]. In addition, bariatric surgery has proved to be the only modality that has the potential to achieve complete remission of type 2 diabetes mellitus (T2DM), and its advantage over conservative therapy is significant^[3-6]. The Diabetes Surgery Summit of experts in Rome in 2007, the American Diabetes Association in 2009, and the International Diabetes Federation in 2011 published guidelines to consider laparoscopic bariatric surgery as a treatment for T2DM^[4,7].

T2DM is a proven risk factor for postoperative complications in other fields of surgery^[8-12]. There is likely no single mechanism to explain this increased risk but wound healing, re-epithelization, angiogenesis, inflammatory response, pain, and coagulopathy are all negatively affected by T2DM. In addition, the risk for renal, cardiovascular, and respiratory failure is increased in T2DM patients undergoing surgery^[13-22]. In bariatric procedures involving stapling of the gastrointestinal tract, specifically gastric bypass, T2DM is a significant risk factor for re-admission and early complications in some reports but not in others^[23-31].

Nevertheless, reports of the contributing effects of T2DM in post-LSG complications are far and few between. As a result, the quoted risk in LSG for the general population was adopted for the T2DM subgroup and described in diabetes literature and guidelines as up to 15% for mild complications, 2%-6% for severe complications, and 0.1%-0.5% for mortality^[32]. Our hypothesis is that postoperative morbidity of LSG in T2DM patients is higher than that of patients without T2DM^[33]. The aim of this study is to examine the prevalence of complications after LSG in T2DM patients in comparison to control non-T2DM patients.

MATERIALS AND METHODS

Settings

A retrospective cohort study including all patients admitted for LSG from January 2008 to May 2015 at the Soroka University Medical Center (SUMC) was conducted. SUMC is a regional academic tertiary 1044-bed medical center in southern Israel, providing healthcare to a diverse population of one million.

Data sources

SUMC has been using digitized records, including in-hospital reports and outpatient follow-up (diagnoses, chronic diseases, surgical reports, BMI measurements, laboratory, and imaging tests) since 2000. After approval from the local ethics committee, data regarding patients in our study were gathered using this database.

Definitions

Complication: Any deviation from normal postoperative course in the first 60 d was defined as an early complication. An admission longer than 5 d and readmissions or reoperations within 60 d after surgery were considered a complication and were reviewed. In such cases, the record was expeditiously inspected in order to classify the exact type (leak, bleeding, stricture, dysphagia, renal and respiratory failure, or other) and the grade of the complication. The complications were graded using the conventional Clavien-Dindo (CD) classification system for postoperative complications^[34]. In this study, severe complications are defined as CD 3b or higher (requiring intervention under general anesthesia, ICU hospitalization, multiorgan failure, or death). Complications graded as CD 3a or below (any deviation from the normal postoperative course requiring any drug therapy, parenteral nutrition, blood transfusion, or intervention not under general anesthesia) are defined as mild.

Type 2 diabetes: Patients are said to have T2DM if one of the following conditions is fulfilled: (1) diagnosis made by the general practitioner or taken from the admission note; (2) a diagnosis of complication of T2DM (ICD-9 code 2504 for diabetes with nephrotic manifestations, 2505 for diabetes with ophthalmic manifestations, 2506 for diabetes with neurological manifestations, 2507 for diabetes with peripheral circulatory disorders) was previously performed; and (3) hemoglobin A1c (HbA1c) level above 6.5% (48 mmol/mol) drawn 2 years prior to the operation.

Preoperative evaluation

In the bariatric practice, every patient with a BMI > 40 kg/m² is eligible for bariatric surgery. Additionally, patients with T2DM are considered for bariatric surgery with a BMI as low as 35 kg/m² or above. In Israel, a patient who is planned for a bariatric procedure needs approval of an institutional multidisciplinary committee that is composed by a bariatric surgeon, anesthesiologist, internist, nutritionist, and social worker. The patient was admitted the day before operation for final evaluation and preparation, which includes blood tests, preoperative anticoagulation therapy, fasting, and IV fluid administration.

Surgical technique

LSG was performed in a standardized fashion. At induction of anesthesia, a first-generation cephalosporin was given for prophylaxis. After peritoneal CO₂ insufflation unto 15 mmHg, 4 to 5 trocars were inserted through the abdominal wall. The greater omentum was dissected away from the gastric greater curvature. A bougie was then introduced by the anesthesiologist and positioned along the lesser curvature of stomach, as a template for gastric resection that starts about 5 cm above the pylorus, up proximally to the gastro-esophageal junction. Resection was performed with laparoscopic staplers fired along the greater curvature close to the bougie. Upon surgeon decision, staple line reinforcement technique was performed (with suture, bio-material, or none). A closed suction drain was positioned along the stomach stump. The bougie that was inserted to the stomach by the anesthesiologist was withdrawn at the end of the operation.

Postoperative care and follow-up

In the first postoperative day the patient was encouraged to drink 300 mL of clear liquid, followed by unlimited drink in the second postoperative day. In the third postoperative day, the patient was instructed to ingest a liquid diet. On the third postoperative day, if vital signs were within normal limits, the patient was in well condition, did not complain of abdominal pain, maintained an acceptable liquid intake, and the suction drain was of serous content under 100 mL a day, he or she was discharged. All patients were advised by a dietitian about the recommended diet for the next month. Every patient was discharged with prophylaxis anticoagulation therapy for the first 30 postoperative days. Follow-up visits at the bariatric clinic for encounter with the surgeon were held at 1 wk, 4 mo, 8 mo, and 1 year post-op.

T2DM patients were routinely followed in the pre-, intra-, and post-operative periods for plasma glucose levels. Insulin therapy was administered if needed in order to control levels below 180 mg/dL.

Statistical analysis

All analyses were performed using IBM SPSS Statistics, version 24.0 (Armonk, NY, United States, IBM Corp). All tests were two-tailed and were considered significant at $P \leq 0.05$. Baseline clinical and demographic variables were compared between study groups by Chi-square for categorical variables or *t*-test for continuous variables. We used chi-square or binomial logistic regression to examine the association between total, mild, and severe early complications as dependent variables and the following

independent variables: diabetes type 2, fasting glucose level, and HbA1c. In addition, the following independent variables were examined as well: age, gender, BMI, previous bariatric surgery, concomitant removal of gastric banding, length of operation, surgeon identity, cumulative surgeon experience for each case, and comorbidities including hypertension, chronic ischemic heart disease, dyslipidemia, smoking status, asthma, sleep apnea, and fatty liver. In the next stage we used multivariate binomial logistic regression with stepwise method to assess the variables presenting statistical significance in the univariate study ($P < 0.05$), and the possible interactions were considered.

RESULTS

In a seven-year study period, 984 patients underwent LSG (66.2% were women). Mean age and BMI were 39.2 ± 12.2 years and 41.7 ± 5.9 kg/m², respectively. There were 143 patients (14.4%) with T2DM. Only one mortality (0.1%) was reported from the whole cohort and occurred at postoperative day 56 in the non-T2DM group. Unfortunately, we could not find any information about any illnesses before the death of the patient, and that raises the suspicion that it was not caused by a medical condition.

Patient characteristics differed among the study and control group in a few variables. The majority of the T2DM patients were male, older, and had a higher prevalence of essential hypertension, dyslipidemia, chronic ischemic heart disease, and sleep apnea.

Fasting plasma glucose (FPG) levels were available for 560 patients (56.9%) of the total study population, of which 112 were T2DM patients (78.3%). Mean FPG levels were 152 ± 51 mg/dL and 96 ± 13 mg/dL in the T2DM group and non-T2DM group, respectively ($P = 0.001$).

HbA1c levels were available for 286 patients (29.1%) of the total study population, of which 115 were T2DM patients (80.4%). Mean HbA1c levels were $7.6\% \pm 1.8\%$ (60 ± 15 mmol/mol) and $5.6\% \pm 0.5\%$ (38 ± 5 mmol/mol) in the T2DM group and non-T2DM group, respectively ($P = 0.001$).

Demographic, biometric, and morbidity characteristics are shown in Table 1. To note, chronic diseases such as anemia, smoking status, dyslipidemia, and chronic ischemic heart disease were reported by the general practitioner and collected from the patient's record.

Table 2 shows the complications by CD Classification. There were 78 early complications in this study (7.9%) with 44 (4.5%) of them categorized as mild and 34 (3.5%) of them categorized as severe. T2DM patients had significantly higher early complications compared to the non-T2DM patients (13.3% *vs* 7.0%, $P = 0.01$). When analyzing the subgroups of mild and severe complications, T2DM patients had significantly more mild complications (8.4% *vs* 3.8%, $P = 0.01$) but not severe complications (4.9% *vs* 3.2%, $P = 0.31$). In a multivariate binomial logistic regression for total early complications, we included T2DM, gender, age, dyslipidemia, hypertension, chronic ischemic heart disease, sleep apnea, and previous bariatric history. After a stepwise procedure, only T2DM was observed to be a significant factor for early complications (Odds ratio 2.031, CI: 1.171-3.522, $P = 0.012$). A similar result was observed for mild complications (Odds ratio 2.316, CI: 1.163-4.611, $P = 0.017$) but not for severe complications ($P = 0.615$).

When analyzing FPG level as an independent variable, it was not found to be significant for early complications ($P = 0.557$), mild complications ($P = 0.668$), or severe complications ($P = 0.701$). When setting a cut point of FPG level below 126 or equal and above 126, we did not find any significant differences in early complications ($P = 0.260$), mild complications ($P = 0.708$), or severe complications ($P = 0.230$).

When analyzing HbA1c as an independent variable, we found that for every elevation of 1% in HbA1c, there was an elevation of 1.314 in the risk for early complications ($P = 0.008$, CI: 1.074-1.609). A similar result of an elevation of 1.407 in risk was observed for severe complications ($P = 0.013$, CI: 1.074-1.843) but not for mild complications. Data on complication subtypes across study population are detailed in Table 3. Due to low event rates in these subgroups, only descriptive statistics are detailed.

DISCUSSION

Reports of early complications after bariatric surgery are abundant, specifically in

Table 1 Patient characteristics

	Non-type 2 diabetes group, n = 841, n (%) or mean (± SD)	Type 2 diabetes group, n = 143, n (%) or mean (± SD)	P-value
Female	573 (68.1)	78 (54.5)	0.02
Age, yr	38 (± 12.0)	48 (± 11.0)	< 0.01
BMI, kg/m ²	41.8 (± 5.9)	41.4 (± 6.1)	0.48
Operative time, min	55 (20.8)	57 (24.6)	0.57
Dyslipidemia	110 (13.1)	75 (52.4)	< 0.01
Essential hypertension	106 (12.6)	63 (44.1)	< 0.01
Chronic ischemic heart disease	10 (1.2)	10 (7.0)	< 0.01
Smoking status	89 (10.6)	22 (15.4)	0.09
Asthma	34 (4.0)	9 (6.3)	0.22
Sleep apnea	36 (4.3)	13 (9.1)	0.01
Anemia	15 (1.8)	4 (2.8)	0.41
Previous bariatric surgery	237 (29.0)	29 (20.3)	0.03
Hemoglobin A1c level % (± SD), mmol/mol (± SD)	5.6 (± 0.5), 38 (± 5.0)	7.6 (± 1.8), 60 (± 15.0)	< 0.01
Fasting plasma glucose mg/dL (± SD)	96 (± 13.0)	152 (± 51.0)	< 0.01

LSG. In large cohorts, the rate of early complications ranges from 5.4% to 7.3% and readmission rate within 30 d is 2.8%^[35-38]. The rate of severe complication in LSG is reported to be 1.2% to 2.2%. Our results show a higher rate of complications (7.9%) when compared to other studies and not only in T2DM patients. This discrepancy could be explained by the difference in definition of complication in each study. In this study we focused on the impact of the complication on general patient health and on the healthcare system rather than on the type of complication. For example, a leak is considered a formidable surgical complication, although in some cases it only moderately affects the patient, whereas a simple postoperative non-surgical complication such as pneumonia can lead to respiratory failure and death. We believe that this holistic approach is more instrumental for a non-surgeon professional, such as a general practitioner, endocrinologist, internist, or dietitian, when considering a bariatric surgery with the patient. In addition, because SUMC is the only medical center in southern Israel, every complicated case and readmission after surgery are seen at SUMC. Therefore, almost every complication is reported in this study. In other studies, it may be difficult to track complications after discharge of the patient due to the patient seeking treatment at a different facility.

For T2DM patients who undergo LSG, we found a significant increased risk for early complications, but this is significant only in the mild group and not in the severe group. This result is aligned with a retrospective Spanish-Portuguese multicenter study by Sánchez-Santos *et al*^[39]. Of 2882 patients, 29.2% of them were T2DM patients, and they found a significantly higher risk for early complications (Odds-ratio 1.48, CI: 1.12-1.95) in the T2DM group compared to the non-T2DM group. Mortality in the group of T2DM was increased as well in this study, but not in our study. In another study based on the American College of Surgeons-National Surgical Quality Improvement Program (ACS-NSQIP) database of 2012-2013, T2DM was associated with increased risk for re-admission during the first 30 postoperative days^[40].

In a study by Creange *et al*^[41] based on the American College of Surgeons-National Surgical Quality Improvement Program database of 2012, 941 out of 6062 LSG patients had T2DM (15.5%). As in our study, the T2DM patient group was more likely to be male and older. In contrast to our results, T2DM was not found to be associated with increased 30 d complication rate.

Aminian *et al*^[42] published an LSG risk calculator based on the same American College of Surgeons-National Surgical Quality Improvement Program 2012 database. In this analysis, type 1 and type 2 diabetes was found as a significant risk factor for 30 d complications. Creange *et al*^[41] state that this difference in results stems from the contribution of type 1 diabetes patients in the Aminian *et al*^[42] report. In our population, all patients with diabetes were diagnosed with T2DM.

This study is the first to assess the effect of FPG levels and HbA1c levels on 30 d complications after LSG. FPG was not found to affect 30 d complication rate, but it was found that any increase of 1% in HbA1c is associated with a significant increase of 31% in the risk for early postoperative complications. This result was maintained

Table 2 Mild and severe early complications (by Clavien-Dindo classification)¹, *n* (%)

Grade	Non-type-2-diabetes group, <i>n</i> = 841	Type 2 diabetes group, <i>n</i> = 143	<i>P</i> -value
1	14 (1.7)	6 (4.2)	0.01
2	15 (1.8)	6 (4.2)	
3a	3 (0.4)	0 (0.0)	
Mild (CD ≤ 3a)	32 (3.8)	12 (8.4)	
3b	15 (1.8)	4 (2.8)	
4a	7 (0.8)	1 (0.7)	0.31
4b	4 (0.5)	2 (1.4)	
5	1 (0.1)	0 (0.0)	
Severe (CD ≥ 3b)	27 (3.2)	7 (4.9)	
Total	59 (7.0)	19 (13.3)	0.01

¹This is a univariate analysis. CD: Clavien-Dindo.

when analyzing the subgroup of severe complications, but not for mild ones. The effect of elevated HbA1c upon early complications after surgery was reported in general surgery as a protective factor in some reports (probably due to heightened postoperative vigilance and lower threshold to treat hyperglycemia), or as risk factor in trauma surgery and several orthopedic procedures in other reports^[43-46]. In our study, the discrepancy between the result that elevated HbA1c is associated with higher risk for early postoperative complications (mild and severe) and the fact the T2DM as a disease by itself is not a risk factor for early complications (mild and severe) could be explained by the fact that many of the T2DM patients have balanced glucose levels, and the more important parameter when evaluating a patient is HbA1c level.

This study has several limitations. First, it is composed of retrospective data. This fact may be tempered by the large number of patients and the wide range of background variables that were collected. Second, despite comprehensive data collection, most patient records were not reviewed individually. Moreover, some data, such as reoperations or readmissions in other medical centers (even if they are part of the same medical insurance organization as our center) were not retrieved. Third, the definition of T2DM is mostly based on patient and primary physician report and not on detailed biochemical evaluation of every patient. This may lead to distortion in the distribution between the two groups. Fourth, only 56.9% of patients had FPG levels drawn and even less had reported HbA1c levels drawn (29.1% of total study population and 80.4% of T2DM group). In addition, this study does not analyze long-term complications in LSG in T2DM patients. The strengths are large sample size and the meticulous assessment of complications. Both of which enabled us to carefully inform the patient of the spectrum of complications that they may face.

Our data show an increased risk only for mild complications of LSG in T2DM patients. Together with extensive data on the chance of T2DM remission^[6], we believe there is good evidence that LSG is a relatively safe and effective option for these patients. In addition, increased HbA1c should be noted as a risk factor for severe complications and further studies are required in order to assess whether strict diabetic control prior to operation may lead to reduced postoperative complications.

Table 3 Early complications (by type), *n* (%)

Early complication by type	Non-type 2 diabetes group, <i>n</i> = 841	Type 2 diabetes group, <i>n</i> = 143
Staple line dehiscence and leak	16 (1.9)	2 (1.4)
Stricture and dysphagia	20 (2.4)	8 (5.6)
Bleeding	7 (0.8)	3 (2.1)
Acute renal failure	0 (0.0)	3 (2.1)
Respiratory failure	4 (0.5)	0 (0.0)
Other	11 (1.3)	3 (2.1)
Total	59 (6.9)	19 (13.2)

ARTICLE HIGHLIGHTS

Research background

Bariatric surgery has been advocated as an effective therapy for type 2 diabetes mellitus (T2DM) in an abundance of studies. Nevertheless, when considering a modality of treatment, its benefits should be weighed against its risks.

Research motivation

The risks that lie in bariatric surgery in the subgroup of T2DM have not been thoroughly investigated. Complications after other types of surgery within this subgroup of patients has led us to believe that post-bariatric surgery complication rates may be elevated in T2DM patients.

Research objectives

The main objectives of the study were to evaluate any kind of postoperative complications in the T2DM group *vs* non-T2DM patients within 60 d of surgery. Any deviation from the normal postoperative course was considered a complication. Further categorization into mild and severe complications was performed. This categorization was based upon Clavien-Dindo classification which is a common postoperative complications grading system.

Research methods

All patients who underwent laparoscopic sleeve gastrectomy performed by three surgeons in a single institute were included. Data was extracted from a digitized database through specific queries regarding length of stay, imaging, reoperations, and readmissions in the first 60 d after the operation. Mortality was extracted from that system as well. Any case of deviation from the average length of stay (more than 3 d after operation), further imaging (no imaging is routinely performed after operation), reoperation, or readmission was studied carefully in order to define the exact type of complication and categorize as mild or severe.

Research results

Nine hundred and eighty-four patients underwent laparoscopic sleeve gastrectomy, among these 143 (14.5%) were diagnosed with T2DM. There were 19 complications in the T2DM group (13.3%) compared to 59 cases in the non-T2DM (7.0%). Out of 19 complications in the T2DM group, 12 were mild (8.4%) and 7 were severe (4.9%). Compared to the non-T2DM group, patients had a higher risk for mild complications (Odds-ratio 2.316, CI: 1.163-4.611, $P = 0.017$), but not for severe ones ($P = 0.615$). Any increase of 1% in hemoglobin A1c levels was associated with a 40.7% increased risk for severe complications ($P = 0.013$, CI: 1.074-1.843).

Research conclusions

In this study, we find that the rate of mild complications is increased in T2DM patients. It means that these patients will suffer more from problems such as dysphagia, surgical site infection, dehydration, pneumonia, and bleeding. But these complications can be treated easily and conservatively without the need for interventions under general anesthesia, reoperations, or prolonged ICU admissions. Together with our knowledge of significant weight loss and reduction in glycemic burden after bariatric surgery, we believe that these complications should be well tolerated in face of the potential long-term benefit of this therapy in this subgroup of patients.

Research perspectives

Another result of our study, that any elevation of 1% in HbA1c levels is associated with a 40.7% increased risk for severe complications should commence a process of evaluating preoperative diabetes control. We believe that in a future study, patients with relatively high HbA1c level (above 9%) should have a short course of pre-operative tight glycemic control tested against patients who do not receive this preoperative intervention. This will also help us understand the pathophysiology of diabetes in surgical patients, and whether complications are driven purely from glycemic control or from chronic micro- and macro-vascular damage associated with diabetes.

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Observational Study

Quantities of comorbidities affects physical, but not mental health related quality of life in type 1 diabetes with confirmed polyneuropathy

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Abstract

BACKGROUND

A large number of adults with long-term type 1 diabetes are affected by symmetrical peripheral neuropathy. These complications increase socioeconomic expenses and diminish the individual quality of life. The 36-Item Short Form Health Survey (SF-36) is a generic patient reported questionnaire, measuring mental and physical health related quality of life. We hypothesized that diabetic neuropathy would decrease physical and mental quality of life measured with SF-36, and that clinical appearance may be associated with the decline.

AIM

To investigate if diabetic neuropathy would decrease physical and mental quality of life measured with SF-36, and if clinical appearance may be associated with the decline.

METHODS

Forty-eight adults [age 50 ± 9 years, 10 females, disease duration 32 (14-51) years] with verified diabetic symmetrical peripheral neuropathy and 21 healthy participants (age 51 ± 6 years, 6 females) underwent standardised nerve

subjects gave their informed consent prior to inclusion.

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conduction testing and completed the SF-36 questionnaire. Furthermore, disease duration, number of comorbidities, both diabetes related and nondiabetes related, vibration perception threshold, number of hypoglycaemic events, HbA1c and administration way of insulin was notified.

RESULTS

In comparison to healthy subjects, patients' mental composite score was not significantly diminished (51.9 ± 8.9 vs 53.1 ± 5.5 , $P = 0.558$), while the physical composite score was (46.3 ± 11.7 vs 54.6 ± 3.3 , $P = 0.002$). As expected, the overall physical health related symptoms in patients were associated to total number of comorbidities ($P < 0.0001$), comorbidities relation to diabetes ($P = 0.0002$) and HbA1c ($P = 0.005$) as well as comorbidities not related to diabetes ($P = 0.0006$).

CONCLUSION

The finding of this study emphasises the importance of focusing on quality of life in adults with diabetes and especially in those with multiple comorbidities as well as the possibility of HbA1c as a biomarker for severe complication.

Key words: Quality of life; 36-Item Short Form Health Survey (SF-36); Diabetes mellitus, Type 1; Diabetic neuropathies; Comorbidity

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Core tip: In this study, we found a diminishment of physical domains more so than mental components from the 36-Item Short Form Health Survey (SF-36), in 48 people with type 1 diabetes and verified diabetic symmetrical peripheral neuropathy when compared to 21 healthy controls. Additionally, this physical diminishment was associated with increases in number of comorbidities and HbA1c. To our knowledge a study of health related quality of life exclusively in people with type 1 diabetes and diabetic symmetrical peripheral neuropathy compared to healthy age-matched controls has not previously been performed, therefore these results are interesting for health care professionals interested in the connection between neuropathy and patient experience.

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INTRODUCTION

Diabetic symmetrical peripheral neuropathy is a frequent complication to type 1 diabetes, although the prevalence varies between countries^[1]. The pathogenesis of diabetic neuropathy is not fully understood; however, it is generally accepted to be a consequence of hyperglycaemic exposure leading to activation of metabolic, biochemical, inflammatory and immune mediated pathways. Clinically, neuropathy can present itself both with and without symptoms, including decreased sensation (numbness) or pain^[2,3]. The severity of peripheral neuropathy is associated with incapacitating complications and a shorter life expectancy for the individual. On top of this, research within the last years has focused increasingly on the physical as well as mental burden of diabetes on quality of life.

It has become more and more acknowledged in clinical practice, to evaluate patient centred outcomes in the measurement of disease burden, progression and treatment outcome and how these impacts fundamental health related quality of life (HRQoL). In an effort to standardise and examine HRQoL the development and validation of instruments, such as the 36-Item Short Form Health Survey (SF-36), enables comparisons of different conditions between diseases, populations and countries^[4]. The SF-36 has proved valid and useful in surveys of general and specific populations comparing the relative burdens of diseases, and in differentiating the health benefits produced by a wide range of treatments. Previous trans-sectional studies have investigated the HRQoL status in diabetes; however, most studies were carried out in

different types of diabetes and with mixed phenotypes. Thus, there is a knowledge gap in characterising the HRQoL in a population with type 1 diabetes and diabetic symmetrical peripheral neuropathy^[5,6].

We hypothesized that the presence of diabetic symmetrical peripheral neuropathy would decrease the individual physical and mental quality of life. Thus, the aim of this study was to compare the HRQoL in adults with type 1 diabetes as compared with healthy age-matched controls using the SF-36 questionnaire. In addition, in patients we wanted to investigate associations between HRQoL and: (1) disease duration; (2) number of comorbidities, both diabetes related and non-diabetes related; (3) vibration thresholds; (4) nerve conduction velocity of the efferent median nerve; (5) nerve conduction velocity of the afferent sural nerve; (6) number of hypoglycaemic events; (7) glycaemic state; and (8) the use of insulin pen or pump.

MATERIALS AND METHODS

Subjects

The study comprised baseline observations of 48 people with long-term type 1 diabetes and verified diabetic symmetrical peripheral neuropathy recruited at the Department of Endocrinology, Aalborg University Hospital, Denmark from June 2014 to March 2016, as part of a clinical trial investigating the effect of liraglutide on neuropathy (TODINELI trial, (EUDRA CT 2013-004375-12). The local ethics committee approved the study protocols (N-20130077). Inclusion criteria were adults (> 18 years) with type 1 diabetes for a minimum duration of two years and diabetic sensory neuropathy verified by nerve conduction velocity testing. Additional criteria has been described elsewhere^[7]. In comparison, 21 adult healthy, age-matched, participants were recruited for comparison. Informed consent was obtained from all individual participants included in the study.

Health quality and pain perception questionnaires

To assess HRQoL, participants completed the SF-36^[8-10]. Even though it is not specific for diabetes, it has been validated and the components are relevant to assess the symptom burden experienced in diabetes^[11]. The SF-36 was developed as a multipurpose eight-scale profile of functional health and well-being scores (physical functioning, role limitation due to physical problems, bodily pain, general health, vitality, social functioning, role limitation due to emotional problems, and mental health), and two summary scores (physical component summary and mental component summary), to explain variations in patient outcomes, covering 4 wk prior to the test^[8,12]. Scores from the 36 items are transformed to a 0-100 scale with higher scores equals better quality of life^[6,12]. A greater than five point change on this scale is considered clinically significant^[6].

Protocol

Health care professionals obtained information about disease duration, comorbidities and way of insulin intake. Vibration perception threshold was measured using a biothesiometer (Bio-Medical Instruments, Newbury, OH, United States) on the distal plantar surface of the big toes. Peripheral nerve conduction testing of the efferent median and afferent sural nerves was evaluated at the elbow and the ankle, respectively, with plastic bar electrodes at skin temperatures above 32 °C. A blood sample was taken for measurement of HbA1c (IFCC) and number of hypoglycaemic events was registered in a patient diary two days prior to the study day.

Statistical analysis

The statistical methods of this study were reviewed by all the authors. Normally distributed data was reported as means and standard deviations, non-normally distributed data as median and interquartile range while categorical data is provided as a percentage. An independent-sample *t*-test was undertaken to determine differences in HRQoL between the two groups. For parametric data, a Pearson's correlation tests were performed to investigate associations between HRQoL and disease duration, HbA1c, average vibration thresholds and nerve conduction velocity for the efferent median and sural nerves. For nonparametric data, a Spearman's correlation tests were performed to investigate associations between HRQoL and number of hypoglycaemic events, total number of comorbidities, diabetes related and non diabetes related comorbidities. Diabetes related includes hypertension, retinopathy, pain, albuminuria, erectile dysfunction and cardiovascular diseases. Non-diabetes related includes hypercholesterolemia, thrombose prophylaxis, operations, reflux, arthritis and arthroses, asthma and allergies, metabolic diseases, vitamin deficiencies and more. Additionally, an independent samples *t*-test was run

to determine if there was a difference between people with diabetes who used insulin pens or pump.

RESULTS

A total of 48 people with type 1 diabetes and 21 adult healthy volunteers were included in and completed the study. The demographic distribution is shown in [Table 1](#) and displays no notable difference in demographic characteristic between the two groups.

Comparison between type 1 diabetics and healthy controls

As seen in [Figure 1](#), when diabetes was present, a numerical decline was observed in every SF-36 domain, compared to healthy subject. Significant differences were found on physical functioning (78.6 ± 27.7 vs 96.7 ± 6.2 , $P = 0.005$), role limitation due to physical problems (82.4 ± 31.7 vs 100 ± 0 , $P = 0.01$), general health (64.4 ± 24.5 vs 85.3 ± 13.1 , $P < 0.001$), vitality (65.5 ± 23.9 vs 78.1 ± 13.9 , $P = 0.03$), role limitations due to personal or emotional problems (87.0 ± 27.3 vs 100 ± 0 , $P = 0.03$) and the physical composite score (46.3 ± 11.7 vs 54.6 ± 3.3 , $P = 0.002$). However, no significance was found looking at bodily pain (76.2 ± 24.34 vs 87.3 ± 17.7 , $P = 0.07$), social functioning (91.9 ± 13.9 vs 95.2 ± 15.0 , $P = 0.39$), mental health (81.2 ± 16.9 vs 86.7 ± 13.6 , $P = 0.20$) and the mental composite score (51.9 ± 8.9 vs 53.1 ± 5.5 , $P = 0.56$).

Associations

There was a negative association between the physical composite score of SF-36 and number of comorbidities ($r = -0.62$, $P < 0.001$), both diabetes ($r = -0.53$, $P = 0.018$) and non-diabetes related ($r = -0.51$, $P < 0.001$), and HbA1c level ($r = -0.41$, $P = 0.005$), as can be seen in [Figure 2](#). However, one of these were associated with the mental composite score of SF-36 ($P > 0.05$).

Additionally, physical parameters of physical function, role limitation due to physical health, bodily pain and general health were all associated to and number of comorbidities ($P < 0.01$), both diabetes ($P < 0.03$) and non-diabetes related ($P < 0.02$), while only physical function and bodily pain were associated to HbA1c ($P < 0.02$). More detail can be found in [Table 2](#).

Disease duration, vibration threshold, nerve conduction velocity of the efferent median nerve and the afferent sural nerve, and number of hypoglycaemic events were not associated with HRQoL scores. Additionally, there was no difference in symptoms between people using standard insulin pens and people using insulin pumps.

DISCUSSION

This study partly confirms our hypothesis, as in particular physical domains and not mental domains were negatively affected in people with diabetes and diabetic symmetrical peripheral neuropathy, potentially limiting the patients in their daily work and social activities. This emphasizes the importance of assessing HRQoL in long-term diabetes. Additionally, increased numbers of comorbidities and high levels of HbA1c, were associated with decreased HRQoL scores.

Decreased physical HRQoL

Decreased HRQoL is of great importance. It has been shown that a 1 point decrease in physical functioning and physical composite scores equals an 9% increase in mortality risk, a 4% increase in the risk of hospitalization within six months, and a 12% increase in the risk of being unable to work^[13]. As preventive medicine may be initiated in order to delay or reverse the negative impact on self-assessed health, the importance of assessing HRQoL is essential in monitoring the self-assessed burden of diabetes. Decreased HRQoL in people with diabetes has previously been shown in studies from Croatia, Norway and Australia^[5,6,14,15]. However, the previously investigated cohorts consisted of both type 1 and type 2 diabetes. As patients with type 2 diabetes often appear with other comorbidities and stereotypical life style, this can have negative impact on the combined HRQoL^[14]. In contrast, a study by Jacobson *et al*^[16] studied HRQoL in people with type 1 diabetes over an average of 23 years and found no decrease in HRQoL scores over time. The present study we looked into HRQoL in patients with type 1 diabetes compared to healthy and we found numerically decreased and clinically relevant declines in HRQoL for all sub-scores, except social functioning and a most significant decrease in the physical components. This finding,

Table 1 Demographics

	Type 1 diabetes (n = 48)	Healthy controls (n = 21)	P value
Gender, male n (%)	38 (79)	15 (71)	0.48
Age	50 ± 9	51 ± 6	0.53
Height	178.4 ± 1.2	179.9 ± 1.9	0.51
Weight	90.0 ± 2.3	87.3 ± 4.5	0.56
Right handed n (%)	41 (85)	17 (81)	0.64
Smoking n (%)	10 (21)	4 (19)	0.87
Disease duration	32 (14-51)		
HbA1c (IFCC), mmol/mol	65.5 ± 9.7		

Numerical data was compared with a *t*-test and categorical data with a χ^2 -test.

related to the mixed cohorts, is possibly because people with type 1 diabetes are found in all social groups, and hence also in all social groups with larger psychological resources in comparison to people with type 2 diabetes. Contrary, compared with the study by Jacobson *et al*^[16], we only had a cross-sectional look at HRQoL and therefore do not know the long-term ramifications for our patient group.

No decrease in mental HRQoL in people with severe diabetic neuropathy

Mental health has received increased recognition in recent years. Studies have shown that people with type 1 diabetes have a three-fold rate of depression in comparison to the general population. However, in the current study we did not find a significant decreased mental composite score, nor in the mental domains of social functioning and mental health.

Comorbidities decrease HRQoL

The presence of physical disabling diabetic complications such as cardiovascular events, gastrointestinal dysfunction and neuropathy with or without pain, have been shown to decrease HRQoL^[6,14,17,18]. In a cohort of people with type 1 diabetes who were followed over 6 years, disease duration and the presence of complications convincingly decreased the physical composite scores^[19], and the presence of neuropathy in type 1 diabetes negatively influenced the physical composite score^[20], in line with the impact on the sensory and motor system. Additionally, a study over 17 years in people with type 1 diabetes showed that development of microvascular complications significantly decreased HRQoL^[16]. We showed no association between the severity of neuropathy and the HRQoL scores, nor with disease severity and duration. These findings are plausibly biased by the fact that all patients were included based on severe polyneuropathy. In a study assessing HRQoL in chronic diseases with the presence of comorbidities, HRQoL was decreased due to the chronicity, but this was exaggerated as the number of comorbidities increased^[21]. In particular, Bjorner *et al*^[13] showed that number of comorbidities did not affect mental composite score. The current finding where HRQoL decreased with an increased number of comorbidities, needs to be considered when preventive medicine and adequate disease management is planned.

HbA1C decreases HRQoL

Even though HbA1c only indicates the preceding 3-mo' glycaemic status, it provides patients and clinicians with an objective measure. Mellerio *et al*^[22] studied the association between HbA1C and HRQoL in adult people with childhood onset type 1 diabetes. They concluded that no metabolic parameters, including HbA1c, was predictive of HRQoL, thereby indicating that social impact was more important than glycaemic control for the well-being^[22]. In contrast, the mean disease duration in this cohort was 32 years and we showed that HRQoL decreased as HbA1c increased, which may reflect lack of life-long tight glucose control. On the other hand, this could also reflect the impact of neuropathy and its effect on the ability to tightly control glucose, thus pointing to HbA1c as a potential marker of complications and not solely of glucose control. The data on the association between HRQoL and hypoglycaemia is conflicting. Hypoglycaemic events affects negatively on the individual health, and quality of life^[20]. However, in a larger cohorts of people with type 1 diabetes no association between hypoglycaemia and HRQoL was shown^[20,23]. In contrast, in younger individuals an association between role limitations due to physical problems and hypoglycaemic events was reported^[18]. Unfortunately, in the current study,

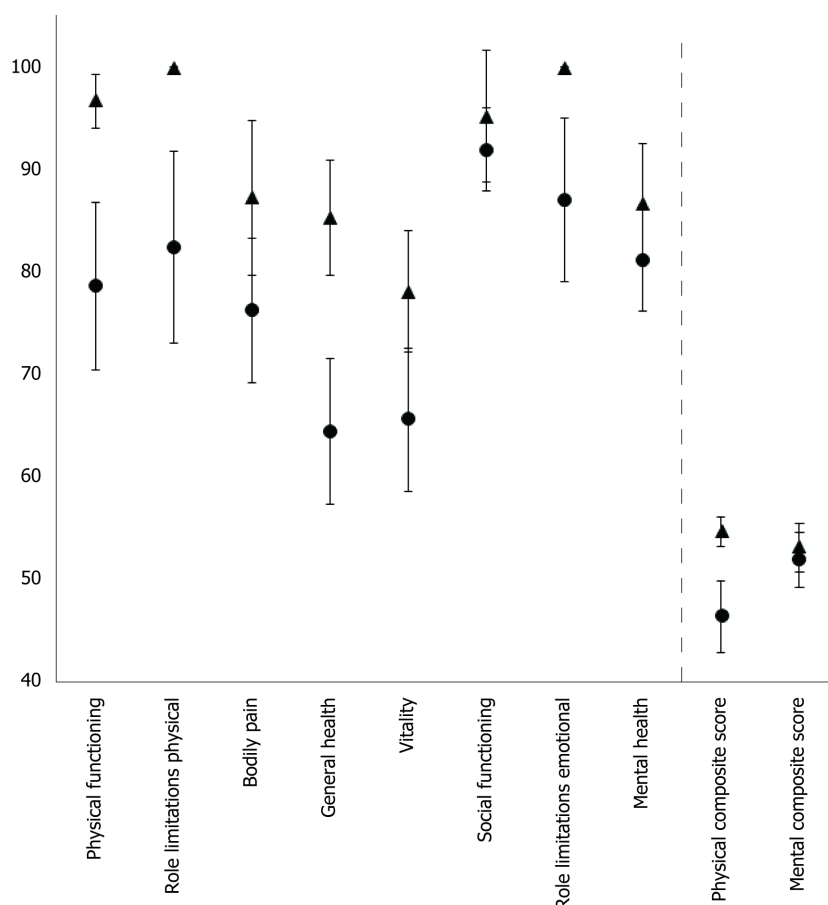


Figure 1 Comparison of mean SF-36 scores with confidence intervals for people with type 1 diabetes (●) and healthy participants (▲).

hypoglycaemic episodes were only sparsely recorded (48 h), and therefore it was not surprisingly, that we did not find any association between the number of hypoglycaemic events and HRQoL.

Insulin treatment is the core of glycaemic control and management of type 1 diabetes and with the rise of new technologies such as continuous glucose measurements combined with improved treatment the last couple of years, this has become easier for the patients. Surprisingly, Hart *et al*^[17] showed that continuous insulin treatment decreased the mental composite score, due to the stress of regular blood glucose monitoring. Such findings were not supported by this study, as no differences in HRQoL were shown between people using insulin pens or pump.

Limitations

This study was not without limitations. Firstly, this study was conducted in a well-defined, middle-aged cohort with verified severe diabetic symmetrical peripheral neuropathy and thus the results cannot be directly generalised to other patient groups. Additionally, these were compared with healthy individuals, and therefore the effects measured may be skewed due to the effect of diabetes alone on SF-36. Secondly, we used the SF-36 to measure HRQoL assessments. Future studies may use the diabetes specific quality of life questionnaires and potentially add more insight into diabetes HRQoL. Lastly, it would have been interesting to study if hyperglycaemic events were associated to HRQoL in this cohort.

Conclusion

In summary, as hypothesised this study showed a decrease in the physical components of the HRQoL in a well-defined cohort of people with type 1 diabetes and severe diabetic symmetrical peripheral neuropathy. To our surprise, no associations were found in the mental components. Furthermore, decreased HRQoL was associated to number of co-morbidities and dysregulated glycaemic control, but not to the severity of neuropathy. This emphasises the importance of considering quality of life in people with diabetes, especially in those with multiple comorbidities. Furthermore, it is important to consider HbA1c as a biomarker for complication and

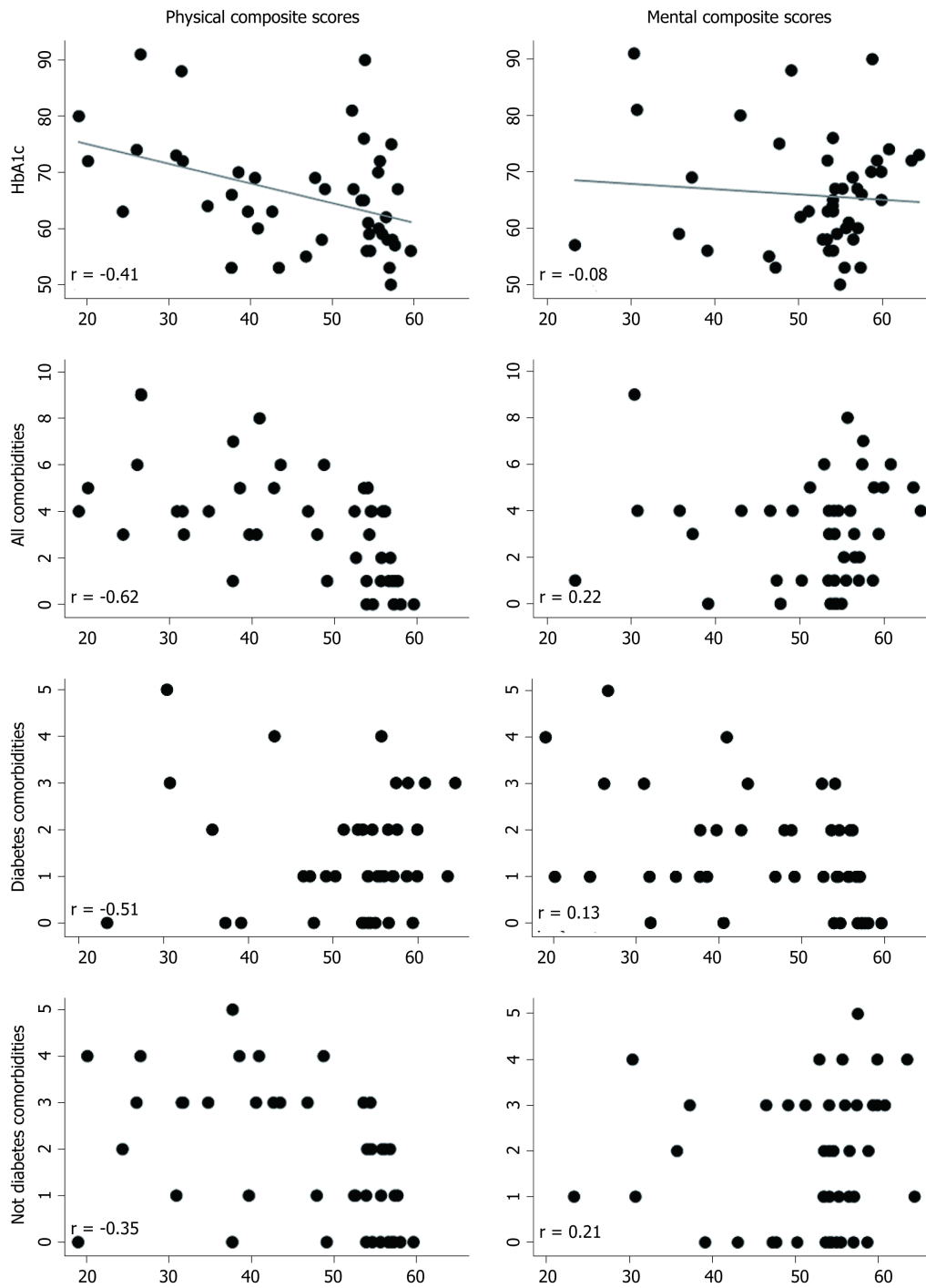


Figure 2 Correlations between physical and mental composite scores, HbA1c and comorbidities.

thereby indirectly for quality of life.

Table 2 Associations between 36-Item Short Form Health Survey and HbA1c, comorbidities, both diabetes and non-diabetes related in patients with type 1 diabetes

		HbA1c	All omorbidities	Diabetes elated	Non-diabetes related
Physical functioning	r	-0.51	-0.58	-0.42	-0.52
	p	< 0.01	< 0.01	< 0.01	< 0.01
Role limitation physical health	r		-0.44	-0.33	-0.38
	p		< 0.01	0.03	0.01
Bodily pain	r	-0.34	-0.48	-0.43	-0.38
	p	0.02	< 0.01	< 0.01	0.01
General health	r		-0.41	-0.34	-0.36
	p		< 0.01	0.02	0.02

Diabetes related comorbidities includes hypertension, retinopathy, pain, albuminuria, erectile dysfunction and heart and vessel diseases. Non-diabetes related comorbidities includes hypercholesterolemia, thrombose prophylaxis, operations, reflux, arthritis and arthroses, asthma and allergies, metabolic diseases, vitamin deficiencies and more.

ARTICLE HIGHLIGHTS

Research background

Diabetic symmetrical peripheral neuropathy is a frequent complication to type 1 diabetes and is associated to incapacitating complication and decreased lifespan, possibly affecting health related quality of life (HRQoL). The 36-Item Short Form Health Survey (SF-36) is a generic patient reported questionnaire, which can be used to evaluate mental and physical HRQoL in patients with diabetes.

Research motivation

HRQoL is an increasingly acknowledged method in clinical practice, to evaluate patient centred outcomes in the measurement of disease burden, progression and treatment outcome.

Research objective

To investigate if diabetic neuropathy would decrease physical and mental quality of life measured with SF-36, and if clinical appearance may be associated with the decline.

Research methods

Baseline data of standardised nerve conduction and SF-36 questionnaire as well as information on disease duration, number of comorbidities, vibration perception threshold, number of hypoglycaemic events, HbA1c and administration way of insulin was collected from 48 adults with verified diabetic symmetrical peripheral neuropathy and 21 healthy participants as part of a clinical trial.

Research results

People with diabetic symmetrical peripheral neuropathy had a significantly decreased physical score, but not mental score compared with healthy. Furthermore, this decrease in physical score was associated with total number of comorbidities, comorbidities relation to diabetes and HbA1c as well as comorbidities not related to diabetes.

Research conclusions

HRQoL is an important tool for evaluate patient centred outcomes in people with diabetes and is decreased with diabetic symmetrical peripheral neuropathy but also with increase in symptoms and suboptimal long-term glucose measures.

Research perspectives

HRQoL is an informative measure for use in investigation of diabetes and related neuropathy or symptoms in the future.

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Effectiveness of royal jelly supplementation in glycemic regulation: A systematic review

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Author contributions: Omer K and Newton G conceptualized research question and created search strategy; Omer K and Gelkopf MJ conducted full text inclusions and risk of bias assessments; Omer K and Newton G conducted GRADE assessment; Newton G reviewed manuscript throughout writing stage; Omer K conducted database search, data extraction and analysis, and manuscript preparation.

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Abstract

BACKGROUND

Royal jelly (RJ) has been observed to have therapeutic properties in diabetic individuals, including the reduction of high blood sugar. This systematic review synthesized existing evidence to investigate the effectiveness of RJ supplementation in managing measures of blood glucose.

AIM

To determine the effectiveness of RJ supplementation on glycemic responses in healthy and non-insulin dependent diabetic adults, as well as animal models of diabetes.

METHODS

This was a systematic review employing the PRISMA strategy. Peer-reviewed, published articles were extracted from several databases using key words related to target population, intervention and outcome and hand-selected for inclusion. Included articles proceeded to data extraction phase, where information on target parameters and effectiveness of treatment was summarized. Following this, the risk of bias for each included study was evaluated. Then, the long-term and immediate effectiveness of RJ supplementation in glycemic control were assessed using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) tool, which rates the quality of evidence.

RESULTS

Of 168 articles extracted from database searching, eighteen were included for analysis in this systematic review. Across the studies, studied populations, intervention styles and outcome measures were largely heterogeneous. Despite this, the results in studies indicate a general trend of positive effect of RJ in glycemic regulation *in vitro* and *in vivo*. Additionally, some dose-dependent glycemic effects were observed, along with some large effect sizes. The risk of bias for human and animal studies is generally low-unclear risk, although lack of

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blinding is a serious concern in both categories. Overall, as per the GRADE tool, the quality of evidence is low, and very low for long-term and immediate effectiveness of RJ, respectively. A major limitation affecting evidence quality is the heterogeneity among included studies. Fasting blood glucose and glucose clearance appear to be most affected by RJ supplementation.

CONCLUSION

Quality of evidence suggesting that RJ is an effective modulator of glycemic regulation is low for long-term effects of RJ, and very low for immediate effects.

Key words: Royal jelly; Type 2 diabetes; Dietary supplement; Glycemic control; 10-hydroxy-trans-2-decenoic acid; Hyperglycemia; Adults; Animals

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Core tip: Royal jelly (RJ) is a promising natural treatment to improve high blood glucose. Insulin sensitivity, fasting blood glucose levels, and rate of glucose clearance are among the glycemic parameters investigated in the current systematic review that are shown to approach normal levels due to regular RJ intake.

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INTRODUCTION

The incidence of type 2 diabetes (T2D) has drastically increased over the past thirty years, making it among the most taxing diseases for health agencies worldwide^[1-3]. As of 2017, projections for global prevalence of diabetes in 2030 have already been surpassed^[4,5]. By 2040, the prevalence of T2D is expected to reach 642 million worldwide^[5]. The epidemic is believed to stem from rapid changes in society since the 1980s that promote long periods of inactivity, energy and nutrient overconsumption; however, further epigenetic and genetic interactions continue to be explored^[1,2]. T2D is heavily associated with cardiovascular disease and obesity, which largely accounts for the morbidity and mortality in patients with the disease^[6]. Thus, to minimize risk of complications in patients, food intake and concentrations of blood glucose must be tightly managed^[6].

Numerous natural health products, including royal jelly (RJ), have been explored as potential hypoglycemic agents^[7,8]. RJ is a yellow, milky substance secreted by worker honey bees (*Apis mellifera*) through cephalic exocrine glands, such as the mandibular and hypopharyngeal glands^[9]. RJ functions to nourish larvae for the first three days after being reared, although larvae destined to be reproductive queen bees are fed the material throughout their entire larval and adult life^[9]. 10-hydroxy-2-decenoic acid (10H2DA), a fatty acid unique to RJ, is a major compound of interest in blood glucose management for its observed hypoglycemic effects^[7]. Takikawa *et al*^[7] found that 10H2DA significantly increases non-insulin dependent phosphorylation of AMP kinase (AMPK) in skeletal muscle, increasing translocation of glucose transporter type 4 (GLUT4) to cell surface and consequently, glucose transport into the cell. An *in vivo* study examining life-extending effects of RJ found that 10H2DA induces upregulation of molecules involved in caloric restriction, reducing energy intake^[10]. 10H2DA has demonstrated interactions with estrogen receptors leading to alterations in gene expression, potentially including those involved in glucose regulation^[11]. The glucose modulating role of 10H2DA is the most well-investigated mechanism by which RJ might benefit patients with T2D, although other RJ components, such as sebatic acid, may also be important.

In addition to *in vitro* studies, RJ administration has demonstrated therapeutic potential in human and rodent diabetic models. In a randomized controlled trial, Khoshpey *et al*^[12] found that daily ingestion of capsules containing 3000 mg RJ for eight weeks significantly decreased fasting blood glucose (FBG) in diabetic individuals compared to a placebo group. However, the effects of RJ administration

on glycemic control outcomes are inconsistent across studies, possibly due to considerable variation in studied population and intervention. For example, while Khoshpey *et al*^[12] found no significant change in carbohydrate (CHO) intake in the RJ-treated group, Pourmoradian *et al*^[13] found a significant decrease in CHO intake in diabetic individuals in response to daily ingestion of 1000 mg lyophilized RJ for eight weeks. Rodent studies have shown a more pronounced effect: Ghanbari *et al*^[14] found that addition of 100 mg/kg RJ in drinking solution improved circulating insulin and FBG in diabetic mice to levels similar to the healthy control group. Zamami *et al*^[15] observed a similar magnitude of effect in insulin-resistant rats following administration of 300 mg/kg enzymatically treated RJ. It is difficult to apply the results of rodent studies into a human context due to variation in physiologic processes, particularly absorption and distribution of nutrients such as 10H2DA^[16]. Presently, there is a lack of synthesis and analysis of these human and animal studies investigating the therapeutic effects of RJ.

This systematic review will investigate the effectiveness of RJ as a therapeutic agent in individuals with T2D. Specifically, we will assess animal, human and *in vitro* studies examining the effects of administration of RJ and its constituents on various outcomes that relate to glycemic control, such as plasma glucose levels, plasma lipid levels and hemoglobin A1c (HbA1c) levels in healthy and diabetic individuals. Included studies will investigate outcomes following both acute and long-term administration of RJ on glycemic control. The synthesis and evaluation of existing trials provides individuals and health care professionals with a resource to make informed decisions regarding T2D therapy. To our knowledge, no systematic review investigating RJ as a treatment for diabetes exists.

MATERIALS AND METHODS

The guidelines of the 2009 PRISMA model strategy were followed throughout this review^[17].

Search strategy

We conducted a systematic search of peer-reviewed articles relating to the impact of RJ on glycemic outcomes. A set of keywords were developed by Kamel Omer and Genevieve Newton to yield trials with study variables that are appropriate for the research question. These were subsequently searched in five databases: Cochrane Library, CINAHL Plus, PubMed (*via* NCBI), Web of Science, and ProQuest. Operator commands were used to yield studies containing at least one keyword for each variable within the title or abstract. A review protocol does not exist for this systematic review.

Selection of articles

Following extraction and compilation of articles from the database results, duplicate studies were electronically removed. Bibliographies of studies were manually scanned to capture relevant studies. Titles and abstracts were manually screened and articles not meeting inclusion criteria were removed. Full text screening was completed for the remaining articles, where studies not meeting the inclusion criteria were removed. This was conducted in duplicate by Kamel Omer and Maxwell J Gelkopf; final decisions were settled by discussion. The resultant articles proceeded to the quality appraisal stage.

Inclusion criteria

(1) Population: Healthy or diabetic human adults or animal models; (2) Intervention: Oral administration of RJ or its constituents; (3) Outcomes assessed: Direct measures of glycemic control or measures pertinent to glycemic control; (4) *In vitro* studies: Effects of RJ (or constituents) administration were investigated on an outcome directly related to glycemic control; (5) Methodology includes control group for comparison with treatment; and (6) Available in English.

Data extraction

Data on study design, subjects, treatment, and relevant outcomes and results were abstracted for each included study, where applicable, qualitative and quantitative details were added to each of these study variables. For subjects, the extracted number of participants was those that completed the study. Data summaries of each included study were then classified into one of three different tables, depending on study population and intervention style. The classifications are: (1) Acute administration (examining immediate RJ effects) of RJ in human trials; (2) Long-term administration (examining long-term RJ effects) of RJ in human trials; and (3) Long-term

administration of RJ in animal trials and *in vitro* trials.

Risk of bias

For each included human and animal study, the Cochrane Collaboration's risk of bias assessment tool was used to determine the risk of bias^[18]. The tool covers five pre-specified areas of bias: Selection bias (allocation concealment and random sequence generation), performance bias (blinding of participants and researchers), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), and reporting bias (selective reporting)^[18]. The tool also includes an "other bias" category for other sources of bias that do not fall within these prespecified areas^[18]. For each area of bias, the tool provides criteria to rate the study as low, high, or unclear risk of bias. A high risk of bias indicates a high possibility of bias that is likely to impact the study results, while a low risk suggests negligible risk^[18]. An unclear risk of bias indicates that insufficient information is provided to determine if results are impacted by bias, but some doubt is raised^[18].

Two authors, Kamel Omer and Maxwell J Gelkopf, assessed all included articles to reach a consensus on the risk of bias for each study. Higgins *et al*^[18] provides a detailed guide on the ranking procedure, which was applied by reviewers throughout the risk of bias assessment. Although the tool is designed for human trials, O'Connor and Sargeant^[19] developed a modified version for use in risk of bias assessment of animal trials. This modified version was used by the reviewers to guide ranking of risk of bias for the included animal studies. Justification was noted for each judgement, including paraphrases and direct quotes from the article when available.

Quality of evidence

Following determination of the risk of bias for each study, the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) tool was used to rate the overall quality evidence across all included studies for a single outcome^[20]. In this review, GRADE was used to assess the quality of evidence for RJ's capacity to manage blood glucose levels following long-term supplementation of RJ as well as acute effects of RJ administration. The GRADE tool encompasses five domains to determine overall quality: risk of bias, indirectness to research question, imprecision of results, inconsistency between studies and publication bias^[20].

To represent the GRADE rating, a summary of findings table was created. Ranking begins at high quality due to the majority of trials being randomized controlled trial but are gradually degraded if serious concern exists in any of the five domains^[20]. Ranking can also be upgraded if there is no plausible confounding bias, or if magnitude of effect is large or dose-dependent^[20]. The overall ranking is determined by number and magnitude of all downgrades and upgrades^[20]. Each of the four possible rankings directly correspond to a certain overall quality of evidence (very low, low, moderate, high)^[19]. Ryan and Hill^[21] provide criteria for what constitutes a concern in these domains, which was used as a guide for reviewers when determining the GRADE ranking. To factor animal studies in, a modified version of GRADE for animal studies developed by Wei *et al*^[22] was used. Kamel Omer and Genevieve Newton reached a consensus on the GRADE score based on criteria and justification was provided for each decision on the summary of findings table.

Summary measures

To determine effect size and the precision of the quantitative data of the study results, appropriate measures were manually calculated. Standardized mean difference (SMD, also known as Cohen's d) was calculated as described by Faraone^[23] using a pooled standard deviation and sample means of the treatment and placebo group to estimate magnitude of treatment effect. A large effect size constitutes a SMD of > 0.8, while a small effect size is considered to have a SMD of < 0.2; all values in between are medium effect sizes^[23]. A negative value indicates that the treatment reduces the parameter being investigated^[23]. The SMD was calculated for values taken at the endpoint of the study. For studies with multiple treatment groups, the SMD was calculated for the group with highest dosage. Where numerical values were unavailable, values were interpolated from provided graphs. If values were reported as medians, these were used in place of the mean throughout the effect size calculation. As part of the GRADE evaluation, 95% CIs of SMDs for long-term, hypoglycemic outcomes were calculated to assess imprecision of the effect sizes. For simplicity of evaluation and comparison, SMDs were reported in this review for statistically significant outcomes only.

RESULTS

Study selection

The pre-specified search strategy was conducted on March 21st, 2018, yielding 168 results. Grey literature databases were searched, but no pertinent articles were found. One study was captured from scanning the bibliographies of collected studies. After removal of duplicates, the total number of unique studies was determined to be 83. These studies went on to the title and abstract screening. Fifty-seven studies were removed due to not meeting the inclusion criteria at this stage. The remaining 26 underwent a full-text screening to determine eligibility. Of these 26, eight were excluded: two because they were not written in English, two for assessing outcomes not related to glycemic control, one because it lacked an oral intervention in treatment groups, one because the study design did not have a control group, one because it lacked an intervention, and one because it did not target the desired population for this review. The resultant 18 articles were included in the systematic literature review for quality of evidence appraisal (Figure 1).

Study characteristics

Following the systematic search, study characteristics, including intervention style, length, participants and results were manually extracted and summarized into the Tables 1, 2 and 3^[24-36].

Risk of bias within studies

For human trials, the pre-specified areas of bias are generally low risk. The biggest source of concern for bias stems from the lack of transparency of measures taken to prevent a given area of bias. This was particularly evident in the allocation concealment category, as evidenced by the high proportion of human trials at high or unclear risk in that category (Figure 2). Moreover, although many studies claimed that participants and personnel were blinded, description of the actual blinding methodology was rarely provided, suggesting that performance and detection bias are considerable concerns among included human studies. The next biggest source of concern for bias is apparent in the “other bias” section-this is largely due to confounding bias arising from the recruitment of participants from a single source (*e.g.*, common hospital). Overall, of the human trials, domains of bias at high risk are relatively few.

Across the animal studies, the risk of bias is a notably bigger concern. One hundred percent of the studies are considered high risk of detection bias (*i.e.*, group allocations known to outcome assessors), potentially due to blinding being uncommon in animal studies (Figure 3). Attrition bias is another serious concern across the animal studies; most included animal studies excluded some individual subjects from analysis without any explanation. Performance and selection bias, however, are well accounted for in the included animal studies, with nearly 100% of both domains at low risk. Like human trials, reporting bias is not a serious concern in the animal trials (Figure 4).

Overall quality of evidence

The quality of evidence was evaluated as per the GRADE criteria with results shown in Tables 4 and 5. This evaluation integrated study results with risk of bias across all studies included in this review.

Effectiveness

Direct measures of glycemic control (FBG, glucose clearance rate, insulin levels) appear to be appreciably impacted by RJ administration. Most of the included studies which examined FBG and rate of glucose clearance observed significant change from baseline due to oral supplementation of RJ. Of these results, the majority had large effect size estimates, substantiating the role of the intervention in the observations. Most of the studies investigating insulin levels also reported a beneficial effect. Furthermore, in studies with multiple experimental groups, a dose-response relationship was observed in plasma insulin levels and rate of glucose clearance, but not FBG levels. Abnormal regulation of these parameters give rise to other secondary conditions associated with T2D, such as high levels of HbA1c.

The beneficial effect of RJ supplementation was demonstrated by improved indirect measures of glycemic control. These indirect measures are precursors (*e.g.*, high circulating fat) or indicators (*e.g.*, HbA1c) of hyperglycemia. The effectiveness of RJ administration in improving these parameters was not as apparent, as there was no clear trend in outcome responses to RJ treatment. However, when significant changes were detected in the experimental group, these results mostly had large effect magnitude estimates. The inconsistency between observed effects may be due to heterogeneity among the included studies.

The wide range of supplementation forms included in the evidence may explain

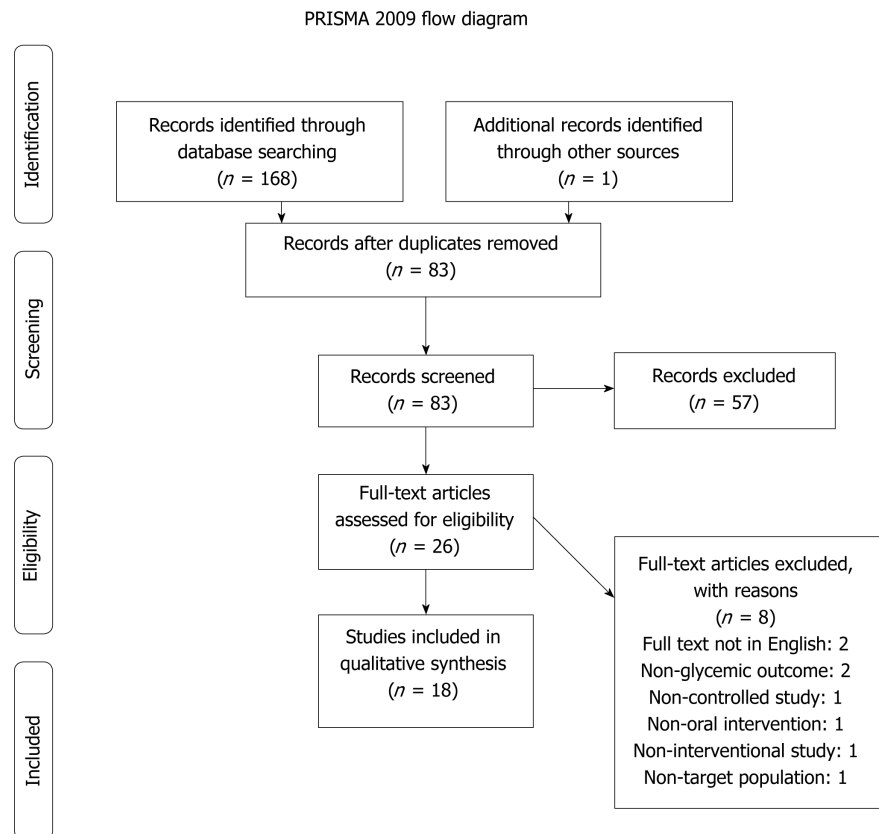


Figure 1 Flow diagram summarizing study selection process.

some of the observed inconsistency between results. For example, many studies used lyophilized RJ, which is known to be chemically different and less bioactive compared to fresh RJ^[27,37]. Numerous clinical trials used enteric coated capsules to deliver RJ, which is known to alter pharmacodynamic properties of compounds^[38]. Some animal and human studies added the supplementation to meals, which may affect effectiveness due to food-drug interactions^[39]. When similar populations were studied, some outcomes showed reduced, negated or contradictory effects in response to RJ as compared to 10H2DA and vice versa, such as between Yoshida *et al*^[36] and Watadani *et al*^[35]. This circumstance indicates the possibility of RJ constituents having interactions amplifying or diminishing effectiveness of certain glycemic outcomes.

Due to the different investigation types and subjects across the included studies, it is important to contextualize the effectiveness for the different populations. The largest improvements to blood glucose were observed in diabetic models, both human and animals. Healthy (human) models had some improvements in blood-glucose parameters; however, for most normal blood-glucose parameters, there is a limit on the possible difference from baseline. Only one *in vitro* was included and was used in this review primarily to elucidate mechanisms and support the *in vivo* results. Thus, the effectiveness of RJ as a glycemic regulator was determined through the lens of diabetic human patients, with the other populations used to support the findings.

Mostly displayed in the rodent trials, there was a marked difference on effectiveness of RJ administration between studies that investigated the effects of RJ between genders, with greater efficacy seen in males. The divergent effects may potentially be due to the estrogenic activity of compounds derived from RJ, particularly 10H2DA and a sterol, 24-methylenecholesterol^[11]. These have weak affinity for estrogen receptors that induce changes in gene expression^[11]. Because these compounds would compete with endogenous estrogen for receptor binding, RJ may not be effective in individuals with elevated estrogen levels (*i.e.*, premenopausal females). Correspondingly, estrogen perfusion has previously been linked to improved hyperglycemic symptoms in postmenopausal females and males, which is consistent with the evidence in this review^[40,41].

Although very few participants in the included study reported undesirable effects of RJ consumption, potential adverse effects in humans are an important factor in their feasibility as a glycemic regulator. One case report found an association between haemorrhagic colitis and daily RJ intake, which had never been documented

Table 1 Human trials examining acute effects of royal jelly treatment

Ref.	Study design	Subjects	Treatment	Outcome measures	Effectiveness
Iaconelli <i>et al</i> ^[24]	Crossover study	N = 10 + 10 healthy individuals and individuals with type 2 diabetes	Each subject went through three studies on different days: 0 g, 12 g, or 23 g of sebacic acid substituted fats in a meal	Glucose clearance: Postprandial Insulin secretion/clearance rate GLUT4 expression in L6 myotube cells	Significantly improved glucose clearance in diabetic subjects only in dose-dependent manner (d = -1.70) Significantly improved GLUT4 expression (d = 0.81) and glucose uptake in L6 cells (d = 0.67) Insulin secretion/clearance decreases significantly in similar fashion between healthy and diabetic patients. Dose-response relationship. For diabetics, d = -1.12
Mobasser <i>et al</i> ^[25]	Randomized controlled trial	N = 20 + 20 adults with type 2 diabetes aged 30-65 in control and treatment groups	15 g of royal jelly ingested orally after overnight fasting	Hyperglycemia: Fasting blood glucose, glucose clearance after royal jelly consumption Hyperinsulinemia: Serum c-peptide and insulin	No significant difference in outcome measures between two groups
Münstedt <i>et al</i> ^[26]	Controlled trial	N = 10 + 10 healthy males, split into experimental and control groups	20 g of fresh royal jelly ingested orally	Glucose clearance: Plasma samples during OGTT Insulin resistance: Serum insulin and c-peptide	Significantly increased rate of glucose clearance (insufficient information for effect size calculation) No significant change in serum insulin profile
Münstedt <i>et al</i> ^[27]	Randomized controlled trial	N = 15 healthy male adults aged 20-34, unspecified distribution between treatment and control groups	0.55 g lyophilized royal jelly in enteric-coated capsule ingested orally	Hyperglycemia: Glucose clearance (OGTT) Insulin resistance: Serum insulin and c-peptide	Improved glucose clearance and decreased plasma insulin, unspecified statistical significance (insufficient information for effect size calculation)

OGTT: Oral glucose tolerance test; GLUT4: Glucose transporter type 4.

previously^[42]. Bronchospasm and anaphylaxis have also been noted in individual cases^[43,44]. The aforementioned cases are possibly due to allergic reactions to RJ proteins^[42,43,44]. Harmful RJ-drug interactions should be considered; consuming RJ while taking warfarin has been associated with hematuria^[45].

DISCUSSION

The present systematic review suggests that RJ has a positive effect on both direct and indirect measures of glycemic control in diabetic and healthy individuals. This general trend was observed in both animal and adult human trials but was more pronounced in the former. In healthy individuals, supplementation of RJ may reduce risk of developing hyperglycemia and insulin resistance. With the evidence presented, RJ is likely more effective as a long-term dietary supplement rather than for acute treatment of hyperglycemia. Effective clinical doses appear to be as low as 1000 mg of fresh RJ daily for diabetic humans, but true values may vary between individuals and supplementation form.

Regulation of glycemic control by 10H2DA

In a normal state, intracellular protein-protein interactions arising from insulin binding to its surface receptor are critical to blood glucose regulation^[46,47]. One major result of the signaling cascade induced by insulin on various tissue types is the translocation of the GLUT4 glucose transporter to the cell surface, which works to import glucose into the cell^[46]. The insulin-dependent pathway also modifies gene

Table 2 Human trials examining effects of long-term royal jelly treatment

Ref.	Study design	Subjects	Treatment	Outcome measures	Effectiveness
Khoshpey <i>et al</i> ^[12]	Randomized double-blind controlled trial	N = 11 females + 12 males aged 20-65 with type 2 diabetes in control group (placebo) N = 13 females + 10 males aged 20-65 with type 2 diabetes in treatment group	3000 mg royal jelly oral capsules once per day for 8 wk. Control received placebo	Macronutrient intake Hyperglycemia: Fasting blood glucose	No significant change in macronutrient intake Fasting blood glucose significantly reduced in comparison to control group (d = -0.87)
Mobasserri <i>et al</i> ^[28]	Randomized controlled trial	N = 25 + 25 females with type 2 diabetes aged 30-65 in control and treatment groups	200 mg royal jelly powder prepared in gel form and served with breakfast for 8 wk. Control group received placebo	Plasma triglyceride	Significantly decreased plasma triglyceride in comparison to control (d = -0.476)
Morita <i>et al</i> ^[29]	Randomized double-blind controlled trial	N = 30 healthy adults 42-83 yr of age in control (placebo) N = 31 healthy adults 42-83 yr of age in treatment group	3000 mg royal jelly in 100 mL liquid daily for 6 mo. Control received placebo identical in appearance	Body weight: BMI Insulin resistance: HOMA-IR Hyperglycemia: HbA1c, fasting blood glucose Plasma triglyceride	Significantly improved fasting blood glucose (d = -0.9596) No significant changes in other outcomes of interest
Pourmoradian <i>et al</i> ^[13]	Human double-blinded randomized clinical trial	N = 23 females aged 30-65 with type 2 diabetes in treatment group N = 22 females aged 30-65 with type 2 diabetes in control group	1000 mg lyophilized royal jelly in soft gel form served after breakfast for 8 wk. Control group received placebo soft gel	Body weight: weight scale before and after study period Macronutrient intake: 24-h recall food questionnaire for 3 d before and after study period	Significantly decreased body weight within same group, before and after intervention (d = -0.3808) Significantly decreased energy intake within same group, before and after intervention (d = -9.52)
Pourmoradian <i>et al</i> ^[30]	Human double-blinded randomized controlled trial	N = 21 females aged 30-65 with type 2 diabetes in treatment group N = 20 females aged 30-65 with type 2 diabetes in control group	1000 mg lyophilized royal jelly in soft gel form served after breakfast for 8 wk. Control group received placebo soft gel	Plasma insulin HbA1c	Significantly decreased plasma insulin and HbA1c and insignificantly decreased fasting blood glucose compared to baseline within same group, before and after intervention. d = 0.016 (HbA1c)
Shidfar <i>et al</i> ^[31]	Human double-blinded randomized controlled trial	N = 23 + 23 adults 25-65 yr old with type 2 diabetes in experimental and control (placebo) groups	1000 mg royal jelly in soft gelatin capsules 3 times daily for 8 wk. Control group received placebo identical in appearance to treatment	Hyperglycemia: Fasting blood glucose Fasting blood sugar Macronutrient intake: 24-h recall diet questionnaire Insulin resistance: HOMA-IR	d = -0.0785 (plasma insulin) Significantly decreased fasting blood levels to more normal range (d = -0.3725) Did not significantly alter macronutrient intake Significantly decreased HOMA-IR: improved insulin sensitivity (d = -0.79)

RJ: Royal jelly; HOMA-IR: Homeostatic model assessment of insulin resistance; BMI: Body mass index; HbA1c: Hemoglobin A1c.

expression and protein activity such as those involved in glycogen breakdown^[46]. Insulin receptor substrate (IRS) proteins are key intermediates in the pathway^[47]. With elevated levels of circulating fatty acids as in diabetic individuals, phosphorylation of IRS proteins is inhibited *via* activation of protein kinase C (PKC)^[47,48]. As a result of decreased sensitivity to insulin, the cellular responses involved in regulating blood

Table 3 Animal and *in vitro* trials examining effects of long-term royal jelly treatment

Ref.	Study design	Subjects	Treatment	Outcome measures	Effectiveness
Ghanbari <i>et al</i> ^[14]	Randomized controlled trial	N = 8 healthy male Wistar rats aged 10-12 wk (control) N = 8 diabetic male Wistar rats aged 10-12 wk N = 8 healthy male Wistar rats aged 10-12 wk receiving treatment N = 8 diabetic male Wistar rats aged 10-12 wk receiving treatment	100 mg/kg BW royal jelly dissolved in 1 mL of water daily for 6 wk	Hyperinsulinemia: ELISA test on plasma sample Hyperglycemia: Fasting plasma glucose	Treatment significantly improved insulin levels (d = 1.67) and hyperglycemic fasting blood glucose (d = -2.72) levels to levels similar to healthy control group
Fujii <i>et al</i> ^[32]	Controlled trial	N = 80 male streptozotocin-diabetic rats aged 5 wk equally split into three experimental groups and one control group	Each experimental group had one of 1, 10, and 100 mg/kg body weight royal jelly administered orally by force for 4 wk. Control group received purified water	Hyperglycemia: Blood glucose (unknown whether fasting) Body weight	Royal jelly administration overall slightly decreased blood glucose levels in non-dose dependent manner (no information on statistical significance) No significant change in body weight between groups
Membrez <i>et al</i> ^[33]	Randomized controlled trial	N = 15 male db/db mice aged 6-8 wk in control group N = 30 male db/db mice aged 6-8 wk equally split in two experimental groups	1 g/kg body weight of sebatic acid was added to chow food in one experimental group, and 10 g/kg body weight SA to second experimental group's chow for 6 wk	Hyperglycemia: OGTT and fasting (plasma samples) HbA1c: Plasma samples Liver gene expression: RNA extracted from liver samples Food intake: Chow consumed	In more heavily supplemented group: Hyperglycemia significantly improved (d = -1.86) and improved glucose clearance (d = -3.20), HbA1c significantly decreased (d = -1.89), ketone bodies significantly increased (d = 1.16), dose response relationship observed, gluconeogenic and lipogenic enzyme expression significantly decreased (insufficient information for SMD estimation), food intake was significantly decreased (d = -1.82).
Takikawa <i>et al</i> ^[7]	<i>In vitro</i>	L6 myotubes grown in cell culture and collected from healthy male mice 7 wk of age	Cell cultured myotubes treated with 10H2DA Mice fed 1.6 mmol/kg 10H2DA	Glucose clearance: GLUT4 translocation to plasma membrane	Significantly improved GLUT4 translocation to plasma membrane in skeletal muscle cells compared to non-treated myotube cells (d = 0.4698)
Yoneshiro <i>et al</i> ^[34]	Controlled trial	N = 8 3-wk old healthy male mice (control) N = 11 3-wk old healthy male mice fed HFD N = 11 3-wk old healthy male mice fed high fat diet with treatment	High fat diet with 5% lyophilized royal jelly powder for 17 wk	Body weight gain Hyperlipidemia: Plasma sample Hyperglycemia: Plasma sample Insulin resistance: HOMA-IR	Body weight gain due to white adipose tissue significantly reduced compared to HFD group (d = -2.82) Significantly decreased levels of NEFA compared to HFD (d = -1.6072) Significantly improved hyperglycemia compared to HFD group (d = -2.04) HOMA-IR significantly decreased compared to HFD group, not significantly different from control group (d = -1.23)

Zamami <i>et al</i> ^[15]	Controlled trial	N = 6 6-wk old healthy male Wistar rats (control, received water)	Two experimental groups: One fed 100 mg/kg and the other 300 mg/kg of dilute enzymatically treated royal jelly supplementation daily for 8 wk	Insulin resistance: HOMA-IR	High fructose diet induced insulin resistance in rats
		N = 5 6-wk old healthy male Wistar rats as vehicle-treated group (received high fructose consumption)		Food intake	Plasma insulin levels and HOMA-IR similar between healthy control group and fructose drinking rats supplemented with 300 mg/kg royal jelly. Dose dependent relationship observed $d = -0.7063$ (effect size of 300 mg/kg royal jelly on fructose drinking rats)
		N = 6 + 6 6-wk old healthy male Wistar rats (received high fructose consumption) in two treatment groups		Body weight	No significant difference in body weight and FBG between groups
				Plasma triglycerides	Plasma triglycerides significantly decreased compared to control dose-dependently ($d = -1.62$)
Watadani <i>et al</i> ^[35]	Controlled trial	N = 7 female KK-Ay mice 5 wk of age in control group	3 mg/kg 10H2DA for 4 wk	Hyperglycemia: Plasma glucose samples collected in intervals after OGTT	Significantly improved glucose clearance ($d = -1.33$) and fasting blood glucose ($d = -1.23$)
		N = 8 female KK-Ay mice 5 wk of age in treatment group		Body weight: Adiposity index of abdominal, mesenteric and retroperitoneal fat tissue	Body weight did not differ between groups
				Insulin resistance: HOMA-IR	Significantly improved insulin sensitivity ($d = -4.44$)
				Glucose regulatory proteins: AMPK, G6Pase, Pck1 levels, GLUT4, GS/GSK in tissue homogenates	Significantly increased levels of G6Pase ($d = 1.22$) and Pck1 ($d = 0.77$) mRNA in liver cells. Significantly increased levels of pAMPK in muscle ($d = 3.13$), but no change in liver. Insignificant increase in GLUT4 in muscle cells. No change in GS/GSK levels between groups
Yoshida <i>et al</i> ^[36]	Controlled trial	16 female KK-Ay mice split into control and experimental groups	10 mg/kg royal jelly in 1/15M phosphate buffer 5 d/wk for 4 wk		Significantly improved rates of glucose clearance ($d = -1.25$)
					Insignificantly decreased body weight
					Significantly increased pAMPK levels in liver ($d = 2.39$) and skeletal muscle ($d = 1.73$). Significantly decreased G6Pase mRNA levels in liver ($d = -1.65$), but no change in Pck mRNA levels. Insignificantly increased GLUT4 levels in skeletal muscle
					Significantly decreased plasma NEFA ($d = -1.42$). No change in plasma TG
					No significant change in plasma insulin

GLUT4: Glucose transporter type-4; 10H2DA: 10-hydroxydecanonic acid; OGTT: Oral glucose tolerance test; HOMA-IR: Homeostatic model assessment of insulin resistance; GS(K): Glycogen synthase (kinase); AMPK: AMP-dependent kinase; NEFA: Non-esterified fatty acids; HFD: High fat diet.

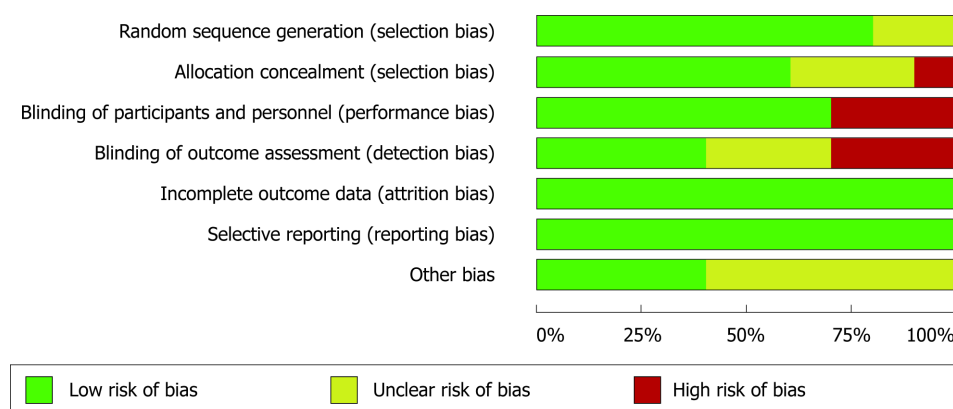


Figure 2 Risk of bias graph showing proportion of bias risk ratings across all included human studies.

glucose are impaired, leading to hyperglycemia^[48].

Intracellular AMPK, which induces similar glycemic responses to insulin in a non-insulin dependent pathway, has been observed to be activated by 10H2DA, the fatty acid derived from RJ that is thought to underlie its glycemic effects^[7,49]. Takikawa *et al*^[7] found AMPK to be activated by 10H2DA *via* activation of CaMKK β (calcium/calmodulin-dependent protein kinase kinase beta). In skeletal muscle, pathways mediated by AMPK have been observed to increase GLUT4 gene expression and translocation^[7,50]. In addition to this, AMPK-mediated regulation of GLUT4 has been observed to improve insulin-stimulated GLUT4 regulation, thus potentially improving response (sensitivity) of insulin receptors bound to ligand^[51]. Moreover, activated AMPK in adipocytes and skeletal muscle are also responsible for enhancing enzymes involved in fatty acid oxidation, thus potentially leading to a decrease in body weight and circulating fatty acids^[36]. Finally, in Yoshida *et al*^[36], increased levels of AMPK following oral administration in mice appeared to stunt activity of glucose-6-phosphatase in hepatocytes, thus suggesting that the kinase can also regulate gluconeogenesis (and therefore glucose export) independently of insulin. By activating an enzyme that works to increase cellular energy levels in the cell, 10H2DA from RJ may mediate the desired hypoglycemic effects in diabetic subjects.

In the hypothalamus, AMPK plays an important regulatory role in food intake^[52]. When activated, a signaling cascade that leads to increased energy intake is initiated^[53]. Downstream of this cascade, mammalian target of rapamycin (mTOR) signaling is known to play a direct and important role^[52]. However, in the presence of 10H2DA, mTOR activity has been observed to be decreased *in vitro*, resulting in decreased energy intake in hypothalamic cells^[10,53]. The beneficial effect of RJ on food intake and body weight is observed in Pourmoradian *et al*^[13], where both parameters were significantly decreased in diabetic female subjects. Following a decrease in macronutrient intake, there is a decrease in fatty acid synthesis and circulating lipids, leading to a decrease in activation of PKC. Thus, RJ's possible action to decrease body weight and food intake is a mechanism that adds to its beneficial effects on glycemic regulation.

As previously mentioned, rodent studies showed a difference between effectiveness of RJ as administered to males compared to females. Fatty acids and sterols in RJ putatively have weak affinity for estrogen receptors, which when activated principally affect gene expression^[11,54]. In addition to affecting transcriptional and translational activity that may regulate glycemic activity, estrogen receptors are able to induce activation of AMPK intracellularly^[54]. Moreover, activated estrogen receptors in skeletal muscle have been shown to amplify GLUT4 translocation^[54]. In the hypothalamus, estrogen suppresses energy intake through mechanisms not fully understood^[54]. Thus, in accord with the evidence collected in this systematic review, activation of estrogen receptors in the body work to increase energy expenditure and decrease energy intake^[54].

Study limitations

Generally, the principal limitation within the included evidence is the wide range of intervention methodology between the included studies. Although this heterogeneity provides data on the various ways to supplement RJ orally (*e.g.*, prandial), there may not be sufficient evidence for one particular intervention method, including duration and dosage of supplementation, to be adopted by health care providers or researchers. As previously described, this variation in intervention methodology also

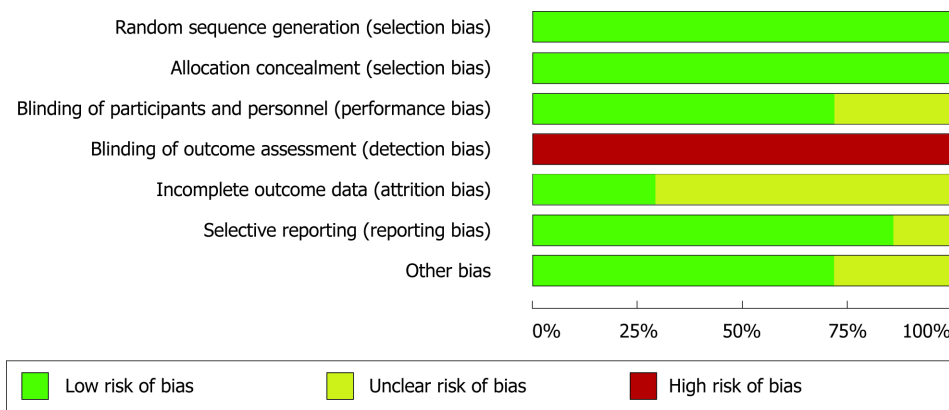


Figure 3 Risk of bias graph showing proportion of bias risk ratings across all included animal studies.

likely contributes to the inconsistency across study results. Moreover, outcomes associated with different interventions at times contradict each other-Watadani *et al*^[35] observed significantly increased expression of G6Pase in liver cells following 10H2DA administration, while Yoshida *et al*^[36] observed significantly decreased expression of G6Pase in liver cells following RJ administration. The source of RJ also differs between studies, which is a potential confounder of the evidence, as the chemical composition of RJ is known to differ between time of year, honeybee age, and geographic location^[55]. Thus, the inconsistency across intervention methods and resultant outcomes is a notable limitation of the overall evidence.

Across human studies, limitations exist largely due to the study populations. Although most of the clinical trials included a balance of male and females, few studies examined exclusively females, and none examined males exclusively. Considering RJ's potential estrogenic activity, a comparison of effects on male and female populations might have provided clearer evidence on the effectiveness of RJ as treatment for diabetes. Notably, the animal studies, which examined exclusively either male or female rodents, displayed differential responses to similar treatment between sexes. Similarly, the age ranges of included subjects in the included trials were relatively wide; inclusion criteria based on tighter ranges (*e.g.*, younger adults, menopausal women) might have determined if certain groups are more affected by the treatment than others, once again potentially due to RJ's activation of estrogen receptors. Moreover, the exclusion and inclusion criteria of the clinical trials affect the generalizability of RJ as treatment for diabetes: For example, many studies excluded individuals who had not taken glucose-lowering medications, or those who had diabetes for a certain length of time. Information on quality of participant dietary patterns and management of diabetes were rarely provided in the included studies but have a large impact on the effect of a supplement such as RJ. These factors are all important when considering the external validity of the synthesized evidence for clinical application.

The main limitation associated with animal studies is the physiological variability between animals and humans. Rodent studies, which comprise a considerable portion of the evidence, are good models of T2D in humans, but lack key pathologies in the disease, including pathologies found in pancreatic islets, the secretion site of insulin^[8]. Moreover, when investigating the effects of an oral agent such as RJ, bioavailability is critical: absorption, distribution and metabolism of nutrients such as fatty acids found in RJ may vary between rodents and humans^[16]. Our preliminary research indicates that bioavailability and metabolism of RJ in human models has not yet been established, so the enhanced response of RJ observed in animal trials may not be applicable to human patients.

Another limitation stems from the similarity between aspects of some studies. A considerable number of the included studies share the same authors, such as Pourmoradian *et al*^[13], Pourmoradian *et al*^[30], Mobasseri *et al*^[28], and Mobasseri *et al*^[25]. As a result, there is noteworthy overlap in the methodology of these studies, although different outcomes are assessed in each. In maintaining very similar study population and methodology, there is the potential for similar undetected bias to affect the results of these studies. Because it presents the risk of the overall evidence misrepresenting RJ's true effect, overlapping methodology between studies is an important factor to consider when interpreting the evidence.

Review limitations

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Fujii 1990	+	+	?	-	+	?	?
Ghanbari 2015	+	+	+	-	+	+	+
Iaconelli 2010	+	+	-	-	+	+	+
Khoshpey 2016	?	?	+	?	+	+	?
Membrez 2010	+	+	?	-	?	+	?
Mobasserri 2014	+	+	+	+	+	+	?
Mobasserri 2015	+	+	+	+	+	+	?
Morita 2012	+	?	+	?	+	+	?
Münstedt 2009	+	+	-	-	+	+	+
Münstedt 2010	?	-	-	-	+	+	+
Pourmoradian 2012	+	+	+	+	+	+	?
Pourmoradian 2014	+	+	+	+	+	+	+
Shidfar 2015	+	?	+	?	+	+	?
Watadani 2017	+	+	+	-	?	+	+
Yoneshiro 2017	+	+	+	-	?	+	+
Yoshida 2017	+	+	+	-	?	+	+
Zamami 2008	+	+	+	-	?	+	+

Figure 4 Risk of bias summary: Judgements about each risk of bias item for all included *in vivo* studies.

The evidence synthesized in this review relied completely on the included studies, which may be unrepresentative of RJ's true effect on the probed outcomes. The exclusion of non-English studies may have removed a considerable number of studies; this is a concern particularly for this topic because almost all the included studies have non-English speaking origins. Also, despite having known insulin-like properties and thus potentially a role in glycemic regulation, no studies on RJ proteins were included in this review^[56]. Lastly, while this review focused on RJ as a dietary supplement, other forms of administration (*e.g.*, topical) may improve effectiveness or bioavailability.

In conclusion, RJ supplementation presents promising potential for treatment of glycemic T2D symptoms. The evidence synthesized in this review complements

Table 4 GRADE assessment for long-term effectiveness of RJ treatment on glycemic control

GRADE criteria	Rating	Support for judgement	Overall quality of evidence
Outcome: Long term glycemic control (<i>n</i> = 14 studies)			
RoB (assessed on Cochrane RoB Collaboration Tool)	No Serious (-1) ¹ Very serious (-2)	Only one study had low RoB for all categories. Most studies had at least one item at high or unclear RoB	High
Inconsistency	No Serious (-1) ¹ Very serious (-2)	Generally, positive clinical effects demonstrated but some studies indicate null effects. There are also large variations in magnitude of effect. Heterogeneity is notable between the studies (in population, intervention and outcome assessment)	Moderate
Indirectness	No ¹ Serious (-1) Very serious (-2)	Evidence synthesized from studies addresses review question with respect to population, interventions and outcome	Low ¹
Imprecision	No Serious (-1) Very serious (-2) ¹	All studies have groups with small sample sizes (≤ 20), with no indication that they meet required sample sizes to detect difference in outcome; observable but statistically insignificant measures in many studies suggest sample sizes were too small to detect difference. 95% confidence intervals of effect size estimates mostly suggest an appreciable benefit for treatment, but there are several that suggest possibility of no meaningful effect	Very low
Publication bias	Undetected ¹ Strongly suspected (-1)	There is chance of publication bias considering the review is entirely "small-scale" trials; this area of research is not well-established and there is potential for publication bias, but none was overtly detected	
Other	Large effect (+1 ¹ or +2) Dose response (+1 ¹ or +2) No plausible confounding (+1 or +2)	Standardized mean difference of studies (effect size) indicates large magnitude of effect of treatment. Dose-response relationships observed	

¹Indicates decision. RoB: Risk of Bias. Adapted from Ryan and Hill^[21] and Wei *et al*^[22].

existing research that demonstrates other therapeutic effects of RJ administration in T2D symptoms, such as oxidative stress, impaired wound-healing and inflammation^[32,57,58]. Future studies should examine the pharmacodynamic properties of RJ, particularly with respect to dosage forms, effectiveness and bioavailability in different populations to further elucidate the effectiveness of RJ as a therapeutic agent of hyperglycemia.

Table 5 GRADE assessment for acute effects of RJ administration on glycemic control

GRADE criteria	Rating	Support for judgement	Overall quality of evidence
Outcomes: Acute glycemic control outcomes (<i>n</i> = 4 studies)			
RoB (assessed on Cochrane RoB Collaboration Tool)	No Serious (-1) Very serious (-2) ¹	Majority of studies had overall high RoB, likely affecting the study results	High
Inconsistency	No Serious (-1) ¹ Very serious (-2)	Outcome effects are somewhat consistent, and studied population are similar enough to not be considered detrimental to evidence quality. Intervention, however, was heterogeneous across all relevant studies	Moderate
Indirectness	No ¹ Serious (-1) Very serious (-2)	Research question is addressed by majority of the animal studies	Low
Imprecision	No Serious (-1) Very serious (-2) ¹	All studies have groups with small sample sizes (≤ 20), with no indication that they meet required sample sizes to detect difference in outcome. For those with calculable effect sizes, the confidence intervals suggest potential for no appreciable benefit	Very low ¹
Publication bias	Undetected ¹ Strongly suspected (-1)	There is chance of publication bias considering the review is entirely “small-scale” trials; this area of research is not well-established and there is potential for publication bias, but none was overtly detected	
Other	Large effect (+1 or +2) Dose response (+1 or +2) No plausible confounding (+1 or +2)	Some dose response relationships observed, however not enough studies to confirm this relationship. Insufficient effect size estimates to determine if effect is large or not	

¹Indicates decision. RoB: Risk of Bias. Adapted from Ryan and Hill^[21] and Wei *et al*^[22].

ARTICLE HIGHLIGHTS

Research background

Existing evidence suggests that royal jelly (RJ) is a promising therapeutic option in hyperglycemic cases. Few studies have specifically examined the clinical viability of RJ as treatment, and no study has critically analyzed the existing evidence. Knowledge of the factors that influence effectiveness of RJ intake provides an alternative treatment for hyperglycemia, which is often associated with diabetes.

Research motivation

This systematic review demonstrated that the intervention style (*e.g.*, length of supplementation, ingestion form) as well as pre-existing patient characteristics may be important factors in its effectiveness, and future research should further investigate these factors to inform patients and health care providers.

Research objectives

This review sought to examine whether there is support for RJ as a glycemic regulator in models of type 2 diabetes as well as healthy individuals. Our analysis found that the existing evidence suggests that RJ is a promising therapeutic option in hyperglycemic cases, with effective doses as low as 1000 mg of fresh RJ daily for diabetic patients.

Research methods

This was a systematic review employing the PRISMA strategy. Five databases were searched using keywords pertinent to the research objectives. Two reviewers conducted full-text screening to select included articles that met eligibility criteria. Relevant information (*i.e.*, intervention style, results, participant characteristics) was extracted from the included articles. Risk of bias was assessed by two reviewers. GRADE, a novel tool developed by Cochrane used to assess overall quality of evidence, was also determined by two reviewers.

Research results

Effective doses of RJ may be as low as 1000 mg of fresh RJ for a diabetic patient. Overall, the quality of evidence for RJ as a treatment is low for long-term effectiveness, and very low for acute effects of RJ consumption.

Research conclusions

Synthesis and analysis of existing studies shows that RJ may be viable as part of a treatment plan in lowering blood sugar. Due to the heterogeneity in studied population and intervention, RJ may have more pronounced effects in certain dosage forms (*e.g.*, fresh RJ) and in certain populations (*e.g.*, postmenopausal females). This information may be useful for individuals and health care practitioners wishing to explore hyperglycemia treatment options.

Research perspectives

Future clinical trials should consider the potential effects of intervention form and length, as well as the effect of participant characteristics to clarify which patient populations or conditions would benefit most from RJ supplementation.

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SGLT-2 inhibitors in non-alcoholic fatty liver disease patients with type 2 diabetes mellitus: A systematic review

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a common comorbidity with type 2 diabetes. The existing therapeutic options for NAFLD are not adequate. Hypocaloric diet and exercise is the cornerstone of therapy in NAFLD. Pioglitazone is the only drug recommended in diabetes patients with biopsy proven non-alcoholic steatohepatitis. The frequent coexistence of NAFLD and type 2 diabetes with their combined adverse health consequences and inadequate therapeutic options makes it necessary to search for newer alternatives.

AIM

To assess the effect of sodium glucose cotransporter-2 (SGLT-2) inhibitors on liver enzymes in type 2 diabetes patients with NAFLD.

METHODS

We searched PubMed/MEDLINE, Cochrane library, Google scholar, and Clinicaltrials.gov for the relevant articles to be included in this systematic review. Human studies done in type 2 diabetes patients with NAFLD treated with SGLT-2 inhibitors for at least 12 wk were included. Data from eight studies (four randomised controlled trials and four observational studies) were extracted and a narrative synthesis was done. A total of 214 patients were treated with SGLT-2 inhibitors in these studies (94 in randomised controlled trials and 120 in observational studies).

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RESULTS

The primary outcome measure was change in serum alanine aminotransferase level. Out of eight studies, seven studies showed a significant decrease in serum alanine aminotransferase level. Most of the studies revealed reduction in serum level of other liver enzymes like aspartate aminotransferase and gamma glutamyl transferase. Five studies that reported a change in hepatic fat exhibited a significant reduction in hepatic fat content in those treated with SGLT-2 inhibitors. Likewise, among the three studies that evaluated a change in indices of hepatic fibrosis, two studies revealed a significant improvement in liver fibrosis. Moreover, there was an improvement in obesity, insulin resistance, glycaemia, and lipid parameters in those subjects taking SGLT-2 inhibitors. The studies disclosed that about 17% (30/176) of the subjects taking SGLT-2 inhibitors developed adverse events and more than 40% (10/23) of them had genitourinary tract infections.

CONCLUSION

Based on low to moderate quality of evidence, SGLT-2 inhibitors improve the serum level of liver enzymes, decrease liver fat, and fibrosis with additional beneficial effects on various metabolic parameters in type 2 diabetes patients with NAFLD.

Key words: Alanine aminotransferase; Hepatic fat; Hepatic fibrosis; Non-alcoholic fatty liver disease; Sodium-glucose cotransporter-2 inhibitor; Type 2 diabetes mellitus

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Core tip: The frequent coexistence of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes, their adverse health consequences, and lack of adequate therapeutic options makes it necessary to search for newer alternatives. Currently, pioglitazone and vitamin E are recommended in addition to lifestyle modifications for the management of NAFLD. Animal studies have shown that sodium glucose cotransporter-2 inhibitors might be beneficial in NAFLD present in diabetes patients. The current systematic review shows that sodium glucose cotransporter-2 inhibitors improve the serum level of liver enzymes, liver fat, and liver fibrosis with additional beneficial effects on various metabolic parameters in type 2 diabetes patients with NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an emerging public health issue worldwide. The prevalence of NAFLD in type 2 diabetes mellitus patients is three times greater as compared to the general population. Its prevalence in diabetic subjects ranges from 69%-87% depending upon the imaging modality used^[1]. The spectrum of NAFLD includes simple steatosis, steatohepatitis, and cirrhosis^[2]. Besides NAFLD is a risk factor for extrahepatic complications like cardiovascular disease, chronic kidney disease, and type 2 diabetes. In addition, the prevalence of both microvascular and macrovascular complications is increased in patients with NAFLD and type 2 diabetes^[3].

The existing therapeutic options for NAFLD are not adequate. Hypocaloric diet and exercise is the cornerstone of therapy in NAFLD. Pioglitazone and vitamin E are recommended only in biopsy-proven non-alcoholic steatohepatitis (NASH), but vitamin E is not recommended in diabetic patients due to inadequate evidence^[4]. The frequent coexistence of NAFLD and type 2 diabetes with their combined adverse health consequences and inadequate therapeutic options makes it necessary to search for newer alternatives. Based on the information from animal studies, sodium glucose

cotransporter-2 (SGLT-2) inhibitors appear promising in the management of NAFLD^[5-7]. This systematic review is an effort to review the available literature on the effect of SGLT-2 inhibitors on NAFLD in type 2 diabetes patients.

MATERIALS AND METHODS

Protocol and registration

This systematic review was performed according to the predefined protocol registered in PROSPERO (Registration ID: CRD42018104572). The protocol can be accessed at the website address <https://www.crd.york.ac.uk/prospéro>. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2009 guidelines for reporting this systematic review^[8]. Ethics committee approval was not required for this systematic review because it was done using published data found in the public domain.

Eligibility criteria

All observational and randomised controlled trials (RCTs) done using SGLT-2 inhibitors among type 2 diabetes patients with NAFLD having both baseline and post-treatment serum alanine aminotransferase (ALT) level data with a minimum follow-up duration of 12 wk were included in this systematic review. The studies with concomitant pharmacological therapy like pioglitazone or α -tocopherol (vitamin E) for treating NAFLD were excluded to avoid the confounding effects of these drugs on liver function tests. Only those studies that were done in humans and published in English were considered for inclusion. We excluded abstract-only articles, case reports, conference presentations, editorials, reviews, expert opinions, and studies with five participants and less.

Primary and secondary outcomes

The primary outcome was the change in serum ALT levels in type 2 diabetes patients with NAFLD treated with SGLT-2 inhibitors. The secondary outcomes were change in serum aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels, hepatic fat, hepatic fibrosis, metabolic profile, anthropometric parameters, and the adverse effects of SGLT-2 inhibitors.

Information sources

PubMed/MEDLINE, Cochrane library, Google scholar, and Clinicaltrials.gov were searched from their date of inception until 31st August, 2018.

Literature search and study selection

The search terms/MeSH terms used were "NAFLD", "Nonalcoholic fatty liver disease", "Non-alcoholic fatty liver disease", "Non alcoholic fatty liver disease", "NASH", "Non-alcoholic steatohepatitis", "Nonalcoholic steatohepatitis", "Non alcoholic steatohepatitis", "Fatty liver", "Type 2 diabetes mellitus", "Type 2 diabetes", "Diabetes mellitus type 2", "Diabetes type 2", "SGLT-2 inhibitors", "Sodium glucose cotransporter-2 inhibitors", "SGLT-2", "SGLT2", "SGLT 2", "Canagliflozin", "Dapagliflozin", "Empagliflozin", "Ipragliflozin", "Luseogliflozin", "Tofogliflozin", "Sotagliflozin", "Remogliflozin", "Ertugliflozin", and "Sergliflozin" (Table 1). The references of the search articles were scrutinised for relevant articles.

Data collection process

The titles and/or abstracts of studies were retrieved using the search strategy and those from additional sources were scrutinised independently by two review authors (HR and JPS) to identify studies that potentially met the inclusion criteria as outlined above. The full texts of these potentially eligible studies were retrieved and independently assessed for eligibility by three review team members (HD, SS, and RP). Any disagreements between the reviewers over the eligibility of particular studies were resolved through discussion with a fourth senior reviewer (SKK). A standardised, pre-formatted excel form was used to extract data from the included studies for the assessment of study quality.

Data items and synthesis of results

The extracted data included the author of the study with year, the study methodology, the recruitment and study completion rates, the types of population, the exposure/intervention (dose of SGLT-2 inhibitor, duration), the results (outcome measures like change in serum ALT, AST, GGT, hepatic fat, markers of liver fibrosis, fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c), lipid profile, homeostasis model assessment-estimated insulin resistance (HOMA-IR), body mass

Table 1 Literature search strategy

S. No	Search terms
1	NAFLD
2	Nonalcoholic fatty liver disease
3	Non-alcoholic fatty liver disease
4	Non alcoholic fatty liver disease
5	NASH
6	Non-alcoholic steatohepatitis
7	Nonalcoholic steatohepatitis
8	Non alcoholic steatohepatitis
9	Fatty liver
10	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9
11	Type 2 diabetes mellitus
12	Type 2 diabetes
13	Diabetes mellitus type 2
14	Diabetes type 2
15	11 OR 12 OR 13 OR 14
16	SGLT-2 inhibitors
17	Sodium glucose cotransporter-2 inhibitors
18	SGLT-2
19	SGLT2
20	SGLT 2
21	Canagliflozin
22	Dapagliflozin
23	Empagliflozin
24	Ipragliflozin
25	Luseogliflozin
26	Tofogliflozin
27	Sotagliflozin
28	Remogliflozin
29	Ertugliflozin
30	Sergliflozin
31	16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30
32	10 AND 15 AND 31

NAFLD: Non-alcoholic fatty liver disease; NASH: Non alcoholic steatohepatitis; SGLT-2: Sodium glucose cotransporter-2.

index (BMI), any adverse effects, information for the assessment of the risk of bias, and sources of funding/support.

The statistical review of the study was performed by a biomedical statistician (SSK). A narrative synthesis of the results of individual studies was done. The change in the difference in means and difference in proportions and the respective *P* values as mentioned in the original manuscripts were tabulated and explained in our study.

Risk of study bias

The risk of bias of the RCTs was done using Cochrane risk of bias tool^[9]. The studies were graded as “good quality” or “fair quality” or “poor quality” according to the level of risk. Methodological Index for Non-Randomized Studies (MINORS) scale was used to assess the risk of bias of observational studies^[10]. A study was considered to be an ideal study if the score was 16 for single arm and 24 for comparative studies.

RESULTS

Study selection

Our literature search from all the aforementioned databases yielded 73 articles (including references of the relevant articles). After eliminating duplicate articles, 55

articles were screened. Eight articles met all of the inclusion criteria (total 214 patients were on SGLT-2 inhibitors) (Figure 1).

Study characteristics

The summary of all studies included in this systematic review is given in Tables 2 and 3. Out of the eight studies, four are RCTs^[11-14] and four are observational^[15-18]. Five studies were conducted amongst the Japanese population. Ipragliflozin was used in three studies whereas canagliflozin and luseogliflozin were used in two studies each, but dapagliflozin and empagliflozin were used in one study each. All studies used one type of SGLT-2 inhibitor except the one authored by Seko *et al*^[16], where both canagliflozin and ipragliflozin were used. The change in serum ALT was a secondary outcome while the effect of SGLT-2 inhibitors on liver fat was the primary outcome in all RCTs.

Risk of bias within studies

The risk of bias of RCTs was assessed using the Cochrane risk of bias tool. Among the four RCTs, the studies done by Kuchay *et al*^[11] and Eriksson *et al*^[14] were of good quality however those done by Ito *et al*^[12] and Shibuya *et al*^[13] were of fair quality (Table 4). The risk of bias of observational studies was assessed using the MINORS scale. All the observational studies were of less than ideal quality (Table 5).

Primary outcome

Change in serum ALT levels: In all of the studies, there was a decrease in serum ALT levels from the baseline in those treated with SGLT-2 inhibitors (Table 6) but in the study done by Shibuya *et al*^[13] it did not reach statistical significance.

Kuchay *et al*^[11] found a significant decrease in serum ALT levels in the empagliflozin arm compared to the control arm at the end of the study (difference between the two arms was -10.9 IU/L, $P = 0.005$). In the study done by Ito *et al*^[12] ALT levels decreased equally in both the groups [Change from baseline in ipragliflozin group: -17.5 (4) and pioglitazone group: -20 (3.4), $P = 0.642$]. Similar results were found in the study by Shibuya *et al*^[13] [Δ ALT in luseogliflozin arm was 9 (-20, 1) and in metformin arm was 4.5 (-5, 9), $P = 0.064$]. Eriksson *et al*^[14] found that the ALT reduction in the dapagliflozin arm was more compared to placebo [Δ ALT in dapagliflozin arm was -8.24 (8.24) and in the placebo arm was -0.18 (8.82), $P < 0.05$]. Seko *et al*^[16] demonstrated that the serum ALT levels in SGLT-2 inhibitor arm was lower compared to the sitagliptin arm at the end of the study [48.8 (5.5) vs 71.1 (10), $P = 0.039$].

Secondary outcomes

Change in serum AST levels: Seven of the included studies had data regarding change in serum AST levels (Table 7). The study done by Shibuya *et al*^[13] did not have data on AST levels. All the studies showed a significant reduction in serum AST levels in those treated with SGLT-2 inhibitors. The decrease in AST with empagliflozin and ipragliflozin was similar compared to placebo and pioglitazone respectively whereas dapagliflozin was better than placebo.

Change in serum GGT levels: Seven studies had data regarding GGT levels. Six studies reported a significant decrease in serum GGT levels in those treated with SGLT-2 inhibitors (Table 8). In the study done by Seko *et al*^[16], there was an insignificant decrease in both the SGLT-2 inhibitor and DPP-4 inhibitor groups. The decrease in GGT with empagliflozin and ipragliflozin was similar compared to placebo and pioglitazone correspondingly while dapagliflozin was better than placebo.

Change in hepatic fat: Kuchay *et al*^[11] and Eriksson *et al*^[14] evaluated hepatic fat using magnetic resonance imaging- derived proton density fat fraction (Table 9). It was found that there was a significant reduction in hepatic fat in the empagliflozin arm compared to the control arm in the study done by Kuchay *et al*^[11]. In the study done by Eriksson *et al*^[14], dapagliflozin or omega-3 carboxylic acid when administered alone or in combination reduced hepatic fat fraction significantly. When compared with placebo, only the combination of both drugs reduced hepatic fat fraction significantly. Sumida *et al*^[18] showed that luseogliflozin significantly reduced hepatic fat fraction using magnetic resonance imaging-hepatic fat fraction. Ito *et al*^[12] and Shibuya *et al*^[13] used liver/spleen attenuation ratio for measuring hepatic fat. They found that ipragliflozin was equivalent to pioglitazone in improving liver/spleen attenuation ratio while luseogliflozin was found to be superior to metformin in the same aspect.

Effect on liver fibrosis indices

Ito *et al*^[12] and Ohki *et al*^[15] evaluated liver fibrosis using the FIB-4 index (Table 10).

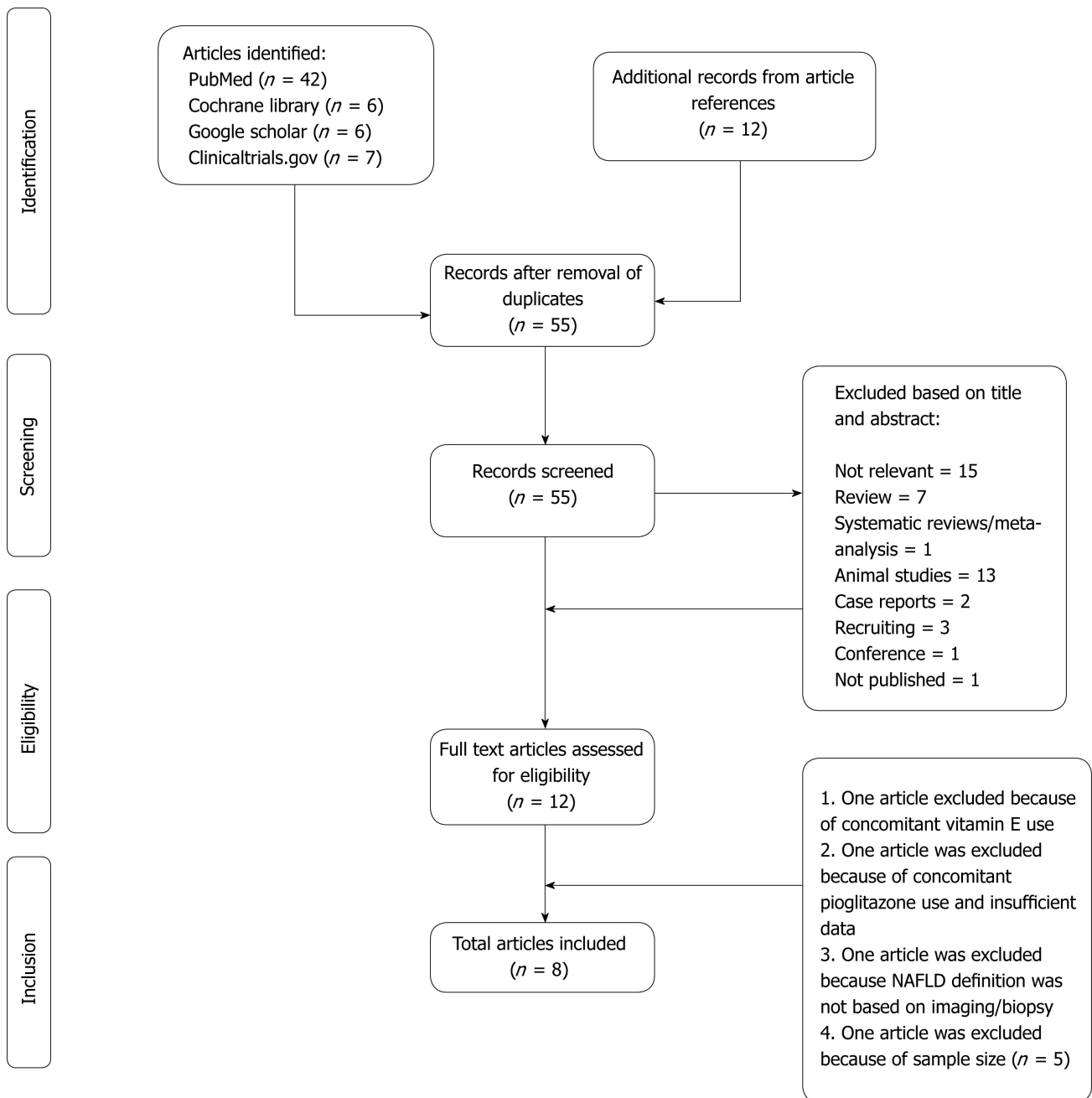


Figure 1 Literature search and study selection.

There was a significant decrease in the FIB-4 index in the ipragliflozin arms compared to baseline. Ipragliflozin was similar to pioglitazone in decreasing the FIB-4 index. Sumida *et al*^[18] used both the FIB-4 index and NAFLD fibrosis score. There was no significant change in either indices.

Change in metabolic and anthropometric parameters

Seven studies reported changes in FPG and HbA1c (Tables 11 and 12). The majority of the studies showed a decrease in FPG and HbA1c.

In the study done by Ito *et al*^[12] there was no difference in the change in HOMA-IR in those treated with either ipragliflozin or pioglitazone ($P = 0.401$) (Table 13). There was a significant decrease in HOMA-IR in those treated with dapagliflozin compared to placebo in the study done by Eriksson *et al*^[14]. Surprisingly there was an insignificant increase in HOMA-IR in those treated with either a SGLT-2 inhibitor or a gliptin in the study done by Seko *et al*^[16].

Six studies included data on the changes in lipid profile (Tables 14, 15, and 16). There was a significant decrease in serum triglycerides in two studies (Kuchay *et al*^[11] and Ito *et al*^[12]). Three studies exhibited an increase in high-density lipoprotein cholesterol levels (Ito *et al*^[12], Ohki *et al*^[15], and Seko *et al*^[16]). Most of the studies (Ito *et*

Table 2 Randomised controlled trials

S. No	Ref.	Inclusion criteria	Age (yr)	Male gender	Intervention arm	Control arm	Follow-up duration	Primary outcome
1	Kuchay <i>et al</i> ^[11] , 2018	Age > 20 yr, hepatic steatosis (MRI-PDFF > 6%), HbA1c > 7.0% to < 10.0%	Intervention arm: 50.7 (12.8) Control arm: 49.1 (10.3)	Intervention arm: 16 (64%) Control arm: 17 (68%)	Standard treatment + Empagliflozin 10 mg daily (<i>n</i> = 25)	Standard treatment (<i>n</i> = 25)	20 wk	Change in liver fat content by MRI-PDFF
2	Ito <i>et al</i> ^[12] , 2017	Age 20-75 yr, HbA1c 7.0-11.0%, BMI < 45 kg/m ² , On diet and exercise therapy alone or with oral hypoglycaemic agents other than SGLT-2 inhibitors and thiazolidinediones and/or insulin, NAFLD, findings suggesting hepatic steatosis and hepatic dysfunction on clinical laboratory tests or on imaging studies (<i>e.g.</i> , computed tomography or ultrasound)	Pioglitazone arm: 59.1 (9.8) Ipragliflozin arm: 57.3 (12.1)	Pioglitazone arm: 18 (53%) Ipragliflozin arm: 14 (44%)	Ipragliflozin 50 mg daily (<i>n</i> = 32)	Pioglitazone 15-30 mg daily (<i>n</i> = 34)	24 wk	Change in L/S attenuation ratio
3	Shibuya <i>et al</i> ^[13] , 2018	Fatty liver diagnosed on the basis of computed tomography or abdominal sonography, HbA1c 6.0%-10.0%, age 20-70 yr	Luseogliflozin arm: 51 (47-62) Metformin arm: 60 (53-66)	Luseogliflozin arm: 10 (62.5%) Metformin arm: 8 (50%)	Luseogliflozin 2.5 mg daily (<i>n</i> = 16)	Metformin 1.5 g daily (<i>n</i> = 16)	24 wk	Change in L/S attenuation ratio
4	Eriksson <i>et al</i> ^[14] , 2018	Age 40-75 yr, treated with a stable dose of metformin or sulfonylurea alone or in combination for at least 3 mo, MRI-PDFF > 5.5%, BMI 25-40 kg/m ²	Dapagliflozin arm: 65 (6.5) Omega 3-carboxylic acid arm: 66.2 (5.9) O + D arm: 65(5.4) Placebo arm: 65.6 (6.1)	Dapagliflozin arm: 16 (76.2%) Omega 3-carboxylic acid arm: 11 (55%) O + D arm: 15 (68.2%) Placebo arm: 17 (81%)	Dapagliflozin 10 mg daily (<i>n</i> = 21) or Omega 3-carboxylic acid 4 g daily (<i>n</i> = 20) or Combination (<i>n</i> = 22)	Placebo (<i>n</i> = 21)	12 wk	Change in liver fat content by MRI-PDFF

MRI-PDFF: Magnetic resonance imaging-derived proton density fat fraction; L/S: Liver/spleen; O + D: Omega 3-carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2; NAFLD: Non-alcoholic fatty liver disease.

al^[12], Eriksson *et al*^[14], Ohki *et al*^[15], Seko *et al*^[16], and Sumida *et al*^[18]) showed no change in serum LDL levels.

Five studies included BMI change (Table 17). There was a reduction in BMI in the SGLT-2 inhibitor arms in all the studies. Empagliflozin was similar to placebo in reducing BMI whereas luseogliflozin was superior to metformin in reducing BMI.

Adverse effects of SGLT-2 inhibitors

Out of the eight studies, six studies reported the adverse effects of SGLT-2 inhibitors. There were a total of 30 reported adverse events in 176 patients taking SGLT-2

Table 3 Observational studies

S. No	Ref.	Design	Inclusion criteria	Age (yr)	Male gender	Sample size	SGLT-2 inhibitor	Follow-up duration
1	Ohki <i>et al</i> ^[15] , 2016	Prospective study	Type 2 diabetes with NAFLD treated with GLP-1 analogues or DPP-4 inhibitors and failed to normalise serum ALT levels	54.2 (49.3-60.1)	19 (79.2%)	24	Ipragliflozin 25-50 mg daily	320 d (302-329)
2	Seko <i>et al</i> ^[16] , 2016	Retrospective cohort study	Type 2 diabetes with NAFLD	SGLT-2 inhibitor arm: 60.3 (1.8) Sitagliptin arm: 59.4 (3.7)	SGLT-2 inhibitor arm: 9 (37.5%) Sitagliptin arm: 8 (38.1%)	24 (SGLT-2 inhibitor); 21 (Sitagliptin)	Canagliflozin 100 mg (<i>n</i> = 18) or Ipragliflozin 50 mg daily (<i>n</i> = 6)	24 wk
3	Gautam <i>et al</i> ^[17] , 2018	Prospective study	Type 2 diabetes with NAFLD	-	-	32	Canagliflozin 100 mg daily	24 wk
4	Sumida <i>et al</i> ^[18] , 2018	Prospective study	Age > 20 yr, HbA1c > 6.5% to < 8.5%, NAFLD	55.4 (13.6)	28 (70%)	40	Luseogliflozin 2.5 mg daily	24 wk

NAFLD: Non-alcoholic fatty liver disease; SGLT-2: Sodium glucose cotransporter-2; GLP-1: Glucagon like peptide-1; DPP-4: Dipeptidyl peptidase-4.

inhibitors (Table 18). The most common adverse event was genitourinary tract infection (10 events).

DISCUSSION

Type 2 diabetes is commonly associated with NAFLD. Serum ALT levels are commonly above the upper limit of normal with AST levels lesser than ALT levels^[19]. Animal studies have shown that SGLT-2 inhibitors decrease liver enzymes (ALT, AST), liver weight, and hepatic steatosis^[20-23]. There are several mechanisms for improvement in serum liver enzymes in the patients taking SGLT-2 inhibitors. These drugs cause hyperglucagonemia by increasing glucagon secretion from the pancreatic α cells. Glucagon stimulates gluconeogenesis and β -oxidation of fatty acids in the liver via stimulation of peroxisome proliferator-activated receptor alpha and carnitine palmitoyl transferase-1^[13]. Thus SGLT-2 inhibitors help to reduce hepatic fat. They reduce collagen deposition and inflammatory cytokine expression in liver^[5,22]. They decrease liver enzymes by additionally improving glycaemic parameters and insulin resistance. Out of eight studies, seven showed a decrease in serum ALT and AST levels in our systematic review. Shibuya *et al*^[13] observed a decrease in ALT that almost reached statistical significance, however data regarding AST was unavailable^[13]. Out of seven studies, six illustrated a significant decrease in GGT levels while in the study by Seko *et al*^[16] the change in serum GGT level almost reached statistical significance.

Liver enzymes are surrogate markers of liver histological response, but an improvement in liver histology is not always associated with a decrease in serum liver enzymes^[11]. The five studies that evaluated changes in hepatic fat showed a decrease in hepatic fat. There was no correlation of a change in ALT with a change in hepatic fat in the study by Shibuya *et al*^[13], however there was a correlation between these two parameters in the study by Sumida *et al*^[18]. The decrease in hepatic fat in the SGLT-2 inhibitor arm was comparable to pioglitazone, which is an approved drug for treatment of NAFLD irrespective of the presence of diabetes. Eriksson *et al*^[14] observed that although the hepatic fat content decreased in the dapagliflozin arm it did not reach statistical significance compared to placebo. The lesser duration of this study (12 wk) compared to other studies may have contributed to this difference.

The progression of NAFLD to cirrhosis is determined to a large extent by the liver histology. Studies with up to 20 years follow-up have shown that the risk of progression to cirrhosis for simple steatosis, NASH, and NASH with fibrosis are 0%-4%, 25%, and 38%, respectively^[24]. The FIB-4 index is a non-invasive tool to assess liver

Table 4 Assessment of study quality of randomised controlled trials

Study	Criteria	Risk of bias	Study quality
Kuchay <i>et al</i> ^[11]	Random sequence generation	Low risk	Good quality
	Allocation concealment	Low risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	
Ito <i>et al</i> ^[12]	Random sequence generation	Low risk	Fair quality
	Allocation concealment	Unclear risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	
Shibuya <i>et al</i> ^[13]	Random sequence generation	Unclear risk	Fair quality
	Allocation concealment	Unclear risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	
Eriksson <i>et al</i> ^[14]	Random sequence generation	Low risk	Good quality
	Allocation concealment	Low risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	

fibrosis^[25]. It is calculated from the patient's age, platelet count, ALT levels, and AST levels. The FIB-4 index was decreased with SGLT-2 inhibitor therapy in two out of three studies. Sumida *et al*^[18] used the NAFLD fibrosis score in addition to the FIB-4 index to assess liver fibrosis. The NAFLD fibrosis score is a composite score of six variables (age, BMI, hyperglycaemia, platelet count, albumin, and AST/ALT ratio)^[26]. There was no significant change in either indices in this study.

It has been shown that NAFLD is more common in those with poor glycaemic control than those with good glycaemic control^[27]. SGLT-2 inhibitors promote glycosuria by inhibiting SGLT-2 in the proximal convoluted tubule. Therefore their action is dependent on blood glucose levels but insulin independent^[28]. They cause a significant reduction in FPG^[29]. A meta-analysis of RCTs has concluded that the average HbA1c reduction at 52 wk of SGLT-2 inhibitor therapy to be 0.6%^[30]. Another meta-analysis has shown that SGLT-2 inhibitor monotherapy is equivalent to metformin monotherapy in reducing HbA1c levels^[31]. However, the decrease in HbA1c was more in the luseogliflozin arm compared to the metformin arm in the study by Shibuya *et al*^[13]. Four out of seven studies and six out of seven studies showed a decrease in FPG and HbA1c, respectively, in the SGLT-2 inhibitor arm. Thus, the improved glycaemic status is one of the mechanisms by which SGLT-2 inhibitors ameliorate NAFLD.

SGLT-2 inhibitors ameliorate insulin resistance in numerous ways. SGLT-2 inhibitors improve obesity associated insulin resistance by regulating macrophage

Table 5 Assessment of study quality of observational studies

S. No	Criteria	Ohki <i>et al</i> ^[15]	Seko <i>et al</i> ^[16]	Gautam <i>et al</i> ^[17]	Sumida <i>et al</i> ^[18]
1	A clearly stated aim	2	2	2	2
2	Inclusion of consecutive patients	0	2	2	1
3	Prospective collection of data	2	0	2	2
4	Endpoints appropriate to the aim of the study	2	2	2	2
5	Unbiased assessment of the study endpoint	0	0	0	0
6	Follow-up period appropriate to the aim of the study	2	2	2	2
7	Loss to follow up less than 5%	2	2	2	2
8	Prospective calculation of the study size	0	0	0	0
9	An adequate control group	NA	0	NA	NA
10	Contemporary groups	NA	2	NA	NA
11	Baseline equivalence of groups	NA	2	NA	NA
12	Adequate statistical analyses	NA	2	NA	NA
13	Total score	10/16	16/24	12/16	11/16

recruitment and altering the proportion of pro-inflammatory and anti-inflammatory macrophages. They enhance fat utilization by promoting β -oxidation of fatty acids and browning of white adipose tissue by inducing the expression of thermogenin leading to an improvement in the lipid profile. Similar to other antidiabetic drugs, SGLT-2 inhibitors reduce insulin resistance by decreasing glucotoxicity. Dapagliflozin has been shown to improve insulin sensitivity by increasing adiponectin and zinc-A2-glycoprotein levels^[32]. Only dapagliflozin was shown to decrease insulin resistance in the study by Eriksson *et al*^[14].

SGLT-2 inhibitors cause weight reduction. The major mechanism that causes weight reduction is the decrease in fat mass. The decrease in fat mass is due to the shift in substrate utilization to lipids instead of carbohydrates^[33,34]. Ito *et al*^[12] and Shibuya *et al*^[13] demonstrated that SGLT-2 inhibitors caused a significant reduction in abdominal visceral and subcutaneous fat area as measured by computed tomography scan. Similarly, Eriksson *et al*^[14] showed that dapagliflozin significantly reduced abdominal visceral and subcutaneous adipose tissue volume as assessed by magnetic resonance imaging. The other mechanisms of weight loss are the urinary glucose loss which amounts to approximately 200 Kcal/d and osmotic diuresis^[33,35]. Unlike the other weight-reducing effects of SGLT-2 inhibitors, which are potentially beneficial, osmotic diuresis is clearly an adverse effect. Seko *et al*^[16] showed that ipragliflozin and canagliflozin significantly reduced total body water in addition to body fat mass as measured by bioelectrical impedance analysis. Five studies showed a significant decrease in BMI in patients on SGLT-2 inhibitor therapy. Thus, the major beneficial effects of SGLT-2 inhibitors on NAFLD are exerted via reduction in hepatic fat and fibrosis, improved glycaemic control, decrease in insulin resistance, and weight loss.

The most common adverse effects of SGLT-2 inhibitors are genitourinary tract infections. In addition, they may cause diabetic ketoacidosis, dizziness, acute kidney injury, lower limb amputations, and bone fractures^[36,37]. A meta-analysis concluded that there was no difference between placebo and SGLT-2 inhibitors for serious adverse events^[38]. Among the 30 adverse events reported in all the studies, the most common was genitourinary tract infections (10 out of 23 characterised events).

The major strength of this systematic review was that the effect of five SGLT-2 inhibitors on NAFLD in patients with type 2 diabetes was evaluated in both RCTs and observational studies. Moreover, liver fat, liver fibrosis, metabolic, and anthropometric parameters in addition to liver enzymes were assessed as outcome variables following SGLT-2 inhibitor therapy. Yet this systematic review has a few limitations. First, most of the studies were done amongst the Japanese population. As a result, the study findings may not be applicable to patients from other ethnicities. Second, the sample size was considerably small and the duration of follow-up was of limited period in most of the studies. Third, the confounding effect of concomitant anti-diabetes drugs like metformin, DPP-4 inhibitors, and glucagon like peptide-1 analogues on NAFLD cannot be ruled out, particularly in observational studies. Fourth, two studies (Eriksson *et al*^[14] and Sumida *et al*^[18]) were funded by pharmaceutical companies, which is a source of potential conflicts of interest.

Summary and conclusion

In conclusion based on the available evidence, SGLT-2 inhibitors were found to

Table 6 Change in serum alanine aminotransferase levels in individual studies

Study	Serum ALT level (IU/L)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	64.3 (20.2)	49.7 (25.8)	0.001	0.005
	Control	65.3 (40.3)	61.6 (38.4)	0.422	
Ito <i>et al</i> ^[12]	Ipragliflozin	57.4 (27.3)	38.2 (20.5)	< 0.05	0.642
	Pioglitazone	53.1 (26.6)	36.8 (15.1)	< 0.05	
Shibuya <i>et al</i> ^[13]	Luseogliflozin	49.5 (31.0, 70.0)	31 (26.0, 55.0)	0.057	0.064
	Metformin	39 (23.0, 56.0)	39 (27.0, 51.0)	0.518	
Eriksson <i>et al</i> ^[14]	Placebo	33.53 (12.4)	-0.2 (8.8) ¹	-	-
	Omega-3 CA	37.65 (14.7)	+5.9 (16.5) ¹	-	Non-significant ²
	Dapagliflozin	39.41 (14.7)	-8.2 (8.2) ¹	-	< 0.05 ²
	O + D	35.88 (17.1)	+0.1 (12.9) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	62 (43.0-75.0)	38.0 (31.0-65.0)	0.01	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	70.8 (8.1)	48.8 (5.5)	0.002	0.039
	Sitagliptin	92.4 (11.2)	71.1 (10.0)	0.012	
Gautam <i>et al</i> ^[17]	Canagliflozin	96 (18.7)	60.0 (17.6)	< 0.00001	-
Sumida <i>et al</i> ^[18]	Luseogliflozin	54.7 (28.2)	42.4 (26.5)	< 0.001	-

¹Change from baseline.²Compared to placebo.

ALT: Alanine aminotransferase; CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

improve serum levels of liver enzymes, liver fibrosis indices, and liver fat without significant side effects in type 2 diabetes patients with NAFLD. They showed additional beneficial effects on obesity, glycaemic parameters, insulin resistance, and dyslipidaemia in these subjects. However, the quality of evidence was low to moderate. Prospective studies, preferably RCTs, comparing different SGLT-2 inhibitors with standard treatments of NAFLD in multi-ethnic populations with a longer follow-up period are needed in the future.

Table 7 Change in serum aspartate aminotransferase levels in individual studies

Study	Serum AST levels (IU/L)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	44.6 (23.5)	36.2 (9.0)	0.04	0.212
	Control	45.3 (24.3)	44.6 (23.8)	0.931	
Ito <i>et al</i> ^[12]	Ipragliflozin	39.7 (16.7)	27.3 (8.9)	< 0.05	0.802
	Pioglitazone	43.3 (20.5)	32.4 (15.4)	< 0.05	
Eriksson <i>et al</i> ^[14]	Placebo	29.4 (13.2)	-1.2 (7.2) ¹	-	-
	Omega-3 CA	30.6 (10.2)	+4.8 (9.0) ¹	-	Non-significant ²
	Dapagliflozin	31.2 (11.4)	-4.2 (5.4) ¹	-	< 0.05 ²
	O + D	30 (10.2)	+1.2 (5.4) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	37 (29.0-52.0)	28 (23.0-31.0)	0.03	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	54.4 (5.6)	38 (3.1)	0.001	-
	Sitagliptin	67 (7.7)	52.5 (7.7)	0.016	-
Gautam <i>et al</i> ^[17]	Canagliflozin	72 (16.7)	53 (10.3)	< 0.00001	-
Sumida <i>et al</i> ^[18]	Luseogliflozin	40.7 (22.2)	31.9 (18.2)	< 0.001	-

¹Change from baseline.²Compared to placebo.

AST: Aspartate aminotransferase; CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 8 Change in serum gamma-glutamyl transferase levels in individual studies

Study	Serum GGT (IU/L)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	65.8 (36.1)	50.9 (24.6)	0.002	0.057
	Control	63.9 (45.3)	60.0 (39.0)	0.421	
Ito <i>et al</i> ^[12]	Ipragliflozin	62.8 (58.3)	44.0 (38.3)	< 0.05	0.642
	Pioglitazone	71.6 (54.1)	48.8 (61.2)	< 0.05	
Eriksson <i>et al</i> ^[14]	Placebo	32.4 (17.4)	+2.4 (9.6) ¹	-	-
	Omega-3 CA	54.0 (57.6)	+2.4 (12.0) ¹	-	Non-significant ²
	Dapagliflozin	58.2 (43.2)	-4.8 (13.8) ¹	-	< 0.05 ²
	O + D	40.2 (14.4)	-0.6 (13.8) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	75.0 (47.0-105.0)	60.0 (40.0-101.0)	0.03	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	61.7 (9.1)	58.7 (11.5)	0.051	-
	Sitagliptin	89.2 (11.8)	82.4 (11.9)	0.36	-
Gautam <i>et al</i> ^[17]	Canagliflozin	75.1 (31.8)	69.2 (26.2)	0.003	-
Sumida <i>et al</i> ^[18]	Luseogliflozin	62.4 (77.1)	48.2 (56.3)	0.003	-

¹Change from baseline.²Compared to placebo.

CA: Carboxylic acid; GGT: Gamma-glutamyl transferase; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 9 Change in hepatic fat in individual studies

Study	Parameter	Group	Baseline	Study completion	P value	P value between groups
Kuchay <i>et al</i> ^[11]	MRI-PDFF	Empagliflozin	16.2 (7)	11.3 (5.3)	< 0.0001	< 0.0001
		Control	16.4 (7.3)	15.5 (6.7)	0.054	
Ito <i>et al</i> ^[12]	L/S ratio	Ipragliflozin	0.8 (0.2)	1.0 (0.2)	< 0.05	0.90
		Pioglitazone	0.8 (0.3)	1.0 (0.2)	< 0.05	
Shibuya <i>et al</i> ^[13]	L/S ratio	Luseogliflozin	0.9 (0.6-1.0)	1.0 (0.8-1.2)	0.0008	0.00002
		Metformin	1.0 (0.8-1.1)	0.9 (0.7-1.0)	0.017	
Eriksson <i>et al</i> ^[14]	MRI-PDFF	Placebo	15.1 (6.5)	-0.6 (1.9) ¹	-	-

Sumida <i>et al</i> ^[18]	MRI-HFF	Omega-3 CA	22.2 (11.0)	-3.2 (2.9) ¹	-	Non-significant ²
		Dapagliflozin	17.3 (9.1)	-2.2 (3.3) ¹	-	Non-significant ²
		O + D	17.8 (9.2)	-3.2 (3.5) ¹	-	< 0.05 ²
		Luseogliflozin	21.5 (7.2)	15.7 (6.8)	< 0.001	-

¹Change from baseline.²Compared to placebo.

MRI-PDFF: Magnetic resonance imaging-derived proton density fat fraction; L/S ratio: Liver/spleen attenuation ratio; MRI-HFF: Magnetic resonance imaging-hepatic fat fraction; CA: Carboxylic acid; O + D: Omega-3 CA + Dapagliflozin.

Table 10 Assessment of liver fibrosis in individual studies

Study	Parameter	Group	Baseline	Study completion	P value	P value between groups
Ito <i>et al</i> ^[12]	FIB-4 index	Ipragliflozin	1.44 (0.64)	1.22 (0.55)	< 0.05	0.596
		Pioglitazone	1.84 (1.13)	1.71 (1.19)	Non-significant	
Ohki <i>et al</i> ^[15]	FIB-4 index	Ipragliflozin	1.75 (0.82-1.93)	1.39 (0.77-1.99)	0.04	-
Sumida <i>et al</i> ^[18]	FIB-4 index	Luseogliflozin	1.63 (1.19)	1.52 (0.92)	0.17	-
	NAFLD fibrosis score	Luseogliflozin	1.61 (0.71)	1.62 (0.88)	0.86	-

FIB: Fibrosis 4; NAFLD: Non-alcoholic fatty liver disease.

Table 11 Change in fasting plasma glucose in individual studies

Study	Fasting plasma glucose (mg/dL)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	173.0 (44.0)	124.0 (17.0)	< 0.001	0.85
	Control	176.0 (57.0)	120.0 (19.0)	< 0.0001	
Ito <i>et al</i> ^[12]	Ipragliflozin	160.1 (38.7)	136.5 (26.7)	< 0.05	0.785
	Pioglitazone	169.4 (50.9)	139.0 (26.6)	< 0.05	
Shibuya <i>et al</i> ^[13]	Luseogliflozin	127.0 (116.0, 136.0)	125.0 (113.0, 138.0)	0.87	0.583
	Metformin	147.0 (126.0, 161.0)	134.0 (122.0, 145.0)	0.32	
Eriksson <i>et al</i> ^[14]	Placebo	169.2 (29.7)	+6.7 (14.8) ¹	-	-
	Omega-3 CA	162.4 (26.6)	+3.8 (19.3) ¹	-	Non-significant ²
	Dapagliflozin	161.8 (33.3)	-17.6 (26.8) ¹	-	< 0.05 ²
	O + D	168.8 (35.5)	-16.4 (36.0) ¹	-	< 0.05 ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	162.0 (135.0-189.0)	135.0 (120.0-166.0)	0.3	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	125.0 (6.0)	116.6 (4.2)	0.07	Non-significant
	Sitagliptin	114.6 (7.0)	134.0 (10.5)	0.067	
Sumida <i>et al</i> ^[18]	Luseogliflozin	142.0 (30.3)	135.4 (25.6)	0.04	-

¹Change from baseline.²Compared to placebo.

CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 12 Change in glycosylated haemoglobin in individual studies

Study	Glycosylated haemoglobin (%)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	9.0 (1.0)	7.2 (0.6)	< 0.001	0.88
	Control	9.1 (1.4)	7.1 (0.9)	< 0.0001	
Ito <i>et al</i> ^[12]	Ipragliflozin	8.5 (1.5)	7.6 (1.0)	< 0.05	0.522
	Pioglitazone	8.3 (1.4)	7.1 (0.9)	< 0.05	
Shibuya <i>et al</i> ^[13]	Luseogliflozin	7.8 (7.2, 7.9)	6.5 (6.4, 7.0)	0.002	0.023
	Metformin	7.4 (6.9, 7.7)	7.3 (6.7, 7.6)	0.362	

Eriksson <i>et al</i> ^[14]	Placebo	7.4 (0.8)	-0.1 (0.4) ¹	-	-
	Omega-3 CA	7.4 (0.7)	+0.1 (0.4) ¹	-	Non-significant ²
	Dapagliflozin	7.4 (0.6)	-0.6 (0.7) ¹	-	< 0.05 ²
	O + D	7.5 (0.8)	-0.5 (0.5) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	8.4 (7.8-8.9)	7.6 (6.9-8.2)	< 0.01	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	6.7 (0.1)	6.5 (0.1)	0.055	Non-significant
	Sitagliptin	7.0 (0.3)	6.9 (0.3)	0.331	
Sumida <i>et al</i> ^[18]	Luseogliflozin	7.3 (0.7)	7.0 (0.7)	0.002	-

¹Change from baseline.²Compared to placebo.

CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 13 Change in homeostasis model assessment-estimated insulin resistance in individual studies

Study	HOMA-IR			P value	P value between groups
	Group	Baseline	Study completion		
Ito <i>et al</i> ^[12]	Ipragliflozin	5.2 (2.5)	4.8 (5.5)	Non-significant	0.401
	Pioglitazone	5.7 (3.4)	4.5 (2.7)	< 0.05	
Eriksson <i>et al</i> ^[14]	Placebo	4.2 (2.4)	-0.2 (1.4) ¹	-	-
	Omega 3-CA	5.4 (2.9)	+0.3 (2.4) ¹	-	Non-significant ²
	Dapagliflozin	4.3 (1.9)	-1.1 (1.4) ¹	-	< 0.05 ²
	O + D	4.4 (1.7)	-0.9 (1.6) ¹	-	< 0.05 ²
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	4.5 (0.5)	7.9 (2.3)	0.955	-
	Sitagliptin	4.4 (0.5)	6.5 (0.8)	0.163	

¹Change from baseline.²Compared to placebo.

HOMA-IR: Homeostasis model assessment-estimated insulin resistance; CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 14 Change in serum triglycerides in individual studies

Study	Serum triglycerides (mg/dL)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	201.0 (124.0)	155.0 (52.0)	0.01	0.678
	Control	212.0 (115.0)	175.0 (43.0)	0.019	
Ito <i>et al</i> ^[12]	Ipragliflozin	166.9 (76.4)	143.4 (81.4)	< 0.05	0.938
	Pioglitazone	188.4 (148.8)	169.3 (131.3)	Non-significant	
Eriksson <i>et al</i> ^[14]	Placebo	169.2 (84.1)	-11.5 (45.6) ¹	-	-
	Omega-3 CA	186.9 (81.5)	-15.9 (47.4) ¹	-	Non-significant ²
	Dapagliflozin	178.0 (103.6)	+14.2 (40.5) ¹	-	Non-significant ²
	O + D	168.3 (72.6)	-25.7 (57.1) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	148.0 (107.0, 222.)	145.0 (114.0, 172.0)	0.75	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	153.8 (15.9)	137.8 (10.5)	0.236	-
	Sitagliptin	193.4 (25.2)	191.1 (23.8)	0.986	
Sumida <i>et al</i> ^[18]	Luseogliflozin	158.1 (110.5)	129.4 (59.5)	0.062	-

¹Change from baseline.²Compared to placebo.

CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 15 Change in serum low-density lipoprotein cholesterol in individual studies

Study	Serum low-density lipoprotein cholesterol (mg/dL)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	112.0 (35.0)	95.0 (22.0)	0.018	0.512
	Control	114.0 (30.0)	96.0 (17.0)	0.001	
Ito <i>et al</i> ^[12]	Ipragliflozin	108.3 (36.2)	110.7 (40.1)	Non-significant	0.057
	Pioglitazone	104.0 (27.9)	114.6 (29.5)	< 0.05	
Eriksson <i>et al</i> ^[14]	Placebo	98.2 (34.4)	+1.6 (15.5) ¹	-	-
	Omega-3 CA	111.8 (34.4)	+2.3 (17.4) ¹	-	Non-significant ²
	Dapagliflozin	109.4 (34.8)	+7.7 (20.5) ¹	-	Non-significant ²
	O + D	88.9 (23.2)	+5.8 (21.7) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	113.0 (89.0-142.0)	103.0 (92.0-122.0)	0.08	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	119.2 (5.8)	119.8 (5.7)	0.943	-
	Sitagliptin	112.9 (4.9)	127.1 (8.8)	0.063	-
Sumida <i>et al</i> ^[18]	Luseogliflozin	101.0 (22.4)	105.0 (24.4)	0.11	-

¹Change from baseline.²Compared to placebo.

CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 16 Change in serum high-density lipoprotein cholesterol in individual studies

Study	Serum high-density lipoprotein cholesterol (mg/dL)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	42.0 (12.0)	45.0 (12.0)	0.087	0.752
	Control	45.0 (15.0)	47.0 (12.0)	0.097	
Ito <i>et al</i> ^[12]	Ipragliflozin	48.9 (9.3)	54.7 (10.4)	< 0.05	0.82
	Pioglitazone	47.4 (11.6)	52.7 (13.5)	< 0.05	
Eriksson <i>et al</i> ^[14]	Placebo	51.4 (14.9)	-0.4 (5.0) ¹	-	-
	Omega-3 CA	49.9 (14.1)	+0.4 (3.2) ¹	-	Non-significant ²
	Dapagliflozin	49.9 (9.5)	+0.4 (4.8) ¹	-	Non-significant ²
	O + D	51.4 (10.2)	+1.6 (5.0) ¹	-	Non-significant ²
Ohki <i>et al</i> ^[15]	Ipragliflozin	42.0 (40.0-50.0)	44.0 (42.0-59.0)	0.01	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	53.9 (2.5)	55.4 (2.6)	0.043	-
	Sitagliptin	54.8 (3.3)	55.6 (2.3)	0.531	-
Sumida <i>et al</i> ^[18]	Luseogliflozin	55.6 (11.7)	57.5 (13.4)	0.062	-

¹Change from baseline.²Compared to placebo.

CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 17 Change in body mass index in individual studies

Study	Body mass index (kg/m ²)			P value	P value between groups
	Group	Baseline	Study completion		
Kuchay <i>et al</i> ^[11]	Empagliflozin	30.0 (3.8)	28.7 (3.5)	0.001	0.124
	Control	29.4 (3.1)	28.8 (2.8)	0.019	
Shibuya <i>et al</i> ^[13]	Luseogliflozin	27.9 (26.2, 28.7)	27.0 (25.6, 28.3)	0.002	0.031
	Metformin	27.2 (24.8, 32.1)	27.3 (24.3, 31.6)	0.646	
Ohki <i>et al</i> ^[15]	Ipragliflozin	30.1 (26.1-31.4)	27.6 (25.3-30.2)	< 0.01	-
Seko <i>et al</i> ^[16]	SGLT-2 inhibitor	29.6 (0.7)	28.3 (0.7)	< 0.001	-
	Sitagliptin	29.2 (1.5)	28.9 (1.4)	0.295	

Sumida <i>et al</i> ^[18]	Luseogliflozin	27.8 (3.6)	27.2 (1.0)	< 0.001	-
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SGLT-2: Sodium glucose cotransporter-2.

Table 18 Adverse effects of sodium glucose cotransporter-2 inhibitors in individual studies

Study	No. of adverse events	No. of patients	Types of adverse events
Kuchay <i>et al</i> ^[11]	3	25	Nonspecific fatigue: 1 Arthralgia: 1 Balanoposthitis: 1
Ito <i>et al</i> ^[12]	9	32	UTI: 3 Increased appetite: 2 Nausea: 1 Headache: 1 Diarrhoea: 1 Vaginal candidiasis: 1
Eriksson <i>et al</i> ^[14]	7	21	-
Seko <i>et al</i> ^[16]	2	26	UTI: 2
Gautam <i>et al</i> ^[17]	1	32	Recurrent UTI with genital candidiasis: 1
Sumida <i>et al</i> ^[18]	8	40	Low blood pressure: 3 Vaginal itching: 2 Constipation: 1 Vertigo: 1 Dehydration: 1
Total	30	176	Most common adverse event: Genitourinary tract infections-10

UTI: Urinary tract infection.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is a common comorbidity with type 2 diabetes. The existing therapeutic options for NAFLD are not adequate. Hypocaloric diet and exercise is the cornerstone of therapy in NAFLD. Pioglitazone is the only drug recommended in diabetes patients with biopsy proven non-alcoholic steatohepatitis. The frequent coexistence of NAFLD and type 2 diabetes along with their combined adverse health consequences and inadequate therapeutic options makes it necessary to search for newer alternatives. This systematic review is an effort to review the available literature on the effect of sodium glucose cotransporter-2 (SGLT-2) inhibitors on NAFLD in type 2 diabetes patients.

Research motivation

Because the existing therapeutic options are not adequate for NAFLD patients, there is a need for finding newer alternatives. SGLT-2 inhibitors have shown promise in the management of NAFLD in animals. Hence, we reviewed the available literature on the effect of SGLT-2 inhibitors in NAFLD in type 2 diabetes patients. This will promote further high quality research on the effect of SGLT-2 inhibitors in NAFLD.

Research objectives

The primary outcome was the change in serum alanine aminotransferase levels in type 2 diabetes patients with NAFLD treated with SGLT-2 inhibitors. The secondary outcomes were change in serum aspartate aminotransferase and gamma-glutamyl transferase levels, hepatic fat, hepatic fibrosis, metabolic profile, anthropometric parameters, and the adverse effects of SGLT-2 inhibitors.

Research methods

This systematic review was registered in PROSPERO and performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. We searched PubMed/MEDLINE, Cochrane library, Google scholar, and Clinicaltrials.gov for the relevant articles to be included in this systematic review. A narrative synthesis of the results of individual studies was done. The change in the difference in means and difference in proportions and the respective *P* values as mentioned in the original manuscripts were tabulated and explained. The quality of the randomised controlled trials and observational studies was analysed using the

Cochrane risk of bias tool and MINORS scale, respectively.

Research results

Eight articles (four randomised controlled trials and four observational studies) were included in this systematic review. A total of 214 patients were treated with SGLT-2 inhibitors. SGLT-2 inhibitors caused a significant improvement in liver enzymes, hepatic fat, hepatic fibrosis, glycaemia, insulin resistance, obesity, and lipid parameters with minimal adverse effects. However, the quality of evidence is low to moderate.

Research conclusions

We found that SGLT-2 inhibitors improved the serum levels of liver enzymes, liver fat, and liver fibrosis with additional beneficial effects on various metabolic and anthropometric parameters in type 2 diabetes patients with NAFLD. However, the number of patients treated with SGLT-2 inhibitors was small. The findings of this systematic review will have impact in choosing anti-diabetes medication like SGLT-2 inhibitors to treat NAFLD associated with type 2 diabetes.

Research perspectives

The studies included in this systematic review were heterogeneous with regard to study design and intervention drugs. Most of the studies were done amongst the Japanese population. Prospective studies, preferably randomised controlled trials, comparing different SGLT-2 inhibitors with standard treatments of NAFLD in multi-ethnic populations with a longer follow-up period are needed in the future.

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Bilateral gangrene of fingers in a patient on empagliflozin: First case report

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Abstract

BACKGROUND

Sodium glucose cotransporter 2 (SGLT2) inhibitors use has been associated with toe amputations and non-healing ulcers and gangrene mostly of lower extremities. There are no case reports about association of Empagliflozin with finger ulcers or gangrene. This is the first case report of Empagliflozin (Jardiance) an SGLT2 inhibitor causing gangrene of fingers and second case in literature about any SGLT2 inhibitor causing gangrene of upper extremity.

CASE SUMMARY

A 76-year-old man with type 2 diabetes mellitus sustained minimal trauma to both middle fingers, which started healing. He was started on empagliflozin a week later for management of type 2 diabetes mellitus and started developing gangrene to both middle finger tips along with neuropathic pain which worsened over the course of next four months. Investigations were negative for vascular insufficiency, infection and vasculitis and imaging of hand was normal. Discontinuation of empagliflozin slowed progression of gangrene and caused symptomatic improvement with reduction in neuropathic pain.

CONCLUSION

This case report suggests possible association of empagliflozin and finger gangrene and recommends that more research and awareness among clinicians is needed in this area.

Key words: Empagliflozin; Finger gangrene; Non-healing ulcer; Type 2 diabetes mellitus; Sodium glucose cotransporter 2 inhibitor; Jardiance; Case report

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Core tip: Empagliflozin can cause finger gangrene in patients with type 2 diabetes mellitus. Empagliflozin has gained popularity recently as a newer anti diabetic agent with improved cardiovascular outcomes and better glycemic control in addition to lowering blood pressure and helping with weight loss. Lack of proper awareness about this condition can lead to progression of disease if not identified early on and can result in amputations. This medication should be used with caution in patients who have high risk of gangrene such as that on prednisone and in those with diabetic neuropathy.

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INTRODUCTION

This is the first ever case reported in literature about empagliflozin (Jardiance) as a possible cause of finger gangrene. Sodium glucose cotransporter 2 (SGLT2) inhibitors inhibit sodium and glucose cotransport at proximal renal tubules. SGLT2 inhibitors have been associated with an increased risk of genital infections secondary to increased glycosuria. According to the results of CANVAS trial, Dapagliflozin, another SGLT2 inhibitor of the same class as empagliflozin, has been shown to significantly reduce the risk of cardiovascular events by 14% but it doubled the risk of amputation in patients with type 2 diabetes mellitus^[1]. In a similar study conducted on patients with type 2 diabetes mellitus at high risk for cardiovascular events, patients were given empagliflozin *vs* placebo and those on empagliflozin had lesser adverse cardiovascular events and lower all-cause mortality. Among patients receiving empagliflozin, there was an increased rate of genital infections but there was no increase in lower limb amputations^[2]. In another study of over eight million case safety reports, increased risk of lower-limb amputations especially toe amputations were reported with empagliflozin^[3].

A data analysis conducted based on data from US Food and Drug Administration adverse event Reporting System showed a total of 66 cases of SGLT2 inhibitor-associated amputations^[3]. Among these, there was only one case of hand amputation which was from Dapagliflozin. All others were lower extremity gangrene and ulcers, most commonly of toes^[4]. There are two case reports of empagliflozin related Fournier's gangrene in literature^[5,6] which pointed the benefit of keeping a high index of suspicion and early cessation of SGLT2 inhibitors could potentially prevent the progression of these infections requiring surgical debridement later. Empagliflozin has also been associated with vulvovaginal candidiasis along with other SGLT2 inhibitors^[7].

SGLT2 inhibitors are used in general, cautiously in patients with vascular insufficiency, neuropathy, risk of amputations and very high hemoglobin A1C over 11. However, there are no case reports to date about an empagliflozin as a possible cause of non-healing finger ulcers or gangrene. Ours is the first reported case of empagliflozin (a SGLT2 inhibitor) as likely cause of gangrene of fingers.

CASE PRESENTATION

Chief complaint

Gangrene both middle fingers.

History of present illness

A 76-year-old man with moderately controlled type 2 diabetes mellitus (HbA1c of 8.6) sustained minor injury to the tip of both middle fingers while doing some mechanical work. He had no burns or exposure to heat. Initially, the fingers were healing well with minimal scarring. A week after the injury, he was started on empagliflozin 10 mg for better glycemic control in addition to his other medications. Three weeks after the injury (two weeks after being started on empagliflozin), he started noticing significant pain on tip of both middle fingers which also started changing color to brown and then to black (Figure 1).



Figure 1 Gangrene tip of fingers while on empagliflozin.

History of past illness

No history of previous vasculitis. He has history of polymyalgia rheumatica and was on prednisone 3 mg daily for the past few years. His other medications included aspirin, atorvastatin, metformin and saxagliptin. No history of diabetic neuropathy.

Personal and family history

He is a nonsmoker with no alcohol use. No family history of diabetes, gangrene or significant illnesses.

Physical examination upon admission

He was seen and evaluated in the emergency room twice in the following four months due to worsening symptoms and investigations were done. On exam during both times, he was afebrile, and physical exam was normal except for gangrenous changes tips of both middle fingers. There was no area of erythema around the region of gangrene on either side. Ankle brachial pressure index was normal and filling pressures were normal in both upper extremities.

Laboratory examinations

Blood counts, erythrocyte sedimentation rate, C reactive protein were within normal limits. Tests for vasculitis were negative including Anti-nuclear cytoplasmic antibody and anti-nuclear antibody.

Imaging examinations

Hand X-rays were normal. Echocardiogram showed no evidence of embolic sources.

FINAL DIAGNOSIS

Possible etiology was concluded to be from microvascular damage of unclear etiology.

TREATMENT

Plastic surgery, vascular surgery, dermatology and rheumatology referrals were completed. Biopsy was withheld as there was no surrounding erythema. Patient was seen in endocrinology outpatient for diabetes management and his endocrinologist suspected empagliflozin as a possible cause and discontinued the medication. He was switched to alternate medications for better glycemic control.

OUTCOME AND FOLLOW UP

After a week of stopping empagliflozin, patient started noticing improvement in his pain as well as slowing of blackish discoloration near tip of fingers.

DISCUSSION

Occurrence of finger gangrene or upper extremity gangrene in individuals with type 2 diabetes on treatment with empagliflozin has not been described previously in the literature. We suggest this adverse event could be under reported due to low index of suspicion.

Patient mentioned in this case presented with gangrene at the same site where he sustained minimal trauma initially, therefore the suspicion was more for vasculitis. But the patient had noticed that the sites were healing well initially. Starting of empagliflozin coincided with onset of symptoms of neuropathic pain and worsening of non-healing ulcers and development of gangrene tip of fingers and vasculitis markers were negative.

Even though this patient has polymyalgia rheumatica and was on prednisone at the time of symptoms, markers for vasculitis were negative and he was on consistent dose of low dose prednisone for few years before onset of symptoms. Addition of empagliflozin again was the only other contributing factor for development of symptoms.

The timing of empagliflozin and onset of symptoms as well as improvement after stopping empagliflozin point towards a likely association of the medication with finger gangrene.

CONCLUSION

This first case report of empagliflozin causing finger gangrene suggests the possibility that upper extremity gangrene with use of empagliflozin could go undiagnosed as occurred initially in this case. Prescribers need to be aware of this association and future studies are warranted to clarify if upper extremity ulcers or gangrene are associated with SGLT2 inhibitor use.

Increased awareness among primary care physicians and surgeons about this association could prevent progression of non-healing upper extremity ulcers, gangrene and resultant amputations.

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Do we need to screen every patient in intensive care unit for diabetes in community with high prevalence of diabetes?

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Abstract

Diabetes mellitus (DM) is marked as global health care challenge with almost 10% of the United States population being diagnosed with DM. A sizeable percentage of patients are oblivious of their disease, in spite of easily accessibility knowledge about its early signs and symptoms and rapid diagnostic modalities. Critically ill patients with undiagnosed DM are likely to have an increased mortality as compared to intensive care unit (ICU) patients with diagnosed DM. DM may have adverse effect on ICU patients causing organ failure and complications. Early Screening of patients at the risk of developing disease may prevent long term complications. Early screening and management may be beneficial as controlled DM patients have similar morbidity as non DM patients in ICU. An intense glycaemic and blood pressure control improves retinopathy and albuminuria, but may not affect the macrovascular outcomes.

Key words: Diabetes mellitus; Intensive care unit; Microvascular; Macrovascular; Diabetes screening

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Core tip: Undiagnosed diabetes mellitus (DM) predisposes critically ill patients to DM complications, which may affect their morbidity and mortality during intensive care unit stay.

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INTRODUCTION

An estimated 30.3 million people of all ages, or 9.4% of the United States population had diabetes mellitus (DM) in 2015. This included 30.2 million adults aged 18 years or older (12.2% of all United States adults), of which 7.2 million (23.8%) were unaware of or did not report having DM. The percentage of adults with DM increased with age, reaching a high of 25.2% among those aged 65 years or older^[1]. In spite of the wide accessibility of knowledge about the early signs and symptoms of DM and ease of diagnostic modalities, many patients are oblivious of their disease^[2]. Worldwide approximately 193 million diabetic patients remain undiagnosed predisposing them to the development of several long-term complications of untreated chronic hyperglycaemia, making this a global health care challenge^[3].

PREVALENCE OF DM IN AMONG CRITICALLY ILL PATIENTS

The complications of DM include both microvascular and macrovascular pathologies and comprise of retinopathy, neuropathy, renal failure, cardiovascular complications and increased risk of death. A study conducted by Tancredi *et al*^[4] concluded that there is 15 fold increases on all-cause mortality in patients with Type-2 DM. These complications have profound physical as well as psychological burden on the patient, the family, and the care givers and on a larger scale they affect the health of the society.

On the other hand, intensive care unit (ICU) admissions with undiagnosed DM have been showing a steady increase in the past few years^[2]. Carpenter *et al*^[2] studies the impact of undiagnosed DM in 9 ICU's. The study reported that patients with undiagnosed DM had an increased mortality as compared to ICU patients with diagnosed DM; and also showed increased trend for higher average blood glucose level and insulin infusion. Thus need for DM screening amongst critically ill patients is paramount.

BENEFIT OF DM SCREENING

A study conducted by Kunthi *et al*^[5] suggested that screening of subpopulations using risk scores can rule in high risk patients and the diagnosis can be confirmed by measurements of fasting plasma glucose or HbA1c concentrations or tests for oral glucose tolerance. Screening of the individuals who are at the risk of developing disease will prevent the long term microvascular as well as macrovascular complications. Early detection also helps in optimal disease management by practicing lifestyle modifications such as weight reduction, quitting smoking and alcohol, increased physical activity and healthy diet^[6]. However, various methods of screening may have used in the different studies including risk score, fasting plasma glucose, HbA1c concentrations or tests for oral glucose tolerance. This questions the applicability of a universal operational definition for DM diagnosis.

CONTROVERSIES AND COST EFFECTIVENESS OF DM SCREENING

Alongside, the various large multicentre studies concluded that macrovascular complications do not show any significant change^[7]. The risk of cardiovascular disease and other macrovascular complications does not improve with intensive management of the screened population; hence the application of universal screening method is not promoted^[7]. The UKPDS researchers^[8] showed that despite an intense glycaemic and blood pressure control macrovascular outcomes were not improved but there was a

significant improvement in retinopathy and albuminuria. Krinsley *et al*^[9] have shown that hyper-glycemia not only affects the morbidity in critically ill patients but also the patients admitted to the general medicine wards. They noted that high glucose variability (CV > 20%) increased mortality in non DM patients in both ICU as well as the floor settings but for the DM patients it was restricted only for ICU. Patients with DM having low HbA_{1c} levels and patients without DM have equal mortality and morbidity risks and hyperglycaemia increases mortality. Siegelaar *et al*^[10], in their meta-analysis showed that the diabetic patients have higher chances of developing complications like sepsis or organ failure and these in turn have increased mortality rate compared to non-diabetic population. However, Diabetes does not serve as an independent factor for ICU mortality and after acquiring complications the mortality rate would be same in diabetic as well as non-diabetic patients^[10].

CONCLUSION

Despite DM being widely prevalent in United States, still substantial numbers of patients in older age are undiagnosed. This predisposes them to micro and macrovascular complications, which in turn may affect their morbidity and mortality during ICU stay. Universal screening of DM has been proved beneficial to prevent microvascular complications but not much difference is seen in the macrovascular maladies. Early screening and management may be beneficial as controlled DM patients have similar morbidity as non DM patients in ICU. DM may be associated with increased mortality in ICU patients. However, how DM intrinsically affects the ICU mortality, is still open for discussion.

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Cataract in diabetes mellitus

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Abstract

Diabetes mellitus (DM) is a chronic systemic disease that has increases in prevalence over time. DM can affect all ocular structures, with cataract being the most common ocular complication. Cataract is the leading cause of blindness worldwide. Due to several mechanisms, there is an increased incidence of cataract formation in the diabetic population. Advancements in technology have now made cataract surgery a common and safe procedure. However, the diabetic population is still at risk of vision-threatening complications, such as diabetic macular edema (ME), postoperative ME, diabetic retinopathy progression, and posterior capsular opacification.

Key words: Diabetes; Cataract; Complications; Surgery

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Core tip: Because the number of people with diabetes mellitus is predicted to increase in the future, cataract surgery will remain an important procedure for diabetic patients. Patients with diabetes have multiple issues which should be evaluated preoperatively, perioperatively, and in the postoperative period. The preoperative, intraoperative, and postoperative factors are of paramount importance in the management of such complications and in improving visual outcomes. This article aims to review diabetic cataracts and related complications, and to outline important management strategies.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing on a daily basis, with the International Diabetes Federation estimating that there will be 439 million DM patients by 2030^[1]. An aging population and longer patient life expectancy also means that the prevalence of DM will exceed 33% by 2050^[2]. DM can lead to pathologies in many tissues in the eye structure, with both a systemic chronic metabolic disease and a microangiopathic character^[3]. Cataract is one of the major causes of visual impairment in diabetic patients^[4]. Patients with DM are reported to be up to five times more likely to develop cataract, in particular at an early age^[5-8]. Due to the increasing prevalence of DM, the incidence of diabetic cataracts has also risen. Cataract extraction is one of the most common surgical procedures among the general population, and the number of cataract surgeries each year also continues to increase. Recent technological advancements in cataract surgery have improved surgical outcomes. However, in diabetic individuals, the scale of improvement is still a matter of debate, and many studies have revealed both the results and complications of cataract surgery in diabetic patients. In the light of these findings, this study will review related articles in order to highlight current developments and controversies regarding cataract surgery management in patients with DM.

BIOCHEMICAL MECHANISMS FOR CATARACT IN DIABETES

Different types of mechanisms have been proposed for the pathogenesis of cataract in cases of DM.

Polyol pathway

It has been suggested that the polyol pathway-*via* which the enzyme aldose reductase (AR) catalyzes the reduction of glucose into sorbitol-is a central part of the mechanism of cataract development^[9-11]. Multiple studies have been conducted to explain the AR pathway's role in this process. The increased intracellular accumulation of sorbitol leads to a hyperosmotic effect, resulting in hydropic lens fibers that degenerate and form cataract^[9,12]. The production of sorbitol in diabetic patients (as compared to nondiabetic patients) takes place more quickly than it can be converted into fructose by the enzyme sorbitol dehydrogenase. Intracellular removal of sorbitol through diffusion is also prevented because of its polar character. A hyperosmotic effect is created when an accumulation of sorbitol results in an infusion of fluid. Finally, animal studies have shown that the intracellular accumulation of polyols causes liquefaction of lens fibers resulting in the formation of lens opacities^[9,10,12,13]. In the study of Oishi *et al*^[13], it was found that AR levels in red blood cells of patients under the age of 60 and with short duration of DM had a positive correlation with the prevalence of posterior subcapsular cataract. Moreover, a negative correlation was reported between the level of AR in erythrocytes and the density of lens epithelial cells, which is known to be lower in diabetics than in nondiabetics. These findings suggest that AR may play a role in this pathomechanism.

Osmotic and oxidative stress

Osmotic stress as a result of extensive swelling of the cortical lens fibers is another compounding mechanism in the rapid development of cataracts, especially in young patients with type 1 DM^[14-16]. Osmotic stress resulting from the accumulation of sorbitol induces stress in the endoplasmic reticulum (ER), the main site of protein synthesis, resulting in the formation of free radicals^[17]. Stress in the ER can also be caused by fluctuation of glucose levels that initiate an unfolded protein response producing reactive oxygen species and cause oxidative stress damage to lens fibers. Moreover, increased glucose levels in the aqueous humor may lead to glycation of lens proteins, a process that results in the formation of advanced glycation end products^[18]. Fenton reactions resulting from elevated levels of hydrogen peroxide (H₂O₂) in the aqueous humor of diabetics also induces the generation of hydroxyl radicals (OH⁻) after entering the lens^[19]. Another factor that is elevated in the lens and aqueous humor of diabetic patients is free radical nitric oxide (NO[•]), which may cause an increase in peroxynitrite formation, which contributes to cell damage due to oxidizing properties^[20,21]. However, diabetic lenses have increased susceptibility to oxidative stress due to their impaired antioxidant capacity. Superoxide dismutase (SOD) is the most predominant antioxidant enzyme in the lens that degrades superoxide radicals (O₂⁻) into H₂O₂ and oxygen. Several *in vitro* and *in vivo* animal studies have shown that SOD has protective properties against cataract development

in the presence of DM^[22-24].

Some studies have shown that osmotic stress in the lens resulting from sorbitol accumulation causes apoptosis in lens epithelial cells and leads to cataract formation^[25]. Rapid glycemic control can also increase these effects in the lens by creating a hypoxic environment that reduces protective enzymes and increases oxidative radicals. High AR expression could constitute a risk factor that predisposes the lens to distortions in signaling through the extracellular signal-regulated kinase and c-Jun N-terminal kinase pathways-involved in cell growth and apoptosis, respectively-thereby altering the balance required for lens homeostasis^[11,26]. These findings show that impairments in osmoregulation may render the lens susceptible to even the smallest increase in AR-mediated osmotic stress, potentially leading to progressive cataract formation.

Autoimmunity

Another recently proposed mechanism is autoimmune hypothesis in acute bilateral type 1 diabetic cataracts^[26]. The authors reported that insulin autoantibodies became positive within three months of beginning insulin treatment, and that this period coincided with cataract formation. Their suggestion that there could be an autoimmune process behind acute bilateral cataract in DM warrants further investigation^[26].

The type of cataract seen in diabetic patients has also been investigated. The most common is the senile type^[10]. However, snowflake cataracts, which are characteristic for DM, are very common in type 1 diabetics. Posterior subcapsular cataracts have also been shown to be significantly associated with diabetes. Increased levels of glycated hemoglobin were demonstrably associated with an increased risk of nuclear and cortical cataracts^[6]. Further analysis revealed that diabetic patients were prone to developing cortical cataracts and that this process was associated with the duration of diabetes^[5,7].

Finally, the initiating mechanism in diabetic cataract formation is the generation of polyols from glucose by AR. However, osmotic stress, apoptosis of the lens epithelial cells, and the autoimmune theories may be confounding mechanisms in the development of the cataract formation in DM.

CATARACT INCIDENCE IN DIABETIC PATIENTS

Several clinical studies have reported that cataract formation occurs more frequently and at an earlier age in diabetic patients than in nondiabetic patients^[7,27-29]. Some studies indicate that cataracts are three to four times more prevalent in patients with diabetes under the age of 65. In patients over 65, cataracts are twice as prevalent^[27,30]. The main risk factors are longer duration of diabetes and poor metabolic control. Although older patients suffer from irreversible cataract formation, good metabolic control may reverse cataract in young diabetics.

Several important study groups have investigated cataract incidence in diabetic patients. The Wisconsin Epidemiologic Study of Diabetic Retinopathy investigated the incidence of cataract and factors associated with a higher risk of cataract surgery^[7]. They found 8.3% of patients suffering from type 1 diabetes and 24.9% of those with type 2 diabetes had a 10-year cumulative incidence of cataract surgery. For type 1 diabetics, they found some risk factors, including age, severity of diabetic retinopathy (DR), and proteinuria; for Type 2 diabetics, risk factors included age and use of insulin^[7].

The Beaver Dam Eye Study also reported an association between DM and cataract formation^[5]. The study took place over five years and consisted of 3684 participants aged 43 and older. It showed an increased incidence and progression of cortical and posterior subcapsular cataracts for DM patients. It also found an increased risk of nuclear and cortical cataracts with increased levels of glycated hemoglobin. Further analysis of the study showed that diabetics had a higher rate of cortical lens opacities and previous cataract surgery than nondiabetics^[6]. A longer duration of diabetes was also associated with increased frequency of both cortical cataracts and cataract surgery.

The Blue Mountains Eye Study aimed to examine the relationship between nuclear, cortical, and posterior subcapsular cataracts^[31]. The study supported the findings of previous research, but also found an association between posterior subcapsular cataracts and DM. In contrast to the Beaver Dam Eye Study, nuclear cataracts showed a weak association with DM.

The Barbados Eye Study evaluated the relationship between diabetes and lens opacities among 4314 black participants^[32]. The authors found that a history of DM

(18% prevalence) was related to all lens changes, especially at younger ages. Another study by Srinivasan *et al*^[33] found, for diabetics, the cumulative incidence of cataracts is much higher than that of progression. Moreover, they indicated that the main risk factor for cumulative incidence and progression of most types of cataract is age, with higher rates of both in older patients.

TIMING OF SURGERY

Approaches to the timing of cataract surgery in diabetic patients seem to be changing worldwide. Where once a more conservative approach was applied, now there is a growing tendency toward early surgery. Pollack *et al*^[34] reported that the main cause of poor visual outcomes is macular edema (ME). For this reason, they do not recommend cataract extraction for eyes with DR until visual acuity has deteriorated to 20/100–20/200. Similarly, Schatz *et al*^[35] stated that diabetic patients with cataracts might wish to postpone surgery, especially if there is any retinopathy present preoperatively.

The growing tendency toward earlier cataract surgery in patients with diabetes has contributed to improved visual outcomes^[36]. This approach facilitates panretinal photocoagulation (PRP) and also allows for the identification and adequate treatment of diabetic macular edema (DME) before cataract surgery. In addition, if surgery is undertaken before lens opacities make it more difficult to detect retinal thickening using macular assessment, then risk of ME decreases and visual outcomes may be considerably improved^[37].

PREOPERATIVE EVALUATION

Preoperative counseling is crucial for diabetic patients. Before surgery, patients should have good glycemic control and no evidence of ocular or periocular infection. Transient refractive changes related to morphologic and functional changes in the crystalline lens should be observed during periods of unstable blood sugar^[38]. Hyperglycemia induces myopia and, when intensive medical therapy is applied, patients tend to become more hyperopic as opposed to hyperglycemia. Changes in corneal topographic parameters during periods of glycemic changes can be a potential source of error in keratorefractive and biometric calculations^[39].

A thorough and comprehensive ophthalmologic examination-including an assessment of bestcorrected visual acuity (BCVA) and relative afferent pupillary defect; using slitlamp biomicroscopy to assess the corneal health and neovascularization of the iris (NVI); and using tonometry, dilated funduscopy, and gonioscopy for the evaluation of neovascularization at the angle-is mandatory. In select cases, advanced diagnostic evaluations such as fluorescein angiography, optical coherence tomography (OCT), and Bscan ultrasonography may be helpful. Due to the range of diabetic anterior segment changes, an experienced surgeon will perform better^[40].

Consultation with vitreoretinal subspecialists is recommended by some authors, especially in complicated cases^[41]. PRP is recommended preoperatively in patients with pre-existing proliferative diabetic retinopathy (PDR), because of its possible rapid progression after cataract surgery. In situations where lens opacity precludes PRP, it can be performed after surgery. Another approach is preoperative panretinal cryopexy or combined cataract surgery with vitrectomy and endolaser photocoagulation, particularly in cases with posterior pole tractional retinal detachment (TRD). ME should be efficiently treated preoperatively, since pre-existing maculopathy may worsen postoperatively and is strongly associated with a poor visual outcome^[42].

Treatment options for ME are laser photocoagulation, pharmacotherapy with intravitreal injections of antivascular endothelial growth factor (anti-VEGF) agents, or steroids^[43,44]. Because preexisting DME can increase the risk of ME progression by 20%–50%, intravitreal anti-VEGF agents are recommended perioperatively^[45,46]. Steroids, on the other hand, have been shown to be effective for persistent or refractory DME^[47]. Dexamethasone implants and fluocinolone implants resulted in significant improvement in clinically significant ME and visual outcomes^[48,49]. It has also been shown that dexamethasone has a potentially lower risk of intraocular pressure elevation and cataract formation compared to fluocinolone acetonide and triamcinolone acetate^[50]. Recently, preoperative use of nonsteroidal antiinflammatory drugs, such as diclofenac and nepafenac, has been examined. Most studies suggested that they did not reduce the chances of postoperative ME in patients with DR^[51–54].

Patients with NVI also need prompt treatment, including PRP. In patients who develop neovascular glaucoma (NVG), medical therapy is the first line of defense, however, it is usually ineffective. Eyes with active NVI are at greater risk for intraoperative and postoperative complications. Anti-VEGF agents such as bevacizumab showed dramatic short-term responses in terms of intraocular pressure reduction and regression of neovascularization in the treatment of NVG^[55,56]. Cataract surgery after administering anti-VEGF agents should be done with or without vitrectomy as early as possible to enable treatment of the posterior segment. When NVG is a problem, a combination of trabeculectomy with phacoemulsification may also be considered after regression of NVI. Despite all these options, the visual outcomes following phacoemulsification in eyes with NVG are generally poor.

CATARACT SURGERY IN DIABETIC PATIENTS

Cataract surgery in diabetic patients yields better results since the introduction of phacoemulsification, when compared to extracapsular or intracapsular cataract surgery^[57,58]. Different options are available during surgery that can lead to better surgical results and improved postoperative retinopathy evaluation. As anterior capsular phimosis is more common in diabetic eyes, capsulorhexis size should be larger than normal but smaller than the intraocular lense (IOL) optic diameter, in order to prevent anterior IOL displacement and posterior capsular opacification (PCO)^[59-61]. However, a large diameter optic is also important for the postoperative diagnosis and treatment of peripheral retinal pathology^[8].

Progression of retinopathy after cataract surgery is another problem in diabetic patients^[62]. The duration and complexity of cataract surgery are the main risk factors for progression of retinopathy^[63]; it is therefore important to reduce the time and complexity of the surgery. Poor pupillary dilatation can be seen in diabetic patients as the result of damage to pupillary parasympathetic supply and elevated prostoglandin levels^[64,65]. This means that pupil dilation is also a problem for these patients. As such, iris hooks, malyugin rings, or other iris expanders should be considered for intraoperative use. In cases with NVI, bleeding in the anterior chamber during or after surgery should also be kept in mind. Photoc retinopathy during cataract surgery, especially surgeries of a longer duration, was also more prevalent in diabetic patients than nondiabetics^[66].

While the presence of DM does not increase complications such as posterior capsular rupture, zonular dehiscence, or vitreous loss, the effect of DM on the entire eye can result in other problems. The effects of DM on the ocular surface include neurogenic effects (subbasal nerve abnormalities) and impaired corneal stem cell and epithelial cell division, which can result in keratoepitheliopathy and lead to corneal epithelial defects/abrasions, which may heal slowly^[40,67]. It has also been shown that corneal endothelial cell loss is higher in people with diabetes than in nondiabetics^[68-70]; this means that routine evaluation of diabetic patients using specular microscopy is recommended. Moreover, surgeons should take greater care in order to reduce endothelial stress during surgery.

INTRAOCULAR LENS CHOICE

The most common problem for diabetic patients is DR. For this reason, optimal visualization and treatment of the retina should be kept in mind during cataract surgery. As the diameter of the lens increases, it will provide a larger optical area-a difference that may be crucial for optimal management of DR.

PCO is another concern following cataract extraction. It has been reported that the development and severity of PCO is increased in DM patients as compared to non-diabetic patients^[60,71]. Several studies have shown a relationship between the development of PCO and lens material type, and that the shape of the lens^[72]. A square edge design seems to inhibit lens epithelial cell proliferation and may therefore prevent PCO formation^[72].

Several studies have evaluated the biocompatibility of three common materials used to manufacture foldable IOLs with diabetic patients. One performed a comparison between hydrophobic acrylic and plate-haptic silicone IOLs in diabetic patients; although PCO developed less frequently with hydrophobic acrylic IOLs, it was demonstrated that this material was associated with a higher risk of anterior chamber flare in the early postoperative period^[72]. In addition, hydrophobic acrylic lenses have the lowest propensity for silicone oil adhesion, meaning that they may be the IOL of choice for diabetic patients. Because diabetic patients may need

vitreoretinal surgery during the course of managing their disease, silicone IOLs that develop condensation during pars plana vitrectomy may be relatively contraindicated in such individuals^[73]. Hydrophilic acrylic IOLs are prone to opacification, particularly in patients with PDR, since elevated levels of phosphorus in the serum combined with the aqueous humor of diabetic patients may lead to opacification. Several reports have proved progressive calcific opacification of hydrophilic acrylic IOLs in diabetic patients^[74-77]. Rodríguez-Galietero *et al*^[78] evaluated contrast sensitivity and color discrimination in diabetic patients and suggested that blue-light filtering IOLs do not cause chromatic discrimination defects, but that they may even improve color vision in the blue-yellow chromatic axis. Multifocal and accommodative IOLs in people with diabetes are controversial. Postoperative laser treatment and fundus visualization during vitrectomy are difficult because of the optics of these types of lenses^[79]. Additionally, the design of multifocal IOLs reduces contrast sensitivity and could be a cause of visual dissatisfaction for patients with preexisting maculopathy^[79].

The implantation site in diabetic patients is also important. For DM patients, the ideal site is the capsular bag, as usual. The use of anterior chamber angle-fixated lenses and sulcus fixated posterior chamber IOLs in diabetic patients is controversial. It is recommended that iris claw lenses be avoided in patients with DM, due to the increased risk of iris neovascularization. The theoretical risk of cystoid ME, ovalization of the pupil, and poor mydriasis are other risk factors for diabetic patients after iris claw IOL implantation.

POSTOPERATIVE MANAGEMENT AND INDICATORS OF POOR VISUAL OUTCOMES

Carefully performed cataract surgery in diabetic patients should yield optimal postoperative results. Patient follow-up should also be done carefully. Preoperatively, patients diagnosed with NPDR who have adequate retinal view should undergo detailed retinal examination within three months of cataract extraction. Patients with PDR or those with inadequate retinal view prior to cataract extraction should be examined closely after surgery in order to evaluate their DR status^[80].

Endophthalmitis is the most serious complication of cataract surgery. The risk of postoperative endophthalmitis in diabetic patients has increased and is associated with a poor visual prognosis.

As previously mentioned, as a patient's age and duration of diabetes increases, there is greater prevalence of corneal epithelial defects and persistent erosions due to impaired corneal innervation^[40,68]. Corneal endothelial cell damage and persistent corneal edema in diabetic patients following cataract surgery have also increased^[81,82]. Specular microscopy should therefore be used to evaluate DM patients and all the necessary precautions should be taken intraoperatively. Also more frequently observed in diabetic patients are severe iritis, posterior synechiae, pupillary block, and pigmented precipitates on the IOL^[83].

The Early Treatment Diabetic Retinopathy Study (ETDRS) outlines the prognostic factors after cataract surgery. The presence of clinically significant macular edema (CSME) at the time of surgery was found to be a predictor of poor final BCVA in cases of uncomplicated phacoemulsification^[84]. Another determinant of poor postoperative BCVA was the severity of DR at the time of surgery. As the severity of retinopathy increased, the risk of macular ischemia or edema also increased^[36,58,85]. More severe retinopathy also correlated with a reduced tendency for spontaneous resolution of postoperative ME, which is itself associated with poor postoperative BCVA. PDR without any treatment prior to cataract surgery is another factor-one which comes with an increased risk of vitreous hemorrhage and TRD following surgery^[86].

COMPLICATIONS

Despite the advancement in phacoemulsification technology, poor visual acuity following cataract extraction is still common in patients with DM. PCO, postoperative cystoid macular edema (CME), DME, and worsening of the DR are the main complications seen in diabetic patients^[87].

PCO formation

PCO is one of the most common causes of decreased vision after cataract extraction. Although modifications in surgical technique and improvements in IOL technology have reduced the incidence of PCO, it is still a problem for these patients. Proliferation of lens epithelial cells and the degree of postoperative inflammation are

associated with development of PCO. Proliferation of lens epithelial cells is affected by several factors, including optic edge design, optic-haptic junction, and IOL material. However, surgical trauma and contact with the IOL can induce inflammation and cause epithelial cells to produce cytokines, which induce collagen production and fibrous metaplasia^[88].

While some studies revealed a higher incidence of PCO in diabetic patients^[60,89], others showed fewer cases of PCO in diabetic eyes, regardless of the retinopathy stage, over the course of two years^[90]. In a study by Hyashi *et al*^[91], the development of PCO was significantly higher in diabetic patients 18 mo after surgery, even though it was similar to the control group for the first 12 mo. Severity of retinopathy did not have an impact on the development of PCO, according to some studies^[92]. **Figure 1** demonstrates PCO development in a diabetic patient six months after cataract surgery.

Macular edema

The development of DME, pseudophakic macular edema (PCME), CME, or Irvine-Gass syndrome are other frequent causes of postoperative vision deterioration among the general population^[93,94]. Altered concentrations of angiogenic factors after cataract surgery may aggravate maculopathy^[95]. OCT imaging has also revealed increased retinal thickness following an uneventful cataract surgery in diabetic eyes without retinopathy as compared to non-diabetic eyes^[96]. Chu *et al*^[93] reviewed 81,984 eyes and reported that, even in the absence of retinopathy, diabetic patients' eyes had an increased relative risk of ME after surgery. In addition, patients with preexisting DR had a higher relative risk of ME, with this risk being proportional to the increasing severity of retinopathy^[93]. **Figure 2** shows the development of CME in a diabetic patient after cataract surgery.

The incidence of CME varied between 0.2% and 20% in older studies. However, recent studies report lower rates of CME, ranging from less than 1% to 2%-3%^[97]. The methods of detection used in these studies have a significant effect on the rate of CME detection. Fluorescein angiography and OCT were more sensitive, for example, reporting higher rates of CME than clinical detection^[97]. It is also important to differentiate DME from PCME (Irvine-Gass syndrome), since the pathogenesis, treatment, natural course, and outcomes for both are very different. While the underlying presence of DR, exudates, and ME point toward DME, if there is minimal or no DR and there are no exudates in the posterior pole, this suggests PCME. When in doubt, fluorescein angiography can help to distinguish; if the angiography shows a petaloid pattern associated with hyperfluorescence of optic disc and there is no retinopathy or microaneurysms, edema may be considered as a result of Irvine-Gass syndrome^[36].

According to Medicare data, the cost of cataract surgery and related patient care in the United States can be doubled due to ME^[98,99]. Therefore, the prevention of CME in diabetic patients is very important. Recently, both prophylactic and therapeutic usage of both topical steroidal and non-steroidal anti-inflammatory eye drops (NSAIDs) has become central to perioperative management of CME in diabetic patients. Especially NSAIDs have been shown to decrease the incidence of CME in the general population. In a systematic review of 15 randomized trials, Kessel *et al*^[100] showed that topical NSAIDs are more efficient in preventing CME than topical steroids. However, the use of NSAIDs did not change the incidence of CME in patients with DR^[101].

In addition to facing a higher risk of CME, diabetic patients with preexisting DME are at an increased risk of worsening edema following cataract surgery^[29,36]. In ETDRS Report 25, the presence of preexisting, CSME-though it showed no statistically significant difference in the prevalence of ME before and one year after surgery-was associated with worse visual outcomes^[36]. Although ME is commonly seen after cataract surgery, it can follow a benign course. The development of postoperative CSME may be the result of the natural progression of the disease rather than a direct effect of surgery on many patients. On the other hand, the clinical course was quite different in eyes with CSME at the time of surgery. None of them resolved spontaneously within a year and the majority showed clinical and angiographic signs of deterioration. Dowler *et al*^[37] have shown that CSME at the time of cataract surgery is associated with worse visual acuity outcomes at one year post-surgery. It seems possible that severe ME after cataract surgery represents a postoperative deterioration of pre-existing ME that was previously untreated because of lens opacity^[36].

Attempts to stabilize and resolve DME will help improve outcomes, if DME is present prior to cataract surgery. Many strategies for the preoperative medical management of DME are available. Postoperative laser photocoagulation for diabetic ME is controversial. The ETDRS established the utility of focal/grid laser photocoagulation for the treatment of ME^[102]. Focal/grid laser treatment (as described in the ETDRS) was considered as first line treatment for CSME, prior to the use of anti-VEGF

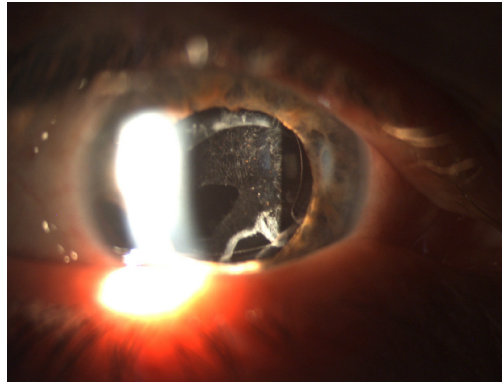


Figure 1 Anterior segment photograph of a diabetic patient who developed posterior capsular opacification six months after phacoemulsification surgery.

agents for central involved DME. It remains an alternative treatment in cases in which anti-VEGFs are not applicable or the center of the macula is not involved^[103]. On the other hand, Pollack *et al*^[34] and Dowler *et al*^[37] showed that ME resolves spontaneously if it arises postoperatively but not when it is present preoperatively. They suggested that early laser treatment is unnecessary for all cases of postoperative DME. Generally, experts do not perform argon laser treatment until six months after cataract surgery.

The advent of anti-VEGF injections has shifted the paradigm in the treatment of DME. Many studies performed on anti-VEGF agents in diabetic patients have shown their effectiveness at preventing and treating CSME^[104-111]. Current opinion supports that anti-VEGF agents are first-line therapy in preoperative treatments, perioperative stabilization of DME, and postoperative management and that they show great success in anatomic recovery and visual function. Focal laser treatment and steroid injections still provide significant additional support.

Progression of retinopathy

Numerous studies have evaluated the effect of cataract surgery on the progression of DR. The progression of DR after intracapsular (ICCE) and extracapsular (ECCE) cataract extraction has been extensively studied^[80,112,113]. Sebestyen *et al*^[112] and Alpar *et al*^[113] demonstrated the progression of retinopathy after ICCE and ECCE, with ICCE showing worse results than ECCE. However, the effect of phacoemulsification is controversial. Modern phacoemulsification procedures are considered faster, safer, and more cost-effective than ICCE and ECCE^[114]. Even with the advances in modern phacoemulsification techniques, some studies have demonstrated a similar trend of DR progression after phacoemulsification surgery; others have reported no significant change^[37,42,115]. **Figure 3** shows the progression of DR in a diabetic patient's right eye after phacoemulsification surgery.

Prospective studies by Dowler *et al*^[37] and Squirrell *et al*^[42] have reported that uncomplicated cataract extraction using phacoemulsification have no effect on the progression of DR. However, Squirrell *et al*^[42] have shown an increased risk of DR progression following cataract surgery in patients with elevated hemoglobin A1c. These studies included one eye that underwent phacoemulsification surgery and one eye as a control. Conversely, some studies that included diabetic patients undergoing phacoemulsification cataract surgery showed a retinopathy progression rate that had nearly doubled at the 12-mo period as compared to unoperated eyes^[84]. Similarly, ETDRS Report 25, which enrolled 3711 patients with a nine-year follow-up period, also showed increased rates of retinopathy progression in cases of phacoemulsification than in unoperated eyes^[36]. A recent study by Denniston *et al*^[116] reported significant postoperative progression of center involving DME, which was associated with the preoperative grade of DR. Shah *et al*^[41] found that recent studies do not support the generalized conclusion that phacoemulsification causes progression of retinopathy and ME in all diabetic patients.

The risk factors for DR progression have also been investigated. In a retrospective study by Krepler *et al*^[115], these included being male, the disease duration, and poor glycemic control. Dowler *et al*^[37] reported that a smaller incision size and shorter surgical duration for phacoemulsification decreased inflammation and may induce less breakdown of the blood-ocular barrier, meaning that uncomplicated phacoemulsification cataract surgery does not accelerate DR progression. Additionally, recent studies suggest that anti-VEGF injections may also affect the incidence of DR

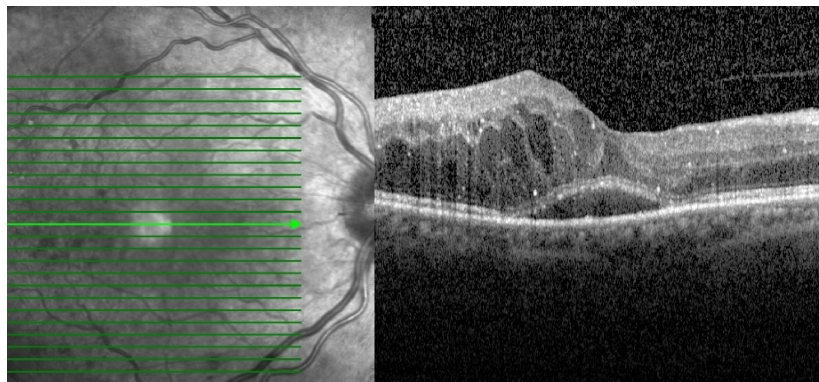


Figure 2 Horizontal optical coherence tomography scan of a diabetic patient, showing the development of cystoid macular edema and serous macular detachment after cataract surgery.

progression^[117]. Despite no current consensus on the prophylactic use of anti-VEGF, their use for patients with more advanced NPDR or PDR and DME should be considered. Other ocular co-morbidities such as vitreous hemorrhage, epiretinal membranes, or TRD may benefit from a combined pars plana vitrectomy and cataract surgery^[118].

CONCLUSION

As the number of people with DM is estimated to continue to increase, cataract surgery will remain important for diabetic patients. Patients with diabetes have multiple issues to be evaluated preoperatively, perioperatively, and in the postoperative period. With the advent of modern surgical and pharmacologic therapies, these patients can, like other cataract patients without diabetes, recover excellent vision. Postoperative monitoring and management of surgical complications will also help to alleviate the risk of vision loss in these patients.

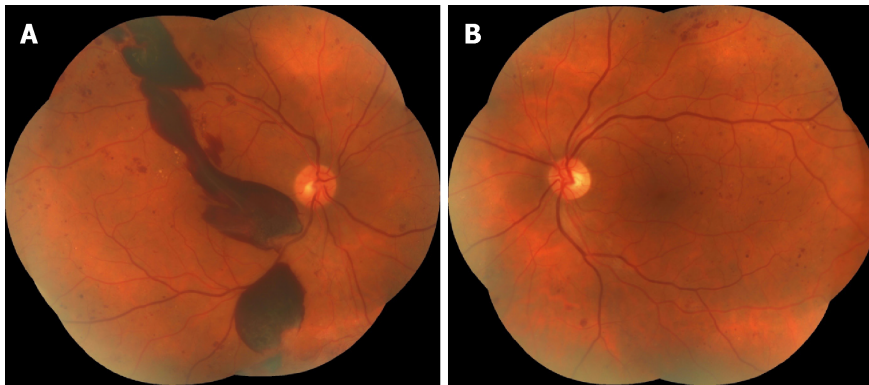


Figure 3 Colored fundus photographs of a diabetic patient reveal the progression of diabetic retinopathy in the right eye after surgery. A, B: Both eyes have dot-blot hemorrhages and hard exudates; two months after surgery, massive preretinal hemorrhages occurred in the right eye (A). A: Right eye; B: Left eye.

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Crosstalk between gut microbiota and antidiabetic drug action

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Abstract

Type 2 diabetes (T2D) is a disorder characterized by chronic inflated blood glucose levels (hyperglycemia), at first due to insulin resistance and unregulated insulin secretion but with tendency towards global spreading. The gut microbiota is recognized to have an influence on T2D, although surveys have not formed a clear overview to date. Because of the interactions between gut microbiota and host homeostasis, intestinal bacteria are believed to play a large role in various diseases, including metabolic syndrome, obesity and associated disease. In this review, we highlight the animal and human studies which have elucidated the roles of metformin, α -glucosidase inhibitors, glucagon-like peptide-1 agonists, peroxisome proliferator-activated receptors γ agonists, inhibitors of dipeptidyl peptidase-4, sodium/glucose cotransporter inhibitors, and other less studied medications on gut microbiota. This review is dedicated to one of the most widespread diseases, T2D, and the currently used antidiabetic drugs and most promising new findings. In general, the gut microbiota has been shown to have an influence on host metabolism, food consumption, satiety, glucose homeostasis, and weight gain. Altered intestinal microbiota composition has been noticed in cardiovascular diseases, colon cancer, rheumatoid arthritis, T2D, and obesity. Therefore, the main effect of antidiabetic drugs is on the microbiome composition, basically increasing the short-chain fatty acids-producing bacteria, responsible for losing weight and suppressing inflammation.

Key words: Type 2 diabetes; Gut microbiota; Metformin; α -glucosidase inhibitors; Glucagon-like peptide-1 agonists; Peroxisome proliferator-activated receptors γ agonists; Dipeptidyl peptidase-4 inhibitors; Sodium/glucose cotransporter inhibitors

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Core tip: Gut microbiota was found to have an influence on host metabolism, food consumption, satiety, glucose homeostasis, and weight gain. Altered intestinal microbiota composition has been noticed in cardiovascular diseases, colon cancer, rheumatoid arthritis, type 2 diabetes, and obesity. Therefore, the main effect of antidiabetic drugs is on the microbiome composition, basically increasing the short-chain fatty acids-producing bacteria, responsible for losing weight and suppressing the inflammation.

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INTRODUCTION

Over the last few decades, diseases related to metabolic processes, such as type 2 diabetes mellitus (T2D), obesity, dyslipidemia, hypertension and cardiovascular diseases (CVD) have become the main health problems around the world^[1]. T2D is a disorder characterized by chronic inflated blood glucose levels (hyperglycemia), at first due to insulin resistance and unregulated insulin secretion but with a tendency towards global spreading. Environmental and genetic factors' contribution are known, but sedentary life style and dietary habits are not the least constituents. Influence of gut microbiota on T2D is also recognized^[2-4], although findings differ between surveys. Because of the interactions between gut microbiota and host homeostasis, gut bacteria are thought to play a great role in diseases, including metabolic syndrome^[5-7].

The composition and richness of the gut microbiota is modulated by diet, host health, age, ethnicity and genetics, and thus are unique and highly variable among individuals^[3,4]. Turnbaugh *et al*^[8] suggest that there is a "core gut microbiome" that could be responsible for proper gut functioning. That core gut microbial profile predominantly consists of bacteria, which belong to the Gram-positive Firmicutes and the Gram-negative Bacteroidetes^[5]. Nevertheless, the increase of intestinal Firmicutes/Bacteroidetes ratio is observed in both obesity and during consumption of energy-rich diets in humans and animal models^[5,9,10]. Similar to obesity outcomes, T2D induces a dysbiosis, mainly by reduction in butyrate-producing bacteria^[11,12] and in *Akkermansia muciniphila*, which is now considered a biomarker for glucose intolerance^[4].

The bacterial phylotypes found to be correlated with weight are associated with the phyla Firmicutes (2 families and 11 genera), Bacteroidetes (1 family and 2 genera) and Tenericutes (1 family and 1 genus)^[3,13]. Among them are five genera affiliated with an increase in weight, including *Erysipelotrichaceae incertae sedis*, *Marvinbryantia*, *Roseburia*, *Candidatus arthromitus*, and *Parabacteroides*^[3,13]. The phylotypes associated with weight loss were of the genera *Lactobacillus*, *Turicibacter*, *Anaerostipes*, *Coproccoccus*, *Blautia*, *Oscillibacter*, and *Clostridium*^[3,13]. For instance, Vrieze *et al*^[14] showed that obesity was associated with modifications in the abundance, diversity, and metabolic function of the gut microbiota, mostly represented as a higher quantity of Firmicutes and a reduced abundance of Bacteroidetes in animal experiments.

Furthermore, one main function of the gut microbiota is to devastate nondigestible carbohydrates into short-chain fatty acids (SCFAs), mostly propionate, acetate and butyrate^[15]. Lines of evidence have suggested that intestinal microbiota and SCFAs exert positive effects on glucose-lowering agents in T2D. Glucose-lowering agents can also alter gut microbiota^[16], thus meliorating glucose metabolism and energy balance; they also have an influence on the production of SCFAs, thereby providing beneficial effects^[12]. Perhaps mechanisms may also affect gene expression, levels of inflammatory cytokines, and the regulation of SCFA synthesis. Furthermore, gut microbiota may attenuate side effects caused by glucose-lowering agents, which is an advantage for diabetic patients. It has even been suggested that human gut microbiota express some enzymes which are capable of binding to and transforming a wide spectrum of bioactive substances^[17,18].

Orally-taken medicines reach the gastrointestinal tract and encounter the intestinal microbiota. It has been shown that microbiota-encoded enzymes have the ability to metabolize xenobiotics and to impact the pharmacogenetics of drugs and their

bioavailability^[19,20]. Accordingly, the gut microbiota may have an influence on drug effectiveness.

Moreover, supplementation with probiotic strains and their combination with nutraceuticals has been demonstrated to provide health benefits in obesity and associated diseases in both animal^[21,22] and human studies^[23-25].

In this review, we will focus on gut microbiota alterations in obese and T2D patients and its response to currently used antidiabetic drugs (Figure 1). Below, we highlight the animal and human research that has begun to elucidate the role of metformin (1,1-dimethylbiguanide hydrochloride), alpha-glucosidase inhibitors (α -GIs), glucagon-like peptide-1 (GLP-1) agonists, peroxisome proliferator-activated receptors (PPARs) activators, inhibitors of dipeptidyl peptidase-4 (DPP-4) and sodium/glucose cotransporter (SGLT-2), and other less studied medications on gut microbiota.

GUT MICROBIOTA AND METFORMIN

Metformin is the most used nonmetabolizable compound from the biguanide class that patients take orally. It is currently the drug of choice recommended by the American Diabetes Association and the European Association for the Study of Diabetes. Metformin has blood glucose-lowering and insulin sensitizing effects and inhibits liver glucose production. Also, this drug modulates the incretin pathway by improving the expression of GLP-1 receptor in the pancreatic islets and raising plasma levels of GLP-1^[5,26,27]. A recent study suggested that inhibition of mitochondrial glycerophosphate dehydrogenase, an enzyme in the glycerophosphate shuttle, could be the main system involved in the metformin-induced inhibition of gluconeogenesis^[28].

Treatment with metformin also alters bile acid recirculation^[6], suggesting that the primary actions of metformin could be in the gut^[11]; however, the absorption of metformin mainly occurs in the small intestine. Moreover, T2D patient treated with metformin can experience improvement in their lipid levels, which would contribute to the reduction of chronic micro- and macrovascular complications. Most of metformin's pleiotropic effects are predetermined by adenosine monophosphate-activated protein kinase (AMPK) activation in the skeletal muscle and liver^[29]. In addition, AMPK activation is known to upregulate autophagic activity through direct phosphorylation of unc-51-like kinase and Beclin 1, key molecules involved in the initiation of autophagy; consequently, metformin can magnify autophagy^[30]. Autophagy is valuable for nutrient supply in the case of energy deficiency, has a significant impact on body metabolism, and is also essential for the proper turnover of organelles, such as mitochondria and the endoplasmic reticulum^[28]. These organelles play critical roles in pancreatic β -cell physiology and insulin sensitivity. Although, a global increase in autophagic activity is likely to improve the metabolic profile under metabolic stress conditions^[28,31], which might be related to attenuation of the chronic low-grade tissue inflammation associated with obesity^[28,32].

Metformin treatment is accompanied by the enrichment of SCFA-producing bacteria, such as *Blautia*, *Bacteroides*, *Butyrivoccus*, *Bifidobacterium*, *Prevotella*, *Megasphaera*, *Butyrivibrio*^[33] or *Phascolarctobacterium*, and has positive effects on the Proteobacteria phylum as well as the *Allobaculum* and *Lactobacillus* genera^[5,32]. Additionally, the composition of the phylum Verrucomicrobia in a high-fat diet (HFD)-Met-treated experimental group was notably raised. Metformin treatment was also shown to have an effect on the gut microbiota in mice on normal diet. Furthermore, the families *Rikenellaceae*, *Ruminococcaceae*, and *Verrucomicrobiaceae*, as well as *Alistipes* spp., *Akkermansia* spp., and *Clostridium* spp., were found to be more abundant with normal diet plus metformin treatment than in the control experimental group^[34].

At the genera level, an increase of *Escherichia* and a decrease of *Intestinibacter* has been detected in a metformin-treated group^[33]. The number of positive connections among microbial genera, especially those within Proteobacteria and Firmicutes, was also found to be increased after 2 mo of metformin treatment^[33]. After 4 mo of treatment with metformin, there were significantly larger increases in fecal concentrations of lactate and a trend toward a larger increase in fecal concentrations of succinate^[33]. In addition, Shin *et al.*^[35] showed significant differences in the abundance of Firmicutes and Bacteroidetes and gut microbiota composition between metformin-treated and non-treated mice but only under HFD conditions. Correspondingly, Lee *et al.*^[34] observed that metformin caused a decrease in bacterial diversity in mice on HFD.

Recently, the degradation of mucin was reported in mice on HFD^[36] and suggested

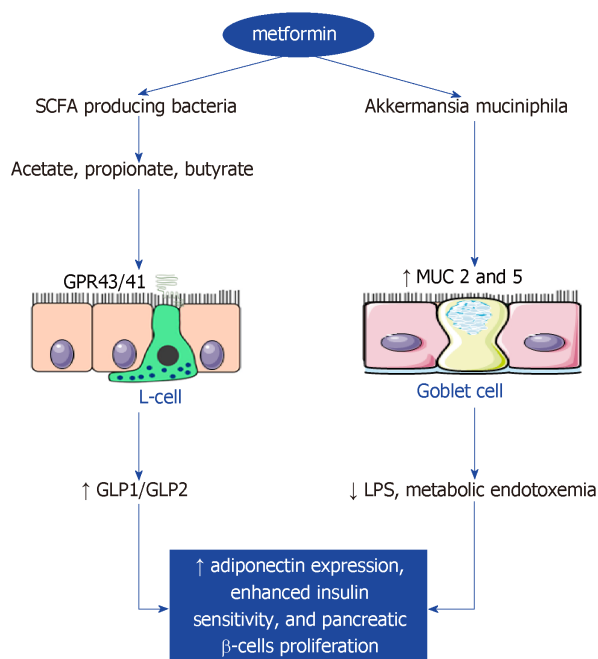


Figure 1 Crosstalk between metformin action and gut microbiota. GLP1: Glucagon-like peptide-1; GLP2: Glucagon-like peptide-2; LPS: Lipopolysaccharide; SCFA: Short-chain fatty acid.

as possibly related to metabolic disorders. After metformin treatment in female mice, but not in male mice, expression of the genes *MUC2* and *MUC5* in the small intestine became remarkably increased^[34]. This is a beneficial effect of the drug, because gastrointestinal mucins produced by goblet cells protect the underlying epithelium from pathogens. Moreover, female hormones are known to exert a protective effect against metabolic disorders^[37] and to be involved in lipid and glucose metabolism. Therefore, the observed differences in the gut microbiota between male and female mice during metformin treatment might be caused by differences in hormone levels, which might be associated with metabolic phenotypes^[34].

Nevertheless, the abundance of *Akkermansia*, which are mucin-degrading bacteria, is positively correlated with the quantity of goblet cells. Studies have highlighted that metformin multiplies the number of goblet cells, irrespective of diet^[5,35]. Another study showed that treatment with *A. muciniphila*, which was identified from enterotype gut microbiota, improved metabolic parameters^[34,36]. Albeit, approximately 30% of patients report experiencing side effects with metformin, including diarrhea, nausea, vomiting, bloating, and lactic acidosis.

GUT MICROBIOTA AND ALPHA-GIs

The α -GIs are oral hypoglycemic antidiabetic drugs that postpone the digestion of carbohydrates such as disaccharides and starch in the small intestine, and which reduce postprandial hyperglycemia^[5] and delay the absorption of glucose, thereby managing blood glucose levels and related complications. To this class belong acarbose, voglibose (also naturally occurring in *Streptomyces*) and miglitol. Thus, α -GIs alter the nutrient sources of bacteria by segregating complex carbohydrates.

Acarbose delays the enzymatic carbohydrates decaying in the small intestine and thereby diminish postprandial hyperglycemia. Clinical studies have shown that acarbose significantly enhances glycemic control and lowers known CVD risk factors, including triglycerides' levels, body mass index, insulin levels, and systolic blood pressure^[38-40]. The prominent mechanisms for this cardiovascular protective function are only partially understood, but they can be attributed to the ability of acarbose to neutralize oxidative stress by increasing H₂ production in the gastrointestinal tract^[38,41]. Panwar *et al.*^[42] found that *Lactobacillus* strains exert effects of glucosidase-inhibitors and regulate blood glucose responses to carbohydrates *in vivo*. As mentioned above, SCFAs play a crucial role in diabetes. In individuals with impaired glucose tolerance, acarbose was found to increase serum butyrate levels. The underlying mechanism for this effect might be that acarbose increases the fermentation of insoluble fibers in the colon. Interestingly, oral supplementation of

butyrate was found to improve insulin sensitivity and increase energy expenditure by enhancing mitochondrial work in mice^[38,43]. To minimize the known gastrointestinal side effects of acarbose (*e.g.*, flatulence, diarrhea, or abdominal cramps), the drug was administered in small proportions. Zhang *et al.*^[39] compared treatment for T2D with acarbose and metformin and showed that both treatments notably increased GLP-1 concentration and decreased glucagon after 24 wk. However, additional benefits of acarbose besides its antidiabetic effect remain unknown.

Acarbose is effective in lowering blood glucose level in patients with T2D by delaying the digestion of complex carbohydrates through the inhibition of pancreatic α -amylase and a variety of α -glucosidases^[38]. Later, microbiota will ferment these carbohydrates, which will alter the composition of the intestinal microbiota. The features of gut microbiota in patients with prediabetes (before treatment) are genera abundance of *Bacteroides* (belonging to Bacteroidetes) and *Faecalibacterium* (belonging to Firmicutes). The most plentiful phyla include Firmicutes (68.53% of all reads), Bacteroidetes (27.85% of all reads), Proteobacteria (1.98% of all reads), and Actinobacteria (0.98% of all reads)^[38].

After the treatment with acarbose, five genera, including *Lactobacillus* and *Dialister*, flourished. In response to acarbose, *Lactobacillaceae*, *Ruminococcaceae* and *Veillonellaceae* increased and six genera, including *Butyricoccus*, *Phascolarctobacterium* and *Ruminococcus*, decreased. Likewise, many of the operational taxonomic units that greatly increased in response to acarbose belong to SCFA-producing taxa, such as *Faecalibacterium*, *Prevotella*, and *Lactobacillus*^[38]. Some species of *Megasphaera* also thrived following acarbose treatment. They can transform carbohydrates into SCFAs, including butyrate, formate, acetate, valerate, and caproate, a process which is valuable for *Lactobacillus* development^[38]. Consequently, the effect of acarbose on body weight might be related to reorganized microbiota structure. It is supposed that SCFAs such as acetate, butyrate and propionate and their concentrations are prognostic of lifespan^[38]. Thus, the increased levels of SCFAs in acarbose-treated mice may lead to the beneficial effect on the lifespan^[44]. However, studies have shown that the weight loss in female mice on acarbose was more dramatic than in males, while the longevity effect is much stronger in males^[45].

Another α -GI, voglibose, changes dysbiosis in diet-induced obese mice^[46]. These changes could increase the production of bile acid metabolites and have an advantageous systemic outcome. Specifically, scientists have found favorable effects of voglibose on several cardiovascular end-points, as it improves glycemic control in mice with cardiac overpressure^[47]. Voglibose has anti-obesity effects on diet-induced obese mice. Possibly, the effects of incretins in voglibose, activation of neuroendocrine linked to leptin, and inducement of the genes responsible for magnified energy metabolism cause the reduction in energy intake and improvement of mitochondrial function^[48]. The reduction in food intake is possibly derived from increased GLP-1 levels due to voglibose supplementation or from direct modulation of hypothalamic genes which lead to the satiety response.

Miglitol shortens the intestinal transit time and suppresses histological and molecular markers of inflammation, for which concentrations are elevated by a high-fat and high-glucose diet and which shifts with the increases in *Erysipelotrichaceae* and *Coriobacteriaceae* induced by the energy-rich diet^[49]. Miglitol is able to alter human gut microbiota because of the transit time reduction.

The development of nonalcoholic steatohepatitis (NASH) could be dependent on the gut environment as well. As α -GIs change the gut environment, they might also protect against NASH development, because of its sensibility to changes in the gut environment^[49]. NASH is characterized by hepatocellular lipid accumulation along with inflammation and fibrosis that is a precondition for oxidative stress, inflammatory cytokines, and endotoxins. There is an essential need for therapeutic interventions considering that NASH can lead to cirrhosis and liver cancer. In addition, acarbose was also demonstrated to have a protective effect against NASH development in HFD-induced obese rats^[50]. However, the underlying mechanisms should be further investigated.

Miglitol was shown to restrain the accumulation of lipid droplets and inflammatory cell infiltration, and to lead to a decrease in the numbers of ballooning hepatocytes as well as to stoppage of the activation of stellate cells, which plays a role in liver fibrosis^[49].

The administration of an α -GI has been found to increase the levels of butyric acid in the intestines of healthy individuals. Indeed, the administration of butyric acid was demonstrated to suppress intestinal inflammation in mice^[51]. These findings suggest that miglitol administration increases the butyric acid level in the intestine and suppresses colon inflammation.

Human gut bacterium *Blaubia* (*Ruminococcus*) *obeum* expresses enzymes, such as α -glucosidases (Ro- α G1), which have specific crystal structures with free active site(s) to

bind and interact with volatile substrates. Therefore, the proposed theory is that α -GIs (acarbose, voglibose, miglitol) can affect the bacterial Ro- α G1 in human gut and exert positive effects or create adverse gastrointestinal symptoms^[52]. The α -GIs bind to the active site of Ro- α G1 and change the enzyme's activity. Acarbose was found to slightly inhibit the gut bacterial α -glucosidases as well as other currently used α -GIs.

GUT MICROBIOTA AND GLP-1 AGONIST

Intestinal endocrine cells (L cells) respond to food ingestion by secreting GLP-1, an incretin hormone^[53]. This hormone can intensify glucose-induced insulin from pancreatic β -cells and suppress glucagon secretion; in addition, it can protect pancreatic β -cells from apoptosis and promote β -cell proliferation. Together, incretins are liable for 50%–60% of postprandial insulin secretion. In addition, GLP-1 plays critical roles in gastrointestinal motility as well as in metabolism; moreover, GLP-1 can possibly suppress gastrointestinal motility, thus affecting the absorption of digested food^[54]. However, the natural GLP-1 is degraded rapidly, primarily through enzymatic destruction by DPP-4. Therefore, another pharmaceutical approach to treat T2D is to increase GLP-1 function, either by the administration of GLP-1 peptide mimetics or suppressing its degradation by DPP-4. GLP-1 expression could be stimulated by binding of SCFAs and secondary bile acids (lithocholic acid and deoxycholic acid) with the G-coupled protein receptor FFAR2 (formerly GPR43)^[53]. Many studies have shown that satiety and glucose homeostasis are modulated by the gut microbiota that induces the secretion of GLP-1^[55,56].

The body weight control induced by GLP-1 is maintained by reduced food intake and inhibition of appetite and gastric emptying^[57]. However, restricted dietary intake supplemented with GLP-1 results in more significant weight loss. Interestingly, the microbial diversity after GLP-1 increment seemed to be dependent on the glycemic state of the mice studied. In normoglycemic mice treated with liraglutide and saxagliptin, bacterial variety significantly decreased, while in transiently hyperglycemic mice it rose to the normal level^[13]. Of late, scientists have proposed liraglutide as a prospective anti-obesity drug because of its additional impact on weight loss in obese and diabetic individuals. The daily injection of liraglutide has been shown to significantly improve glucose tolerance and insulin tolerance in diabetic rats rather than in nondiabetic rats. Although, it was found to alter the microbial composition in both simple obese and diabetic obese rats. The genera *Candidatus*, *Roseburia*, *Arthromitus* and *Marvinbryantia* may promote weight gain, while the genera *Coproccoccus* and *Lactobacillus* are associated with weight loss^[58].

Liraglutide administration has been shown to decrease the relative abundance of all of the obesity-related phylotypes (such as *Romboutsia*, *Ruminiclostridium*, and *Erysipelotrichaceae*) and to enrich the lean-related genera *Blautia* and *Coproccoccus*^[59]. After liraglutide intervention, the abundance of Firmicutes was also found to tend towards decrease in obese rats, whereas the finding was contrary when the study was carried out in human volunteers under field conditions without restriction^[60]. Patients with long duration of T2D show a significantly reduced *Akkermansia* variety. After comparison of the gut microbiota of subjects receiving a GLP-1 agonist and metformin, higher *Akkermansia* abundances were detected in the liraglutide-treated patients^[61]. At first, the genus *Akkermansia* and some genera in the family *Christensenellaceae* increased prominently under liraglutide, unlike that seen under metformin, with the latter of which leading to a greater expansion of *Dorea* and *Sutterella* genera.

GLP-1 receptors are placed on neurons innervating the portal vein, on β cells of the pancreas, and the central nervous system^[62]. GLP-1 after its release can affect afferent neurons innervating the gastrointestinal tract which signal to the caudal brainstem or enteric neurons, and/or they can enter the circulation to functionate centrally, or on peripheral targets to regulate metabolic disorders^[59,63]. Thus, the weight-loss and glucose-controlling effects of liraglutide are possibly mediated by the gut-brain axis. In addition, the GLP-1 analog liraglutide reduces visceral hypersensitivity and acts as a sort of pain-killer^[64]. ROSE-010, another GLP-1 analog, has been shown to diminish visceral pain in patients suffering from irritable bowel syndrome^[65].

Long-term HFD intake has been shown to result in a lack of energy substrates, reduced acetylcholine synthesis, membrane deterioration and oxidative stress, and consequently is valid in intestinal myenteric neurons loss in mice^[66]. Grasset *et al.*^[67] revealed that gut microbiota dysbiosis causes the loss of enteric neurons, attenuated nitric oxide production and following GLP-1 resistance. Nitric oxide produced by nuclear nitric oxide synthase has been generally expected to have a protective effect on enteric neurons that is greater than its damaging effect. The GLP-1 secretagogue l-

arginine oral administration has also been shown to improve glucose tolerance by influencing GLP-1R signaling^[68]. L-arginine is a substrate of nuclear nitric oxide synthase which improves GLP-1 sensitivity in HFD-fed mice and increases the glucose-induced insulin secretion. In consequence, L-arginine supplementation could have beneficial effects on postprandial GLP-1 response and GLP-1 sensitivity in patients with T2D.

GUT MICROBIOTA AND DPP-4 INHIBITORS

Protein CD26 present on the lymphocyte cell surface was first described to have a proteolytic activity in 1966 and was later named as DPP-4. The potential substrates of DPP-4 activity are gut hormones, like incretins, GLP-1 and gastric inhibitory polypeptide, neuropeptides, chemokines, and dietary proteins^[69]. Through the cleavage of key hormones and peptides, the DPP-4 activity influences behavioral, intestinal and metabolic disorders^[70]. DPP-4 can enhance the agonistic activity of gut hormones, like neuropeptide Y and peptide YY (PYY), by cleaving off the N-terminal dipeptide. As PYY has an influence on the ileal and colonic brake of digestion and on the induction of satiety *via* activation of hypothalamic Y2 receptors, these effects could be enhanced by DPP-4^[71]. However, the capacity of DPP-4 in the regulation of satiety has not yet been fully elucidated.

An experiment on Dpp-4 knockout mice showed an increased GLP-1 level and improved glucose tolerance associated with it. This effect was achieved in humans by administration of DPP-4 inhibitors, antidiabetic agents that maintain incretins in their active form^[72]. Moreover, Ahmed *et al*^[73] revealed that overweight and obese patients, in comparison to normal-weight patients, have increased DPP-4 activity, which decreases the activity of GLP-1. The administration of prebiotics led to decreased DPP-4 activity, which was explained by double concentration in the active form of GLP-1^[74].

Some studies have acknowledged that some commensal bacteria, such as *Prevotella* or *Lactobacillus*, can express human DPP-4 homologs^[70,75]. Two groups of scientists published findings of DPP-4 expression being higher in gnotobiotic mice colonized with feces of a lean subject than in germ-free mice, which strongly indicates that their intestinal microbiota produced DPP-4-like activity^[70,76]. The overall literature affirms that DPP-4 encoded by the gut microbiota could compose an innovative mechanism to alter protein digestion, host metabolism, and behavior.

The novel type of DPP-4 inhibitors are sitagliptin, saxagliptin and vildagliptin, which are administrated orally. Sitagliptin increases insulin and suppresses glucagon secretion. In patients with T2D, therapies which include DPP-4 inhibitors promote healing of colitis and diminish depression symptoms. DPP-4 was proposed as a possible target for treating autoimmune diseases, including inflammatory bowel disease as it has an influence on the immune system, particularly on T cell function^[77]. Obese dams with adverse pregnancy outcomes have been reported as having decreased *Lactobacillus spp.* compared with dams with normal litters. Secondly, maternal obesity and reduced fertility are related to bad pregnancy outcomes, gestational diabetes, and preeclampsia^[78]. Sitagliptin or prebiotic consumption during pregnancy could normalize gestational weight gain, increase *Bifidobacterium spp.*, reduce fasting glucose levels, and possibly alleviate pregnancy termination associated with maternal obesity while improving offspring metabolic health and composition of the intestinal microbiome^[79].

Saxagliptin, another DPP-4 inhibitor, appears to act only on a small target group of gut microbes, mainly on the Firmicutes/Bacteroides ratio. It has been shown to enhance the development of the genus *Lactobacillus* within the class *Lactobacillaceae*, the genera *Allobaculum* and *Turicibacter* within class *Erysipelotrichaceae* and suppresses the genus *Bacteroides* within the class *Bacteroidaceae*, and the genus *Prevotella* within the class *Prevotellaceae*. As compared to the GLP-1 agonist liraglutide, saxagliptin reduced the enrichment of the genus *Blautia* and of the genus *Coproccoccus*. Moreover, despite the fact of similar food intake reduction, saxagliptin had a neutral effect on body weight, with subjects on liraglutide having significantly lower weight regardless of their glycemic control^[83].

Another DPP-4 inhibitor, vildagliptin, affects the gut microbiota composition and its metabolic activity. Vildagliptin has been shown to reduce the fasting blood glucose and HbA1c levels. In obese murine models, vildagliptin reduced *Ruminococcaceae*, such as the genera *Oscillibacter*, *Ruminiclostridium_6*, *Anaerotruncus*, and *Ruminococcaceae_UCG_007*, as well as the families *Planococcaceae*, *Christensenellaceae*, and *Prevotellaceae*. The enriched phylotypes were of the *Streptococcaceae* family, the genera *Bacteroides*, and the family *Bacteroidaceae*. In general, it modified the

Firmicutes/Bacteroides ratio, reduced DPP-4 activity in the portal vein and increased the concentration of active GLP-1, improving gastrointestinal function according to AMPs' expression restoration and the depth of the crypts in the ileum^[80]. Vildagliptin also has a potential effect on inflammation, due to reduction of TLR and cytokine expression. Zhang *et al.*^[80] suggested that vildagliptin enriches SCFA-producing bacteria and ameliorates gastrointestinal health and could ultimately mediate their beneficial effects on the host, especially in diabetes.

The DPP-4 inhibitor PKF-275-055 improves glucose/cholesterol metabolism, decreases Firmicutes/Bacteroidetes ratio, and drops mass gain and mesenteric adipose accumulation. Moreover, mice on HFD with PKF-275-055 treatment showed enriched butyrate-producing *Ruminococcus* and of the acetogen *Dorea* compared to the control group^[81].

GUT MICROBIOTA AND SGLT-2 INHIBITORS

The sodium/glucose cotransporters SGLT1 and SGLT2 are generally expressed in the small intestine and are regulated by a sodium gradient created by Na⁺/K⁺ ATPase. SGLT1 transports glucose and galactose across the apical membrane of enterocytes, whereas SGLT2, and at some extent SGLT1, reabsorbs glucose in the renal tubule. SGLT2-selective inhibitors are a new class of treatment for T2D^[82]. SGLT2 differs from other antidiabetic medications because it ameliorates vascular function and thus has advantageous effects on CVD. Among the numerous T2D consequences, CVD is the most widespread and dramatic. Data characterize the gut microbiota as an important regulator of vascular function^[83]. Indeed, people with diabetes have vascular dysfunction and heightened risk of CVD. Distinct signs of diabetes-related CVD are arterial stiffness, endothelial dysfunction, and vascular smooth muscle functional disorder. Reduction in endothelium-independent dilation is present in T2D patients and is a marker of cardiovascular complications.

After 8 wk of treatment with a selective SGLT2 inhibitor dapagliflozin, diabetic mice showed lower arterial stiffness and blood glucose level, and improvements in endothelial and vascular smooth muscle dysfunctions compared to nontreated diabetic mice. In addition, reductions in circulating inflammatory markers, such as MCP-1, IL-1 β and IL-6, and hyperglycemia improvement were detected^[84]. Animals with diabetes treated with dapagliflozin showed decreased Firmicutes/Bacteroidetes ratio and Oscillospira, and increased *Akkermansia muciniphila*.

The dual SGLT1/2 inhibitor has been shown to reduce blood glucose levels and HbA1c and to increase total GLP1 in mice fed a high-sucrose diet^[85]. The higher doses of SGLT1/2 inhibitor accelerated body weight gain and increased Bacteroidetes and decreased Firmicutes quantity, but the *Akkermansia* spp. was not modified^[82]. SGLT1/2 inhibitors or SGLT2-selective drugs like canagliflozin guarantee intestinal SGLT1 inhibition in T2D^[86]. They enhance GLP-1 and PYY secretion and delay the glucose excursion after carbohydrate intake. The dual SGLT1/2 inhibitor LX4211/sotagliflozin, in clinical testing, has the same effects as the medicine described directly above^[87]. However, SGLT1 cannot be completely inhibited as long as changed Na⁺ homeostasis and elevated colonic carbohydrates can lead to the opposite gastrointestinal effects and diarrhea.

GUT MICROBIOTA AND PPAR

The PPARs belong to the nuclear receptor family of regulatory factors that are ligand-activated transcription factors. There are two PPAR γ isoforms: PPAR γ 1 and PPAR γ 2. They form a heterodimeric complex with the retinoid X receptor, which binds to PPAR-responsive elements and then regulates transcription. Its main functions are linked to maintaining homeostasis in the intestine, inducing adipocyte growth and differentiation^[88], cellular apoptosis^[89], regulation of genes involved in glucose and lipid metabolism^[90], and inflammatory responses. PPAR γ is expressed mainly in several tissues such as of the lungs, breast, ovaries, placenta and at most in the colon, where it regulates colonocyte metabolism and cell cycle^[91].

PPAR γ is considered to have anti-inflammatory effects and to be a molecular target for cancer chemoprevention^[92]. It also enhances insulin sensitivity and regulates the genes involved in hypertension and contributing to atherosclerosis. There are lines of evidence indicating reduction of intestinal inflammation and colon cancer development and T2D^[93] by specific PPAR γ agonists.

Nepelska *et al.*^[91] engineered a colonic epithelial HT-29-PPAR γ reporter cell line to control the influence of bacterial metabolites on transcriptional activity of PPAR γ .

Two main metabolites of intestinal bacteria, butyrate and propionate, were linked to activation of PPAR γ transcriptional activity. Notwithstanding, phylogenetic affiliation of the strains were found to not rigorously correspond to reporter gene activities, among them the most general stimulating effect was noticed for Firmicutes and Fusobacteria, while Actinobacteria exerted moderate or no modulation.

The strongest potential of PPAR γ activation is exerted on *Roseburia hominis*, *Roseburia intestinalis*, and *Fusobacterium naviforme*. These are well-known producers of butyrate, therefore the response pattern of PPAR γ reporter cells is exposed to the composition of organic acids of conditioned media. Gene regulation in intestinal epithelial cells is known to be regulated by the SCFAs, especially butyrate^[94]. Acetate negatively affected the PPAR γ reporter system, demonstrating a reverse correlation. Moreover, butyrate and propionate stimulated PPAR γ activity, even at such low concentrations as 0.5mM. Acetate, however, showed an insignificant activation starting from 8 mM, and lactate did not affect the activity but was cytotoxic from 2 mM, which lead to cell detachment^[95]. In general, high concentrations of all organic acids, especially acetic and lactic, have a deleterious effect on the viability of cells, possibly because of decrease in pH.

Some species can activate the expression of PPAR γ target genes even without presence of butyrate and propionate in their conditioned media. To them belong *Atopobium parvulum* and *Prevotella copri*. These bacteria were shown to increase ANGPTL4 and ADRP expression in HT-29 cells. Their underlying mechanisms are probably different, because stimulation with conditioned media of *A. parvulum* showed its influence after 6 h and *P. copri* in 12 h^[91]. The induction of ANGPTL4 by the PPAR γ -specific ligand troglitazone was weaker in either case. In addition, *A. parvulum* and *P. copri* promote PPAR γ phosphorylation *via* ERK1/2^[91]. Studies confirmed that bacterial upregulation of PPAR γ in enteral epithelial cells occurs by phosphorylation^[96]. Though, high levels of *P. copri* and *A. parvulum* in the intestine has been connected to arthritis^[97] and linked to periodontitis^[98] accordingly.

However, the elevated risk of cardiovascular ischemic events is affiliated with the use of such PPAR γ ligands as rosiglitazone. PPAR γ agonists are used in clinics despite the fact that they have serious adverse effects, such as heart failure, weight gain, and increased bone fracture^[99]. At present, natural products could be found as a source of drugs^[100].

Fish oil has established favorable effects in diabetes, CVDs, autoimmune inflammatory diseases, and inflammatory bowel diseases. Neschen *et al.*^[101] revealed advantageous effects of fish oil on glucose and lipid metabolism, improvement of insulin sensitivity, and reduction of triglycerides. Additionally, the n-3 polyunsaturated fatty acids of fish oil, eicosapentaenoic acid and docosahexaenoic acid, are endogenous ligands for PPAR; consequently, even small changes in their structures affect PPAR activation. These fatty acids adjust the insulin-sensitizing, anti-inflammatory and lipid-lowering properties of fish oil^[102].

GUT MICROBIOTA AS A TREATMENT OPTION FOR T2D: FUTURE PERSPECTIVES

Over the past 10 yr, an increasing body of literature has suggested that the gut microbiota plays a crucial role in the host immune system, modulation of inflammatory processes, extraction of energy from the host diet, and alterations of human gene expression, and is considered to make an important impact on obesity/insulin resistance development. Several mechanisms that contribute to explaining the link between altered gut microbiota and pathogenesis of insulin resistance have been described^[7]. They control the fermentation and absorption of dietary polysaccharides to produce SCFAs, which may explain their importance in the regulation of fat accumulation. SCFAs can stimulate the secretion of GLP-1 and GLP-2, thus increasing insulin and adiponectin expression, which might contribute to enhanced insulin sensitivity and pancreatic β -cells proliferation^[103,104]. Another mechanism by which the microbiome may contribute to insulin resistance is compromised gut barrier function with an increased intestinal permeability, accumulation of lipopolysaccharide, and metabolic endotoxemia development^[105].

Lactobacillus and Bifidobacterium are commonly used as probiotics and are the most studied strains in the treatment and prevention of obesity-associated disorders^[15]. Moreover, several potential bacterial candidates, such as *Saccharomyces cerevisiae* var. *boulardii*, *Parabacteroides goldsteinii*, *Enterobacter halii* or *Akkermansia muciniphila*, have been identified and innovative mechanisms of action overriding their beneficial effects for insulin resistance/obesity have been elucidated^[106,107].

The abundance of *Akkermansia muciniphila*, which is a mucin-degrading bacterium

that resides in the mucus layer, has been found to be decreased in obese/T2D and inversely correlated with body weight in both rodents and humans^[34]. Metformin treatment^[35,36], consumption of oligofructose^[108], dietary concord grape polyphenols^[109], and gastric bypass surgery in humans^[110] and mice^[111] leads to a marked increase in *A. muciniphila* abundance with subsequent weight loss and reversed metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance^[34].

F. prausnitzii plays an important role in preserving the gut barrier and controlling inflammation and T2D progression^[112]. A traditional Chinese berberine-containing herbal formula given to T2D patients^[112,113] changed the gut microbiota by increasing *F. prausnitzii*, which was negatively correlated with fasting blood glucose, HbA1c and postprandial blood glucose levels, and positively correlated with homeostasis model assessment of β -cell function (commonly known as the HOMA-B).

Parabacteroides goldsteinii is a commensal bacterium with reduced level in HFD-fed mice. Oral treatment of HFD-fed mice with live *P. goldsteinii* reduced obesity and was found to be associated with increased adipose tissue thermogenesis, enhanced intestinal integrity, and reduced levels of inflammation and insulin resistance^[106].

Identifying the most important microbiota-related metabolic pathways could lead to the development of integrated strategies using new prebiotics or beneficial bacterial strains to prevent and treat these metabolic disorders in the near future^[112].

CONCLUSION

This review is dedicated to one of the most widespread diseases, diabetes, and the currently used antidiabetic drugs and possible promising new findings in this field. The gut microbiota has been found to have an influence on host metabolism, food consumption, satiety, glucose homeostasis, and weight gain. Altered intestinal microbiota composition has been noticed in CVDs, colon cancer, rheumatoid arthritis, diabetes, and obesity. Therefore, the main effect of antidiabetic drugs is thought to be on the microbiome composition, basically increasing the SCFA-producing bacteria responsible for losing weight and suppressing inflammation. Scientists have found that some drugs for T2D also elicit favorably effects on several cardiovascular end points and have anticancer as well as anti-aging effects. However, further detailed experimental and clinical investigations should be conducted to gain a deeper understanding of antidiabetic drugs' functions.

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Antidiabetic treatment on memory and spatial learning: From the pancreas to the neuron

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Abstract

The detrimental effects of constant hyperglycemia on neural function have been quantitatively and qualitatively evaluated in the setting of diabetes mellitus. Some of the hallmark features of diabetic encephalopathy (DE) are impaired synaptic adaptation and diminished spatial learning capacity. Chronic and progressive cognitive dysfunction, perpetuated by several positive feedback mechanisms in diabetic subjects, facilitates the development of early-onset dementia and Alzheimer's disease. Despite the numerous clinical manifestations of DE having been described in detail and their pathophysiological substrate having been elucidated in both type 1 and type 2 diabetes mellitus, an effective therapeutic approach is yet to be proposed. Therefore, the aim of this review is to summarize the growing body of evidence concerning the effect of current antidiabetic treatment options on diabetic and non-DE.

Key words: Memory; Spatial learning; Cognitive; Neural remodeling; Type 2 diabetes mellitus; Antidiabetic drugs

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Core tip: In this review, we aim to create a concise overview of the effects exerted by hyperglycemia on neural tissue, while describing the potential of each antidiabetic drug to improve functional and cognitive capacity in subjects with diabetic encephalopathy.

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INTRODUCTION

Diabetic encephalopathy (DE) is defined as a complex combination of central nervous system (CNS) structural and functional changes, stemming mostly from oxidative stress and chronic inflammation of the neural tissue in the setting of long-standing hyperglycemia. While several mechanisms have been proposed for the explanation of cognitive decline in diabetic subjects, the intricate interplay of various signaling pathways along with the numerous co-morbidities of patients with diabetes do not allow for a definite pathogenetic model to be proposed. Moreover, the pathophysiological substrate of type 2 diabetes mellitus (T2DM) encephalopathy appears to be different from that of T1DM DE^[1]. Currently, despite the abundance of evidence of the subject, the molecular mechanisms implicated in the development of DE and its rate of progression have not been clarified, resulting in a subsequent lack of treatment options for interruption or reversal of the cumulative neuronal damage and functional decline of patients. The purpose of our review is to summarize and describe the interaction between the various antidiabetic substances and DE, in order to facilitate the possible development of a therapeutic algorithm for affected patients.

ENCEPHALOPATHY IN T2DM

Several studies in T2DM subjects have confirmed the dysfunction of cognitive capacity, both in executive and processing tasks, when compared to healthy controls^[2-5]. While the decline of neural capacity in T2DM is described as a multifactorial process, it is evident that tissue insulin resistance (IR) plays a pivotal role in the pathogenetic process. Insulin receptors are expressed in all major components of the CNS (neurons, microglia, astrocytes, oligodendrocytes and vascular system) in varying degrees. The downstream effects of insulin signaling in neural tissue include neurogenesis, apoptosis inhibition, cytokine release, attenuation of inflammatory response, vasodilation and glucogen uptake and storage^[6]. While some researchers have proposed the possibility of de novo insulin synthesis in the CNS, current experimental data support the fact that the majority of centrally-acting hormone is produced at the pancreatic β -cells and subsequently transported through the blood brain barrier *via* the systemic circulation, with vascular endothelium significantly affecting the process^[7]. The role of other peripherally-acting hormones such as glucagon-like peptide-1 (GLP-1), leptin or ghrelin on insulin transport and potency in the CNS has not been described so far. IR, defined as a dysfunction on any of the several stages preceding or during the signaling cascade activated by the insulin-receptor complex formation, can affect the homeostasis of all the processes described above that are mediated by the hormone.

ANTIDIABETIC TREATMENT AND NEURAL FUNCTION

Biguanides

The information surrounding metformin and its effect on cognitive impairment is contradictory and highly complex, varying between different types of test subjects and changing in accordance to different treatment dosages and pathophysiological substrates studied. On a cellular level, metformin exhibits pleiotropic effects, including interaction with multiple signaling pathways such as those of mitogen-activated protein kinases (MAPK) and mammalian target of rapamycin complex 1, that are closely linked to proliferation and apoptosis. Given the relative safety of the substance and its role in cellular turnover, the possibility of repurposing it for use in neurofunctional disorders is currently being investigated^[8]. Chemical derivatives of metformin, such as HL271, induce comparable neuromodulatory effects, without any metabolic action, an indication that the drug effects may be only partially related to glucose homeostasis as is suggested in most of the experimental studies discussed on the following paragraphs^[9].

Ou *et al*^[10] designed an Alzheimer's disease (AD) model in an effort to elucidate the anti-neuroinflammatory properties of metformin. APPswe/PS1 Δ E9 mice underwent treatment with the biguanide, resulting into overall neuroprotective effects, with attenuation of spatial memory impairment, neural cellular proliferation, decreased local inflammation (both inflammatory cells and cytokines) of the brain cortex and the hippocampal region, as well as, reduced amyloid- β plaque deposition. The study results were attributed to drug-induced altered regulation of AMPK, mTOR, ribosomal protein S6 kinase, p65 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathways^[10].

Type 1 and 2 diabetes, induced in animal models through streptozotocin and high-fat diet respectively, have been linked to aberrant hippocampal neuroarchitecture with accompanying inflammation. Long-term metformin administration was shown to have a positive effect on hippocampal neural proliferation and memory function, despite the achieved hypoglycemic effect, a pathway mediated through interaction with insulin receptor substrate-1 *via* adenosine monophosphate (AMP) -kinase phosphorylation cascade activation^[11].

Following a similar pattern of beneficial neural effects, on a diabetic rodent model where both memory and spatial recognition were evaluated with passive avoidance tasks and Y maze spontaneous alternation tests, metformin administration appeared to reverse the diabetes-induced functional decline^[12]. Passive avoidance assesses the capacity of test subjects to avoid certain choices linked to painful stimuli, by use of their previous memory of similar situations, while the Y maze trial recruits several neural compartments and reviews the tendency of a subject for exploring new pathways, a process inherently linked to cognition. The treatment-mediated effects were attributed to numerous metabolic effects including achievement of normoglycemia, upregulation of vascular endothelial nitric oxide production, attenuation of oxidative damage and increased anti-apoptotic potential.

On a study including subjects with non-dementia vascular cognitive decline with impaired glucose homeostasis, the efficacy of donepezil when combined with either metformin or acarbose was evaluated as to the possible achievement of functional improvement. Carotid artery intima-media thickness (CA-IMT), cognitive capacity and IR were assessed at baseline and at 12 mo. The metformin-donepezil group showed superiority in the functional tests administered, a fact that can be attributed to the slower CA-IMT increase and decreased IR indexes when compared to the acarbose group, allowing for better neural tissue perfusion and metabolic signaling, respectively^[13].

One of the several pathogenetic mechanisms explored in relation to DE, among other neurodegenerative processes, is autophagy dysfunction, leading to tissue-accumulation of non-functional peptides, in the form of aggregates. Chen *et al*^[14] attempted to elucidate the effect of metformin administration on the regulation of misfolded polypeptide clearance, by treating diabetic mice with an eight-week regimen of intraperitoneal metformin and/or chloroquine. Neural capacity was evaluated by the Morris water maze (MWM) test, while the presence of aggregates or abnormal tissue architecture were examined by histological preparations and immunohistochemistry. Biguanide treatment had a positive overall effect with enhancement of autophagy, reduction of hyperphosphorylated tau proteins and improved cognitive functionality, when compared to the control group^[14].

Different treatment regimens comprising of metformin and ursolic acid combined or as monotherapy, as well as gliclazide were used by Mourya *et al*^[15] in rodents with metabolic and cognitive impairment due to chronic restraint stress (containment for 2 h/d for 30 d). A total of 60 subjects were subdivided into 10 groups according to treatment protocol, with several metabolic parameters relative to cardiovascular function and IRs were observed. Behavioral and neurological performances were assessed by the MWM test. While insulin sensitivity and cognition were improved in all treatment groups, the most marked anti-inflammatory and neuroprotective effects were produced by the combination of metformin with ursolic acid, suggesting the existence of a synergistic effect between the two^[15].

Metformin-induced neuromodulation has been studied on non-diabetic subjects as well, as is the case in the study of Fatemi *et al*^[16], including a population of ovariectomized mice as the treatment group. Post-surgical subjects presented with cognitive impairment, anxiety disorders and reduced brain-derived neurotrophic factor (BDNF). Treatment with metformin had beneficial effects on behavioral dysfunction and BDNF reduction on both of the treatment groups (Group A: 7 mg/kg and Group B: 15 mg/kg). Reinforcing the idea that the neuroprotective effects of the biguanide class are not solely the result of metabolic normalization of glucose homeostasis^[16].

As opposed to the aforementioned studies, Wennberg *et al*^[17] found no correlation between metformin or other anti-diabetic treatments and cognitive capacity. T2DM subjects with lack of functional impairment at baseline ($n = 508$) were followed-up for a mean duration of 3.7 years. Mild cognitive impairment (MCI) diagnosis was defined as difference equal to or greater than 1 standard deviation than the age-specific mean score of the general population on each test administered. The study population was divided into 4 groups according to treatment type as following: insulin monotherapy, metformin monotherapy, other oral agents as monotherapy or diet and exercise without pharmacological intervention. A universal lack of positive effect on cognition was observed among all groups, with patients on metformin treatment having higher rates of MCI diagnosis at follow-up. The latter was attributed to vitamin B12

reduction secondary to long-standing metformin administration. While the results are validated by the size of the study population and the numerous validated cognitive tests performed, notable limitations such as partial correction of treatment group differences despite covariate consideration and propensity score utilization and lack of B12 measurement should be taken into consideration when evaluating the research conclusions^[17].

Correspondingly, a study conducted on C57BL/6 mice of different age groups yielded neutral results concerning the effect of metformin on metabolic parameters while a negative, age-dependent, impact was observed on both spatial memory and visual acuity of the test subjects. Treatment regimen comprised of 2 mg metformin /mL of drinking water, which is analogous to a human dose of 1500-2000 mg/d (when converted in a body-weight dependent manner), for three months^[18]. While, the contradicting results could be partially attributed to the short study duration there is further research with similar conclusions, in which metformin attenuated memory dysfunction in female subjects and amplified it in males, on an experimental model of AD^[19].

The relationship between the class of biguanides and functional neural capacity remains unclear due to several relevant research projects with controversial results. At the same time, the underlying pathophysiological mechanisms by which metformin exerts its effects on neural tissue have not been, as of yet, entirely elucidated. While there appears to be a positive predilection towards the exploration of metformin administration as a form of neuroprotection, mainly due to its potency in altering a multitude of signaling pathways in the cell cycle, further research is needed in order to clarify whether it is truly efficacious in the clinical setting on patients with diabetes-induced cognitive decline.

Alpha-glucosidase inhibitors

Some of the main representors of the class of alpha-glucosidase inhibitors (α -GIs) are acarbose, miglitol and voglibose. Yan *et al*^[20] administered acarbose to SAMP8 mice for a period of 6 mo. The study population was divided into 3 groups, including the acarbose group ($n = 9$, 9-mo old), young ($n = 11$, 3-mo old) and old controls ($n = 8$, 9-mo old). An age-dependent cognitive decline was observed when the control groups were compared, while the acarbose group showed attenuation of this decline, accompanied by higher levels of insulin, insulin receptors and acetylated histone H4 lysine 8 (H4K8ac). The altered functional phenotype of the acarbose group (less memory impairment, improved spatial recognition) was attributed to both the changes in the concentration of insulin and its receptor and the H4K8ac increase. Higher levels of the latter have been linked to ameliorated long-term memory formation^[20].

Since the data concerning the neurological effect of α -GIs is scarce, with no relevant research including miglitol or voglibose, safe conclusions cannot be currently drawn for their possible actions on neural tissue.

Sulphonylureas

As far as the class of sulphonylureas (SUs) is concerned, there appears to be a lack of relevant clinical studies discussing their effects on the homeostatic regulation of the nervous system. Given their mode of action, through binding on adenosine triphosphate-sensitive potassium channels and the subsequent activation of voltage-gated calcium channels, their possible use for inducing and regulating neuroexcitatory potentials is an interesting perspective. Currently available research discussing the role of SUs in the setting of cognitive decline is centered on the use of glimepiride and glibenclamide.

Ishola *et al*^[21] administered glimepiride on a rodent model of paraquat-induced Parkinsonism with subsequent functional and molecular assessment of the treatment-induced changes. Sulphonylurea treatment attenuated oxidative stress and activation of inflammatory cascades in the neural tissue, while, simultaneously, improving the paraquat-induced memory dysfunction and cognitive performance on the rotarod, open field and Y-maze trials^[21].

Glibenclamide has been shown to exert long-term protective properties on the hippocampal cortex in the setting of traumatic brain injury (TBI)^[22]. Moreover, the aforementioned exerted a beneficial effect when used on an experimental AD model, *via* regulating the activity of the hypothalamic-pituitary-adrenal axis and alleviating AD-related mood-disorders^[23].

Thiazolidinediones

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor (PPAR) agonists, also widely known as glitazones, have been established to interact with the cell cycle and inflammatory cascade.

Pioglitazone was administered as monotherapy and in combination with simvastatin, on a model of lipopolysaccharide (LPS)-induced cognitive dysfunction secondary to amyloid deposition and inflammation. While LPS exacerbated neural oxidative stress, amyloid A β deposition, glutamate tissue-levels and memory impairment, both simvastatin and pioglitazone mitigated the changes. The subjects performance on both the neurobehavioral tests chosen (Y-maze and novel object recognition) did not differ significantly between the combination therapy or the monotherapy group for each treatment alone, a fact possibly explained by both the substances exerting their anti-inflammatory properties on the same pathway of NF- κ B signaling^[24]. In a different study, pioglitazone was administered on subjects with LPS-induced febrile seizures and subsequent memory deficits. On the treatment groups, proinflammatory markers, such as tumor necrosis factor alpha (TNF- α) and interleukine-1 β (IL-1 β), along with oxidative stress were reduced in the hippocampal neural tissue, with accompanying partial resolution of memory impairment and cognitive dysfunction^[25]. Moreover, a meta-analysis performed by Cao *et al*^[26], on the efficacy and tolerance of antidiabetic treatment as adjunct therapy on AD indicated that pioglitazone (15 to 30 mg) was the most beneficial agent (when compared to placebo) in improving cognitive capacity.

Kushwaha *et al*^[27] have indicated the existence of a rosiglitazone-induced anti-apoptotic effect on cerebral cortical tissue of high-fat-diet diabetic mice, for which the underlying mechanisms have not been clearly established. PPAR- γ mediated epidermal growth factor signaling appears to be the most probable pathway by which both glial and neural cells are affected. In a similar fashion, on a model of spontaneously hypertensive rats with consequent brain damage, rosiglitazone exerted a neuroprotective effect by mediating oxidative stress and affecting the levels of apoptotic cellular pathway mediators, independent of blood pressure correction^[28].

Although the anti-apoptotic effects of TZDs on neural tissue are both supported by their mode of action and have been recreated in the experimental setting, there is a current lack of clinical correlation with the molecular findings. In order to establish the possible treatment benefits of this class in DE or other neuropathologic states, there is a definite need for further studying the performance of TZD-treated subjects on functional tests assessing both cognitive capacity and memory impairment.

Incretins

The two antidiabetic drug classes acting on the metabolic pathway of incretin hormones are glucagon-like peptide-1 receptor agonists (GLP-1 RA) and dipeptidyl peptidase-4 inhibitors (DPP-4i). GLP-1 is a hormone with multiple effects in the gut, pancreas and neural tissues, affecting processes such as gastric motility, appetite, insulin and glucagon secretion, while DPP-4 is the enzyme that deactivates it.

DPP-4 inhibitors

Sitagliptin, vilaglipitin, saxagliptin, linagliptin and alogliptin are the current DPP-4is being used for treatment of T2DM^[29].

APP/PS1 mice having been treated with sitagliptin (20 mg/kg for an 8-wk period) underwent neurofunctional assessment with the MWM test. The treatment group presented with ameliorated functional potential attributed to upregulation of BDNF and activation of tyrosine receptor kinase B (TrkB) signaling^[30]. Through similar mechanisms of BDNF and tyrosine hydroxylase upregulation, sitagliptin administered on a model of Parkinson's disease moderated memory deficits, in addition to cellular density increase of dendritic spines in the CA1 region of the hippocampus^[31]. Male Wistar rats with cisplatin-induced neurotoxicity further confirmed the neuroprotective effect of sitagliptin on both the molecular level and motor-cognitive performance, accredited to attenuation of drug-induced cerebellar damage^[32].

As far as vildagliptin is concerned, upon administration in an Alzheimer's experimental model, the substance exhibited anti-apoptotic action in the hippocampal tissue with accompanying attenuation of memory deficits, changes associated with reduced tau phosphorylation and increased expression of neurotrophic proteins. An important mediator pathway and possible treatment target, identified in the above study, was that of phosphorylated protein kinase B/p-glycogen synthase kinase 3 β (Akt/GSK3 β)^[33]. The exact same treatment signature was observed when vildagliptin was used on a model of streptozotocin-induced T2DM with diabetes-related cognitive decline^[34]. Fibroblast Growth Factor 21 (FGF21) has shown superiority when compared to vildagliptin with the study therapeutic end-points being improvement of metabolic function and neuroprotection. Despite both the substances having insulin-sensitizing, anti-apoptotic, mitochondrial and cognition-sparing properties, they differed on several other measurements. FGF21 was a more potent regulator of metabolic parameters and synaptic plasticity in the hippocampus^[35].

Saxagliptin (0.25/0.5/1 mg/kg for 60 ds) has shown neuroprotective properties on streptozotocin-induced AD rats by increase of hippocampal GLP-1 levels, decrease of amyloid plaque formation and deposition^[36]. A slightly different rat model of AD disease, with cognitive deficits produced by D-galactose treatment, was used as grounds for comparing the efficacy of saxagliptin and metformin on learning and memory impairment secondary to aberrant insulin signalling. Several parameters on the MWM test were improved by antidiabetic treatment, along with oxidative biomarkers, tau phosphorylation products being normalized and insulin levels dropping with concurrent insulin receptor elevation^[37]. On the contrary, saxagliptin in an experimental model of Parkinson's (produced by 6-hydroxydopamine administration) showed no cognitive- or motor-sparing properties but produced an interesting functional deterioration in the sham group, deeming it a possible candidate as post-traumatic stress disorder adjunct treatment^[38].

Similar to other members of the DPP-4i class, linagliptin treatment has a beneficial role in ameliorating the progression of neural dysfunction on models of AD disease *via* numerous mechanisms such as amyloid plaque clearance, down-regulation of tau hyperphosphorylation, reduction of oxidative stress and mitochondrial dysfunction^[39-41]. In T2DM test subjects, the neuroprotective attributes of the substances have been linked to changes in cerebral perfusion. In one study, linagliptin treatment post-carotid inclusion related transient cerebral ischemia attenuated cerebral damage unrelated to glucose homeostatic regulation, by mediating oxidative stress and blood brain barrier permeability^[42]. Further, Hardigan *et al*^[43] studied the effects of a 4-wk treatment regimen with linagliptin on vascular remodeling and flow properties of the middle cerebral arteries with beneficial effects being observed on the treatment group. The neuromodulatory role of linagliptin when compared to glimepiride is being studied by use of a composite 3-trial score (Mini-Mental State Examination, Trail Making Test, Verbal Fluency Test) in the cognition sub-study of double-blind, randomized Cardiovascular Safety of Linagliptin (CAROLINA) trial, including 4335 participants with T2DM^[44].

Much like linagliptin, alogliptin has been shown to exert an effect on the architectural and functional integrity of cerebral vasculature. In a mice model of middle cerebral artery occlusion, the treatment group mediated the results of tissue ischemia and restored the defects of the blood brain barrier *via* altering the expression patterns of metalloproteinases and their inhibitors along with occludin and zona occludens-1 proteins^[45]. On another model of diabetic nephropathy with silent cerebral infarcts, the combination of alogliptin and hyperbaric oxygen treatment had a beneficial restorative effect on neural function^[46]. Additionally, on high-fat fed doubly-negative apolipoprotein E mice with resultant cognitive decline, alogliptin up-regulated BDNF and calcineurin hippocampal production with accompanying higher performance on MWM and novel object recognition test than controls^[47].

GLP-1 receptor agonists

The currently approved GLP-1 agonists are exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide and semaglutide.

In three studies (two included subjects with AD and one with T2DM) where the long-lasting GLP-1 analogue exenatide was used, restored BDNF signaling resulted into improved neurocognitive capacity by inhibiting neural apoptosis, in a manner analogous to that discussed on previous segments^[48-50]. Bader *et al*^[51] used a sustained-release preparation of the substance, named PT302, in order to study the role of exenatide treatment in TBI. Subjects in the treatment group presented with a down-regulation of pro-inflammatory markers in the neural tissue, prolonged cellular survival and reversal of functional impairment^[51]. Similarly, on the topic of TBI, Rachmany *et al*^[52] administered exenatide on a similar study population of mice with mild TBI, measuring both the neurofunctional changes and levels of synaptophysin (a biomarker for the viability of presynaptic neurons), pre- and post-trauma, with treatment ultimately attenuating the effects of the injury. Other changes following exenatide treatment include remodeling of hippocampal tissue architecture and diabetes-related deficits reversal, reduction of cortical TNF- α levels, preservation of brain choline acetyltransferase activity and improved amyloid oligomer clearance with subsequent decreased deposition^[53,54]. A novel dual incretin agonist with combined gastric intestinal peptide and GLP-1 activity, the latter in the form of exenatide, has shown similar neuroprotective actions like memory refinement and hippocampal neurogenesis and synaptic remodeling along with a positive metabolic profile^[55].

While liraglutide has been shown to effectively attenuate memory and functional deficits in subjects with various AD or similar pathology patterns in neural tissue, through mediating tau hyperphosphorylation and amyloid deposition^[56-58], contradicting research does exist, in which 12-wk liraglutide treatment was not

superior in cognitive function improvement when compared to placebo^[59]. In the setting of cognitive decline following mood disorders, the GLP-1 RA improved performance in the Trail Making Test-B and composite Z-score of several neuropsychiatric scales measured, a change attributed to IR attenuation and other metabolic parameter modification^[60]. Post-treatment behavioral normalization was also noted in a study by Koshal *et al.*^[61] including mice manifesting with depression secondary to seizure activity. Some of the other changes in the treatment group were the reduction of oxidative stress and seizure activity^[61]. Cognitive-deficient rodents with T2DM treated with liraglutide presented with ameliorated functional potential as a result of activation and modification of downstream signaling pathways of AMPK, mTOR and phosphoinositide 3-kinase (PI3K)^[62]. The involvement of the mTOR pathway in the neuroprotective action of liraglutide was further confirmed in a study of streptozotocin-induced T2DM^[63].

In a manner similar to other GLP-1 RAs, a pattern of reduced proinflammatory mediators and increased amyloid plaque clearance in APP/PS1/tau mice models of AD is observed with the administration of both lixisenatide and dulaglutide, resulting in improved neurocognitive potential. The pathways involved include those of p38-MAPK, protein kinase A and Akt/PI3K^[64-66]. Both the neuroprotective attributes of semaglutide and its superiority to liraglutide in improving cognition have been observed in mice models of Parkinson's disease caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine^[67,68].

SGLT-2 inhibitors

Sodium-glucose cotransporter 2 (SGLT2) inhibitors exert their actions on several tissue types, with their potency as antidiabetic substances stemming from their ability to hinder renal glucose reabsorption in the proximal tubule of the nephron. Members of this class currently in use are canagliflozin, dapagliflozin, empagliflozin, ertugliflozin, ipragliflozin, luseogliflozin, tofogliflozin with sotagliflozin, a dual SGLT1/SGLT2 inhibitor, in phase III clinical trials. The relationship between neural functional capacity and memory integrity and SGLT2 inhibition has been explored in studies utilizing canagliflozin, dapagliflozin and empagliflozin.

Arafa *et al.*^[69] studied the effects of canagliflozin treatment on memory dysfunction secondary to scopolamine administration. As an end-result of SGLT2 inhibitor treatment, neural tissue monoamine and acetylcholine levels were increased with M1 receptor activity, a biochemical shift culminating into improved cognitive function on MWM and Y maze trials^[69]. Similar patterns of altered acetylcholine signaling post-canagliflozin treatment were described on a similar study with diabetic rodents that included a metformin treatment group as well^[70].

Dapagliflozin both as monotherapy and in combination with liraglutide has shown beneficial effects on memory and cognition, following remodeling of neural tissue with increased expression of doublecortin and synaptophysin (biomarkers of neural proliferation and synaptic formation respectively), as well as reduced IR^[71].

The effect of empagliflozin on cognitive function was documented in the study by Lin *et al.*^[72], after a 9-wk regimen on db/db mice. Assessment with the MWM test and immunohistological examination of cortical tissue was subsequently performed. The cognitive function of the treatment group was superior to that of age-matched controls, with concurrent attenuation of oxidative stress and increased BDNF levels^[72].

Given the relative lack of data for this antidiabetic class, combined with the fact that the possible mediating mechanisms, either direct molecular or indirect *via* modification of hemodynamic parameters, for their action on neural tissue have not been elucidated as of yet, there is definite need for further research on the subject.

Insulin

Neural tissue IR is an important substrate for the cognitive decline observed on diabetic subjects, especially in the hippocampal region. Numerous architectural and molecular changes fuel the pathologic process, including increased amounts of oxidative stress, activation of inflammatory cascades, peptide formation and aberrant deposition, commonly in the form of amyloid, as well as dysregulation of the hypothalamic-pituitary-adrenal axis^[73]. As would be expected, since the basis of diminished functional capacity in T2DM is formed on the existence of IR, treatment with insulin, in many forms, has proven to be beneficial in ameliorating the relevant pathophysiological alterations.

Several studies have emerged, exploring the use of insulin *via* intranasal delivery, so as to bypass the blood brain barrier. This route allows for rapid achievement of therapeutic concentrations in the target tissue and treatment effectiveness, with accompanying cognitive improvement post-therapy^[74-76]. Some of the proposed mechanisms for explaining the attenuation of neurofunctional deterioration caused by T2DM include altered activation of electrolyte channels (mostly calcium-related),

neuropeptide expression pattern differentiation, increased clearance of peptides (hyperphosphorylated tau and A β) that deposit as neurofilaments, synaptic remodeling and activation, upregulation of N-methyl-D-aspartate receptors turnover and improvement of hemodynamic parameters such as neural tissue perfusion^[75].

On a study performed by Maimaiti *et al*^[75], short-acting insulin lispro (Humalog) and long-acting insulin detemir (Levemir) were administered intranasally on a rat model of age-related mental impairment. Both the long- and short-acting compounds were equally effective in improving memory recall, matching the performance of aged members in the treatment group, to that of young rodents in the control group^[75]. Slightly different results came from the study of Benedict *et al*^[77] where despite both regular and fast-acting insulin improving cognition when compared to the control group, the short-acting insulin aspart was more efficient than regular insulin in memory recall testing.

In a different research project, long-acting insulin analogs (glargine, detemir, degludec) were compared to regular insulin by use on cultured cortical neurons of rodents. Glargine, detemir and regular upregulated cortical BDNF, and activation of the Akt signaling cascade, with degludec having marginally inferior efficacy. Furthermore, regular and glargine ameliorated memory and cognition (as estimated by performance on the Y maze), showing superiority over detemir^[78].

Finally, many of the physiological actions of insulin in the neural system are mediated by insulin-like growth factor-1 (IGF-1) receptors. Due to the aforementioned, similarly to insulin, use of neurostimulating factors with analogous activity on target tissues, such as IGF-1 has yielded promising results in the setting of neural proliferation and damage recovery post-trauma^[79,80], neurodevelopmental disorders^[81], neurovascular dysfunction^[82] and IR^[83].

Research data pertaining the use of insulin in the setting of cognitive decline, confirm the relationship between IR and mental deterioration, a state reversible by treatment with insulin or insulin-sensitizers. Further research could provide insight on the appropriate insulin delivery methods for achieving maximum therapeutic concentrations and treatment efficacy while minimizing risk, so as to fully utilize the potential of this therapeutic approach for diabetic and non-DE. A brief table containing all the aforementioned cognitive capacity experimental tests used on rodents is provided below (Table 1).

CONCLUSION

DE is term describing a multifactorial state of neural dysfunction resulting from T2DM and its hallmark, IR. Current antidiabetic regimens appear to have a beneficial effect on cognitive decline and memory impairment secondary to diabetes and other causes. Most of the research data on the subject derives from studies on metformin, TZDs and incretins, with further elucidation being required for the role and mechanisms of sodium-glucose cotransporter inhibition on neural functionality. As has been shown by the intranasal delivery of insulin, the development of vectors allowing for direct access to the CNS without inhibition from the blood-brain-barrier could open up some very interesting perspectives for repurposing the antidiabetic therapy as means to effectively treat mental dysregulation states. Moreover, the extensive elucidation of the underlying pathophysiology allowing for oral antidiabetic medication to affect neural functionality could provide insight on the reasons behind cognitive impairment in T2DM, while also allowing for formulation of proper guidelines for hinderance of its development and ultimately, treatment.

Table 1 Experimental trials for the evaluation of cognitive capacity and memory impairment on rodent study populations

Type of test	Method of operation
Y-Maze Spontaneous Alternation	Evaluates willingness of subjects to explore new paths on a 3-arm structure with each pathway angled at 120°
Morris Water Maze	Evaluates spatial and long-term memory by testing the escape capacity and velocity of subjects on a water tank
Passive avoidance	Evaluates learning and memory integrity by introducing aversive stimuli
Rotarod	Evaluates balance and motor coordination by assessing the ability of the subject to remain standing on a rotating cylinder
Open field	Evaluates willingness of subjects to explore new paths, anxiety and motor coordination though observing the subject's movement patterns on a walled-off area
Novel object recognition	Evaluates recognition memory though habituation of test subjects with novel objects and subsequent evaluation of their capacity to discriminate between familiar and unfamiliar.

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Case Control Study

Screening the RFX6-DNA binding domain for potential genetic variants in patients with type 2 diabetes

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statement: The study was ethically approved by the IRB board of Jordan University Hospital (JUH) No. 10-2017-1737, Decision No. 2017-134. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee as well as the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent statement:

Informed consents were obtained from human participants in this

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Abstract

BACKGROUND

The regulatory factor X6 (RFX6), a member of regulatory factor X family, is known to play a key role in the development and differentiation of pancreatic beta cells as well as insulin production and secretion. However, the potential role of RFX6 in type 2 diabetes (T2D) is still unclear.

AIM

Recent studies have indicated that RFX6 binding to DNA could be disrupted in diabetes. Therefore, in this study we investigated whether genetic mutations are present in the DNA binding domain of RFX6 gene that could abrogate its function in T2D.

METHODS

A cohort of T2D patients was enrolled in this study, and the gene encoding the DNA binding domain of RFX6 was amplified by polymerase chain reaction and then analysed by direct DNA sequencing.

RESULTS

The DNA sequence analysis revealed the absence of any exonic mutation. However, we have identified a new heterozygous single nucleotide polymorphism (IVS6+31 C>T) in the intronic region of DNA binding domain gene that is present in 9.2% and 8.5% of diabetic and control people, respectively ($P = 0.97$).

research.

Conflict-of-interest statement: The authors declare no conflict of interest.

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CONCLUSION

We report the absence of any significant genetic variant that could affect the function of RFX6-DNA binding domain in T2D.

Key words: Regulatory factor X6; Genetic variant; Diabetes; DNA binding domain

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Core tip: Regulatory factor X6 (RFX6) protein plays a key role in the differentiation of pancreatic beta cells as well as insulin production and secretion. Several lines of evidence have indicated that RFX6 binding to DNA could be disrupted in diabetes; however, the mechanism underlying this process is still unknown. In this case-control study, we analysed the genotype of RFX6-DNA binding domain in diabetes patients in comparison to healthy controls. Our results indicate the absence of any significant genetic variant in the DNA binding domain that could affect the function of RFX6 in type 2 diabetes.

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INTRODUCTION

Diabetes mellitus is a group of glucose metabolism disorders characterized by high levels of blood glucose. The disease affects millions of people worldwide, which is usually associated with serious complications that affect various systems of human body^[1]. Type 2 diabetes (T2D) is mainly manifested by low insulin production by pancreatic cells and/or the produced insulin does not function effectively^[2]. Alterations in both beta cells average mass and function have been observed and reported in people with diabetes^[3-5].

Regulatory factor X (RFX) proteins constitute a family of DNA binding proteins that is conserved in the eukaryotic kingdom^[6]. In humans, RFXs act as regulatory transcription factors that bind to a conserved *cis*-regulatory element called the X-box motif, which is typically 14-mer DNA sequences located in specific promoter regions of the genome^[7]. The function of mammalian RFX proteins has only recently started to emerge, and has shown to play an important role in regulating growth and development, immune response and endocrine secretions^[7]. The RFX family has seven members of RFX1-7 in mammals, which have wide expression in various tissues and organs. RFX6 is a main member of RFX family that is predominantly expressed in pancreatic islets and encoded by a gene on chromosome 6^[7]. RFX6 possesses a highly conserved DNA binding domain which is critical for binding of RFX6 to X-box promoter motifs and thus regulating their function. Recent studies conducted in mice demonstrated that RFX6 is specifically required for pancreatic beta cells differentiation during embryonic development^[8]. Moreover, RFX6 was shown to be an important factor to maintain key features of functionality of mature beta cells, and RFX6 gene deletion in adult mice beta cells was shown to disrupt glucose homeostasis and caused glucose intolerance, impaired beta cell glucose sensing and defective insulin secretion^[9].

In 2017, Varshney *et al*^[10] published an interesting study in the Proceedings of the National Academy of Sciences, where they performed an integrated analysis of molecular profiling data of the genomic DNA, epigenome and transcriptome in diabetic pancreatic beta islets, to understand the potential connections between genetic variants, chromatin landscape, and gene expression in T2D. The study showed that most of the reported genetic variants in T2D are enriched in regions of the DNA where RFX transcription factors are predicted to bind. The study also concluded that these genetic variants that increased the risk of T2D are predicted to disrupt mainly the binding of RFX6 to genomic DNA^[10], indicating that RFX6 binding to X-box promoter motifs could be disrupted in T2D.

In this study, we sought to investigate if any structural genetic defects could be

present in the RFX6-DNA binding domain in T2D patients that could potentially inhibit its function in diabetes.

MATERIALS AND METHODS

Patient and control samples

Initially, a total of 98 blood samples (49 samples from T2D patients, 49 from healthy volunteers (control group)) were collected from Jordanian population (Table 1). The study was then extended to investigate the association between the identified intronic variant (IVS6+31 C>T) and diabetes. A total of 283 blood samples (141 from T2D patients, 142 from healthy volunteers) were included in the extended study (Table 1). Diabetic participants who enrolled in this study were Jordanian adults (age \geq 20 years), including both females and males, with known history of diabetes and recruited from Jordanian medical centres during the time period between Dec 2015 and July 2017. Controls were unrelated to diabetic patients and had no history of diabetes, as determined by history and lab examination. All blood samples were collected according to protocols approved by the Institutional Review Board, and informed consents were obtained from participants included in the study.

Biochemical examination

The levels of blood glucose and glycosylated Hb (HbA1c) were evaluated in the participants of this study. Blood glucose level was measured by the glucose oxidase method using Cobas c111 analyzer (Switzerland). The percentage of HbA1c in blood was determined using ion-exchange high-performance liquid chromatography (D-10™ Bio Rad, United States).

Molecular techniques

For molecular assays, DNA was extracted from venous whole-blood samples, and then collected in EDTA containing tubes, using Wizard genomic DNA purification kit (Promega, United States). Extracted DNA was stored at -20°C . Polymerase chain reaction (PCR) was used to amplify genomic DNA encompassing the coding sequences and intronic borders of exons 3, 4, 5 and 6 of the *RFX6* gene (NCBI: NG_027699.1). The primers used for PCR assay were as described in (Table 2). The PCR amplification reactions were performed in a total volume of 25 μL in 0.2 mL PCR tubes containing 200 ng of genomic DNA, 5 μL of 5 \times FIREPol® Master Mix (Solis BioDyne) with 7.5 mmol MgCl_2 and 10 pmol of each primer (Gene Link, United States). All PCR reactions were performed using C1000 Touch™ Thermal Cycler (Bio-Rad; United Kingdom) and the reaction conditions were as follows: initial denaturation of 5 min at 95°C , followed by 29 cycles of 30 s at 95°C , 30 s at specific annealing temperature (Table 2), 1 min at 72°C and a final extension of 6 min at 72°C . All PCR products were checked by gel electrophoresis to verify correct product size, then purified and sequenced using the same forward primer used for the gene amplification. DNA sequencing reactions were performed at Macrogen Inc., South Korea, using BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, United States) and the ABI PRISM 3730XL analyzer (Applied Biosystem, United States).

Statistics

Data analyses were performed using IBM Statistical Package for the Social Sciences software (SPSS, version 19). Pearson's chi-squared or Fisher's exact tests were used to test an association between categorical variables (*i.e.*, gender and genetic variant) and case-control status. Student t-test was used to assess the significance of difference of means of age, blood glucose and glycosylated Hb between case and control groups. A 95% confidence interval for the odds ratio was calculated and used to describe the results. For sample size calculation, the Cochran's formula was used^[11].

RESULTS

In this study, the screening for structural genetic variants in specific regions of *RFX6* gene was carried out in T2D patients in attempt to discover new potential mutations that could mediate the pathogenesis of T2D. Evaluation of levels of blood glucose and HbA1c of enrolled subjects was performed to discriminate between diabetic and non-diabetic (Table 1). The DNA sequence analysis of exons 3, 4, 5 and 6 of *RFX6* gene in 49 diabetic patients revealed the absence of any genetic mutation. However, the DNA sequencing of introns borders revealed the presence of a heterozygous genetic variant

Table 1 Demographic and clinical characteristics of diabetic patients and healthy controls

	Control group	Diabetic patients	P-value
Primary screening, (n = 98)			
Male, n (%)	32 (65%)	24 (49%)	0.285
Age (mean ± SD)	50.1 ± 12.3	54.7 ± 10.7	0.149
Female, n (%)	17 (35%)	25 (51%)	0.217
Age (mean ± SD)	47.7 ± 15.5	56.2 ± 13.2	0.065
FBG (mg/dL)	97 ± 10	192 ± 94	< 0.001
HbA1c (%)	5.4 ± 0.4	8.2 ± 2.2	< 0.001
Screening the IVS6+31 C>T, (n = 283)			
Male, n (%)	78 (55%)	59 (42%)	0.105
Age (mean ± SD)	48.9 ± 15.2	57.4 ± 11.3	0.001
Female, n (%)	64 (45%)	82 (58%)	0.136
Age (mean ± SD)	51.8 ± 15.2	57.1 ± 11.5	0.024
FBG (mg/dL)	98 ± 10.4	177 ± 80	< 0.001
HbA1c (%)	5.4 ± 0.5	7.8 ± 1.9	< 0.001

P-value is significant at ≤ 0.05 . FBG: Fasting blood glucose; HbA1c: Glycosylated haemoglobin.

(IVS6+31 C>T) within the intron 6 of *RFX6* gene, where C is substituted by T, 31 nucleotides downstream of the end of exon 6 (Figure 1). To investigate the significance of the identified intronic variant in T2D, the study was extended and the IVS6+31 C>T was screened in 283 samples (141 diabetic and 142 healthy controls). DNA sequence analysis of the samples revealed the identification of the heterozygous IVS6+31 C>T in 9.2% and 8.4% of diabetic and control groups respectively, with no significant association in genotype or allele frequency between diabetic and control groups (Table 3).

The demographic characteristics of the study population (age and gender) are shown in Table 1. In primary screening study, there was no statistically significant difference in male/female proportion ($P = 0.285$ and $P = 0.217$, respectively) or age of gender ($P = 0.149$ and $P = 0.065$, respectively) between case and control groups. As for the extended screening study of IVS6+31 C>T variant, no statistically significant difference in male/female proportion was found between case and control groups ($P = 0.105$ and $P = 0.136$, respectively). However, there was a significant difference in age of male/female of case-control status ($P = 0.001$ and $P = 0.024$, respectively).

DISCUSSION

Recently, it has been proposed that a loss of pancreatic beta-cells mass, differentiation and function is a hallmark of T2D^[1,2]. Concurrently, the regulation of beta cells growth and differentiation has been under intensive investigation, and several lines of evidences have indicated the role of key transcription factors in controlling the function state of beta cells. For example, evidences coming from loss-of-function studies in adult mice beta cells have revealed that transcription factors such as NeuroD1^[12], Nkx6.1^[13] and Pdx1^[14] are important in maintaining the differentiation and function state of pancreatic beta cells. Thus, it appears that loss of function of key beta cell transcription factors results in the loss of both beta cell identity and function. More recently, RFX6 transcription factor has been shown to play a key role in regulating the state of pancreatic beta cells differentiation and function^[9].

RFX6 contains a highly conserved DNA binding domain that facilitates their binding to X-box promoter motif of certain genes, which is essential to regulate the transcription of *RFX6*-target genes^[7]. It has been shown that genetic alterations in the RFX6-DNA binding domain could be associated with neonatal diabetes. In fact, mutations in the RFX6-DNA binding domain are assumed to be the cause of neonatal diabetes in Mitchell-Riley syndrome, through the production of a defective RFX6 protein^[15]. In this project, we sought to detect if any genetic mutation could be present in the RFX6-DNA binding domain in T2D. Based on our findings we conclude that structural mutations in the DNA binding domain of RFX6 are unlikely to exist in T2D. However, another large-scale study could increase the statistical power of our results. In addition, it is noteworthy to mention that RFX6 proteins contain other conserved

Table 2 Primer sequences used in DNA amplification of DNA binding domain sequence of *RFX6* gene

Primer	Sequence (5'-3')	Ta (°C)	Size (bp)
RFX6-3	F: 5- CTT ATG TCT ACT CAT TAC CTC -3	50	306
	R: 5- TCA TGC TAT CTG CCT GAC -3		
RFX6-4	F: 5- CAG TTC ATT CAG AGT TCA AC -3	56	216
	R: 5- CTT CAT GCA CAA GAG CAG -3		
RFX6-5	F: 5- GTC ATC AGG GTT TGC AGT TC -3	50	258
	R: 5- ATT CAA TAG GTA TCA TGC -3		
RFX6-6	F: 5- GTA AGT TGA GAA AGA TGC -3	56	258
	R: 5- CAT GTA TTG CTC AGC TTG -3		

Ta indicates annealing temperature.

regions including B, C, and D domains^[6]. These domains are thought to be involved in RFX6 oligomerization which are required for DNA binding and activation^[7]. Therefore, screening the other functional domains of RFX6 may provide more insights into the potential mechanism by which RFX6 binding to DNA is abrogated in diabetes.

Table 3 Association between the variant IVS6+31 C>T investigated in *RFX6* gene and type 2 diabetes risk

	Control, n (%)	T2D, n (%)	P-value	OR	95%CI
genotype					
C \ C	130 (91.5)	128 (90.8)	0.97	0.9	0.39-2.06
C \ T	12 (8.5)	13 (9.2)		1.1	0.48-2.50
T \ T	0	0		1	0.02-51.1
Allele					
C	272 (95.7)	269 (95.3)	0.82	0.9	0.41-2.03
T	12 (4.3)	13 (4.7)		1.1	0.49-2.44

P-value is significant at ≤ 0.05 . OR: ODDs Ratio; CI: Confidence Interval; T2D: Type 2 diabetes.

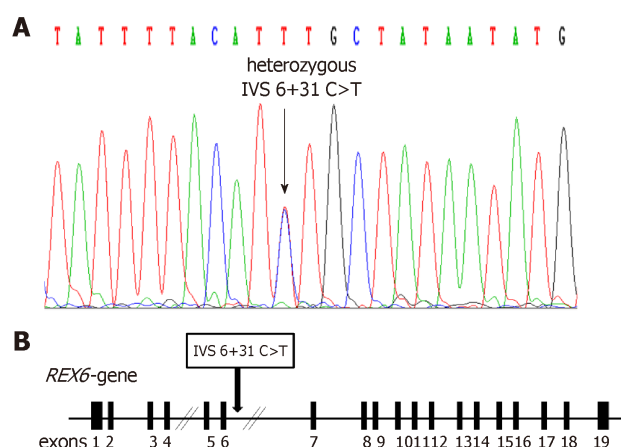


Figure 1 Regulatory factor X6 intronic genetic variant. A: The heterozygous IVS6+31 C>T as determined by automated DNA sequencing analysis; B: *RFX6* gene structure and the location of the IVS6+31 C>T genetic variant (as indicated by the black arrow).

ARTICLE HIGHLIGHTS

Research background

Diabetes mellitus is a global health challenge, which is usually associated with the loss/dysfunction of insulin-producing pancreatic beta cells. Hence, understanding the molecular mechanisms that control beta cells differentiation and function represents a major interest in the medical field. Regulatory factor X6 (RFX6) is DNA binding protein that is predominantly expressed in pancreatic islets of human and plays a key role in regulating pancreatic beta cells differentiation and insulin production, and it has been recently. RFX6 contains a highly conserved DNA binding domain which is critical for binding of RFX6 to DNA and consequently regulates the amount of messenger RNA produced by the gene. Several lines of evidence have indicated that RFX6 binding to DNA could be disrupted in diabetes. However, the mechanism by which this could happen is still unknown.

Research motivation

The presence of genetic mutations in the gene coding for the RFX6-DNA binding domain could result in inhibition of binding of RFX6 to DNA and consequently loss of function. Defining such genetic mutations will provide valuable information to diagnose, treat, prevent and cure type 2 diabetes (T2D).

Research objectives

In this study, we sought to investigate if any structural genetic mutations could be present in the RFX6-DNA binding domain in T2D patients and whether they are associated with diabetes.

Research methods

A case-control study was conducted in T2D patients and healthy volunteers. The DNA was extracted from all subjects and polymerase chain reaction (PCR) was used to amplify genomic DNA encompassing the coding sequences and intronic borders of exons 3, 4, 5 and 6 of the *RFX6* gene, then PCR samples were analysed by DNA sequencing.

Research results

Our data showed the absence of any mutation in the exons coding for the RFX6-DNA binding

domain. However, we have identified a new heterozygous single nucleotide polymorphism (IVS6+31 C>T) in the intronic region of DNA binding domain gene that is present in 9.2% and 8.5% of diabetic and control people, respectively ($P = 0.97$).

Research conclusions

We conclude that genetic mutations in the DNA binding domain of RFX6 are unlikely to exist in T2D.

Research perspectives

RFX6 binding to DNA is mediated by multiple of domains. Indeed, RFX6 proteins contain other conserved regions, including B, C, and D domains, which play a critical role in oligomerization of the protein and are required for DNA binding and activation. Thus, testing the other functional domains of RFX6 in future will provide more insights into the role of RFX6 in diabetes.

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Retrospective Cohort Study

Targeted genotyping for the prediction of celiac disease autoimmunity development in patients with type 1 diabetes and their family members

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Abstract

BACKGROUND

Patients with type 1 diabetes (T1D) and their first-degree relatives (FDRs) have an increased risk of developing celiac disease (CD) compared to the general population. This is largely explained by the shared association with major histocompatibility class II human leukocyte antigen (HLA) DQ2 and/or DQ8 between the two disease states.

AIM

To describe the frequency of CD autoimmunity (CDA) and the distribution of HLA and haptoglobin genotypes in patients with T1D and their FDRs. Additionally, we aimed at identifying predictors associated with an increased risk of developing CDA in patients with T1D and their family members.

METHODS

We obtained clinical information and blood samples from 1027 participants (302 with T1D and 725 FDRs) over a five-year period. Samples were tested for autoantibodies associated with CD, HLA-DQ alleles, and haptoglobin genotype.

STROBE statement: The authors have read the STROBE Statement – checklist of items, and the manuscript was prepared and revised according to the STROBE Statement – checklist of items

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We fit univariate and multiple logistic regression models for CDA separately for subjects with T1D and for FDRs of subjects with T1D.

RESULTS

Implementation of a screening program increased the frequency of CDA by 2-fold in participants with T1D and 2.8-fold in their FDRs. Multivariate analysis found that, in participants with T1D, having both DR7-DQ2 and DR4-DQ8 was associated with an increased frequency of CDA. In FDRs of T1D patients, reported CD in the family was associated with an increased frequency of CDA during screening. Haptoglobin 2 genotype was not associated with developing CDA in the multivariate analysis.

CONCLUSION

Patients with T1D and their FDRs have a high frequency of CDA. Carrying both DR7-DQ2 and DR4-DQ8 was associated with development of CDA in patients with T1D.

Key words: Screening; Gluten; Diabetic; Coeliac; Haptoglobin; Human leukocyte antigen

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Core tip: Serological screening for celiac disease (CD) autoimmunity in subjects with type 1 diabetes (T1D) and their first-degree relatives (FDRs) found an underestimation of CD by 2 fold in T1D patients and 2.8 fold in their FDRs. Participants with T1D who carry DR7-DQ2/DR4-DQ8 were more likely to screen positive for CD autoimmunity. There was no association between carrying zonulin genetics and an increased risk of developing CD in our cohort. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population and, given the often-asymptomatic nature of disease, physicians should have a low threshold for screening.

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INTRODUCTION

Celiac disease (CD) is an autoimmune enteropathy that occurs in genetically susceptible individuals in response to ingestion of gluten. While the worldwide prevalence of this condition is estimated at 1%, prevalence is known to vary among geographical locations and ethnic groups^[1]. The prevalence of CD and other autoimmune conditions appears to be on the rise, and yet most patients with CD remain undiagnosed^[2,3]. Many of these individuals may be asymptomatic and identified through the screening of high-risk groups. Patients with type 1 diabetes (T1D) constitute a high-risk group given their risk of CD is reported at 3-8 times higher than that of the general population^[4-7]. This increased risk is likely due to a shared genetic predisposition with the major histocompatibility (MHC) class II human leukocyte antigen (HLA) DQ2 and/or DQ8 between the two disease states^[8,9]. Recent evidence suggests that, like T1D patients, relatives of those with T1D have an increased risk of autoimmune disease. Screening studies detecting celiac-associated antibodies have found a prevalence of CD in relatives of those with T1D ranging between 2.5% and 6%^[7,10].

Both T1D and CD are diseases for which increased permeability is crucial to the pathogenesis^[11,12]. Zonulin, a family of proteins belonging to the serine proteases group, is a master regulator of paracellular permeability and works through reversibly altering intercellular tight junctions^[13-15]. Serum zonulin levels correlate with increased intestinal permeability and have been associated with many chronic inflammatory disorders, including CD and T1D^[14-16]. One of the zonulin isoforms is the precursor of haptoglobin-2 (HP2)^[17]. In humans, haptoglobin (HP) exists as two

common alleles, *HP1* and *HP2*, giving rise to three different *HP* genotypes (*HP1-1*, *HP2-1*, *HP2-2*). The *HP2* allele is found only in humans and only individuals who possess an *HP2* can produce zonulin. Worldwide, the frequency of *HP1-1* varies from 0.07-0.70^[18]. In the United States, the frequency ranges from 0.31-0.55, with a frequency of 0.41 reported in Caucasians^[19]. Presence of the *HP2* allele has been shown to influence the course of inflammatory disease due to differences in antioxidants, scavenging, and immunomodulatory properties^[18]. Previous work has shown that the zonulin gene (*HP2*) is more frequent in chronic inflammatory diseases such as inflammatory bowel disease^[20], CD^[21], and lupus^[22], and that homozygosity for *HP2-2* is associated with more severe clinical manifestations of inflammatory conditions^[21]. In patients with T1D, the frequency of *HP2-2* and *HP2-1* genotypes is increased compared to that reported in the general population^[23]. Further, the *HP2-2* genotype has been associated with increased risk of coronary artery disease in patients with type-2^[24] but not type-1^[25] diabetes. In patients with T1D, *HP2-2* has not been significantly associated with diabetic nephropathy^[23], but has been associated with an increased risk of cardio-renal mortality^[26], including a decline in kidney function and progression to end-stage renal disease^[27,28]. Overall, the contribution of *HP* genotype to development of other autoimmune diseases, specifically CD, has not been evaluated. However, given the role of zonulin in the pathogenesis of both T1D and CD, we postulated that patients with T1D or their first-degree relatives (FDRs) would be more likely to develop CD if they carried the *HP2-2* genotype.

Since approximately half of T1D patients who are diagnosed with CD present asymptotically, and there are no universally accepted screening guidelines to evaluate for CD in patients with T1D or their FDRs, we employed a prospective program to serologically screen patients with T1D and their FDRs for CD^[29,30]. We aimed at identifying predictors that may increase the risk of developing CD in patients with T1D, and to understand which individuals from these high-risk families were more likely to develop CD to identify which subjects may benefit most from screening. We hypothesized that patients with T1D who develop CD are younger at the age of T1D diagnosis, more likely to carry DR3-DQ2/DR4-DQ8 and more likely to have zonulin genetics, *HP2*, than patients with T1D without CD. We also hypothesized that FDRs of subjects with T1D will be more likely to develop CD if they are female, complain of GI symptoms, carry DR3-DQ2, and carry the zonulin gene *HP2*.

MATERIALS AND METHODS

Setting

This study was performed during the Children with Diabetes (CWD) annual conference. CWD is a United States based organization that provides educational and social support for families of children with T1D. We conducted serological screening for CD at CWD's annual conference over five consecutive years (2013-2017).

Subjects

Children and adults attending the CWD conference diagnosed with T1D or with a FDR (parent, child, or sibling) with T1D were eligible for participation. Participants self-selected to participate by visiting our "booth" to conduct study procedures. Written informed consent was obtained from all participants. All study procedures were reviewed and approved by the Partners Human Research Committee Institutional Review Board.

Clinical information

Participants and, when necessary, their caregivers (on behalf of a child), completed a brief self-report clinical questionnaire targeted to assess the family history of T1D and CD, presence or absence of CD-associated symptoms in the individual, current diet, and other pertinent medical information.

Serology

All subjects underwent venipuncture with an on-site phlebotomist. A minimum of 8 cc of blood was collected from each participant. Serum was evaluated for antibodies to IgA tissue transglutaminase (tTG) and IgG deamidated gliadin peptide (dGP) using QUANTA Lite Rh-tTG IgA ELISA (INOVA Diagnostics, San Diego, CA, United States) on the BioFlash platform. Individuals found to have IgA tTG levels above the kit reference value (> 20 CU) were subjected to confirmatory testing for IgA endomysial antibodies (EMA) using the NOVA Lite Monkey Oesophagus IFA Kit (Inova Diagnostics, San Diego, CA, United States). Subjects found to have elevated IgG dGP in the absence of elevated IgA tTG were further evaluated for potential IgA

deficiency. Serum samples for these individuals were sent to an outside lab (LabCorp, Burlington, NC, United States) and a total IgA level was performed using immunoturbidimetric methods.

HLA determination

HLA was determined from whole blood samples using the DQ-CD Typing Plus (BioDiagne, Palermo, Italy) according to the manufacturer's instructions.

HP genotyping

HP genotype was determined by either PCR or immunoblot depending on availability of biological samples. For determination by PCR, genomic DNA was extracted from venous blood using QIAamp DNA kit (Qiagen, Hilden, Germany). The genotypes were determined by a novel one step PCR method using primers designed with Primer3 in exon 2 and exon 5 of *HP1* corresponding to exons 2 and 7 of *HP2*. The primers were designed as follows: forward: TTTCTGGCTGCTAAGTTG and reverse: AATGTCTTTCGCTGTTGC. The PCR was performed in 10 uL reactions containing 100 ng purified DNA, 5 uL of 2× MyTaq Red Mix (Bioline, Taunton, MA, United States), and 300 nM of each primer. After PCR, the amplicons were electrophoresed on a 1% agarose gel and read under a UV bulb. The duplication in *HP2* results in a size difference of the PCR products (2.5 kb *HP1* and 4.3 kb *HP2*) allowing for differentiation of the two genotypes.

Following screening, all participants were informed of their serological status and genetic compatibility (in regard to HLA only). In the event of abnormal serological findings, patients were instructed regarding necessary follow-up procedures with a local physician or specialist.

Definition of CD, Celiac disease autoimmunity (CDA) and IgA deficiency

Participants who self-reported a diagnosis of CD prior to screening were classified as "previous CD" if their diagnosis was based on biopsy or "history of CDA" if their diagnosis was based on bloodwork alone. Patients with positivity for both IgA tTG and IgA EMA at screening were considered, for this study, positive for CD. In the absence of IgA EMA (IgA tTG elevated alone), subjects were classified as demonstrating CDA. Patients with elevated IgG dGP in the absence of elevated IgA tTG were evaluated for potential IgA deficiency. Serum IgA levels less than 7 mg/dL were regarded as IgA deficient. Individuals found to have elevated IgG dGP and IgA deficiency were classified as CDA. For the purposes of the univariate and multivariate analyses, all patients with CD and CDA were combined and are referred to having CDA.

Statistical analysis

Categorical data are presented as frequency (percentage). Continuous data are described as mean \pm SD if normally distributed and median (interquartile range; IQR) otherwise. All tests of significance were two-sided with $\alpha = 0.05$, and all analysis was performed with SAS 9.4 (Cary, NC). We fit univariate and multiple logistic regression models for CDA, separately for subjects with T1D and for FDRs of subjects with T1D. We included all covariates that had *P*-values less than 0.10 in univariate analyses and in the multiple regression models. We used generalized estimating equations to account for the correlation within families in all analyses; we used an exchangeable working correlation matrix, except for the multiple regression models for individuals with T1D for which we used an independence working correlation matrix due to convergence issues.

RESULTS

Demographics

Demographic data for participants with T1D, T1D and CDA (T1D+CDA), and FDRs of T1D patients with (FDR + CDA) and without CDA are shown in [Table 1](#). The majority of patients in the study were female, White and not Hispanic. As expected, since screening took place at a conference for children with T1D and their family members, participants with T1D were younger than their FDRs at the time of screening. More than 50% of participants reported being asymptomatic at screening. Participants with CDA prior to or at the time of screening had a higher frequency of reporting a relative with CD and a higher frequency of reporting a relative diagnosed with any autoimmune disease.

HLA and haptoglobin genetics

[Table 1](#) demonstrates the frequency of the HLA and haptoglobin genetics for

Table 1 Demographic data: Participants with type 1 diabetes and their first-degree relatives *n* (%)

Demographics	T1D only (<i>n</i> = 280)	T1D + CD (<i>n</i> = 22)	FDR only (<i>n</i> = 689)	FDR +CDA (<i>n</i> = 36)
White	249 (88.9)	22 (100)	629 (91.3)	35 (97.2)
Not hispanic	188 (67.1)	15 (68.2)	451 (65.5)	27 (75.0)
Female	178 (63.6)	17 (77.3)	409 (59.4)	26 (72.2)
Age at screening (yr), median (range)	19 (2-72)	14.5 (7-43)	40 (1-74)	39.5 (3-55)
Age at diagnosis of T1D (yr), median (range)	10 (0.1-64)	6.5 (1-21)		
Presence of gastrointestinal symptoms (GI sx)	113 (40.5)	6 (27.3)	216 (31.4)	13 (36.1)
Presence of extraintestinal symptoms (Ex sx)	78 (27.9)	3 (13.6)	176 (25.5)	7 (19.4)
Both GI and Ex symptoms	56 (20)	3 (13.6)	98 (14.2)	4 (11.1)
Asymptomatic	144 (51.6)	16 (72.7)	394 (57.3)	20 (55.6)
Human leukocyte antigen (HLA) genotype				
DR3-DQ2	49 (18)	2 (9.1)	148 (21.8)	10 (28.6)
DR3-DQ2 homozygote	18 (6.6)	3 (13.6)	25 (3.7)	3 (8.6)
DR7-DQ2	12 (4.4)	1 (4.6)	41 (6.1)	2 (5.7)
DR7-DQ2 homozygote	2 (0.7)	0 (0)	13 (1.9)	0 (0)
DR3/DR7-DQ2 homozygote	1 (0.37)	0 (0)	20 (3.0)	3 (8.6)
DR4-DQ8	83 (30.5)	5 (22.7)	205 (30.2)	10 (28.6)
DR3-DQ2/DR4-DQ8	70 (25.7)	7 (31.8)	50 (7.4)	6 (17.1)
DR7-DQ2/DR4-DQ8	6 (2.2)	4 (18.2)	33 (4.9)	0 (0)
DQ2/DQ8 negative	31 (11.4)	0 (0)	142 (20.9)	1 (2.9)
Haptoglobin genotype (HP) (Zonulin)				
HP 1-1	46 (16.4)	2 (9.1)	120 (17.4)	8 (22.2)
HP 2-1	118 (42.1)	10 (45.5)	278 (40.4)	21 (58.3)
HP 2-2	116 (41.4)	10 (45.5)	290 (42.2)	7 (19.4)
Any HP2	234 (83.6)	20 (90.9)	568 (82.6)	28 (77.7)

T1D: Type 1 diabetes; CDA: Celiac disease autoimmunity; FDRs: First-degree relatives; GI sx: Gastrointestinal Symptoms; Ex sx: Extraintestinal Symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

participants who underwent screening. All individuals with known or newly diagnosed CDA, except for one, carried HLA-DQ2 or 8. The T1D + CDA and FDR + CDA groups demonstrated a higher frequency of HLA DQ2 compared to T1D and FDRs without CDA. Overall, participants in this cohort had a higher frequency of carrying *HP2* (in heterozygosity or homozygosity) than previously published work reporting the frequency of *HP* genotypes in the general population^[18,19].

Prevalence of CD and CDA

Table 2 reports the prevalence of CD and CDA in the screened participant cohort. Prior to our screening program, 3.7% of participants with T1D and 1.8% of FDRs reported a diagnosis of CD or history of CDA. After screening, the estimated prevalence of CDA in our cohort increased by two-fold in patients with T1D and by 2.8-fold in FDRs. One participant in the cohort was found to have elevated IgG dGP and IgA deficiency and was classified as CDA.

Univariate and Multivariate Analysis

Participants with T1D: The univariate analyses of participants with T1D (**Table 3**) showed that an older age at study entry and older age of onset of T1D are associated with lower risk of screening positive for CDA. In addition, the following characteristics (in T1D patients) were associated with higher risk of CDA in our cohort: absence of symptoms, carrying DR7-DQ2/DR4-DQ8, first-degree relation to an individual with CD and first-degree relation to an individual with thyroid disease and/or any autoimmune disease. In multiple regression analysis of subjects with T1D (**Table 3**), carrying DR7-DQ2/DR4-DQ8 remained highly significantly associated with screening positive for CDA.

FDRs of Participants with T1D: The univariate analyses of FDRs of participants with T1D (**Table 4**) showed that absence of DQ2/DQ8 and presence of *HP2-2* are both associated with lower risk of CDA. Carrying DR3-DQ2 in homozygosity, DR3-DQ2/DR4-DQ8, and *HP2-1* are all associated with a higher risk of screening positive

Table 2 Cohort prevalence of celiac disease and celiac disease autoimmunity

	<i>n</i>	CD	CDA	CD at screening	CDA at screening	CDA prevalence estimation
Type 1 diabetes	302	8 (2.7)	3 (1.0)	8 (2.5)	3 (0.7)	22 (7.3)
First-degree relative	725	9 (1.2)	4 (0.6)	18 (2.7)	5 (1.0)	36 (5.0)

T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity.

for CDA. Risk of CDA was increased in those who reported a diagnosis of CD in a FDR and a history of any other autoimmune disease in a FDR. In multiple regression analyses of FDRs of subjects with T1D (Table 4), including the significant predictors from the univariate analyses, only reporting CD in a FDR is highly significantly associated with screening positive for CDA.

DISCUSSION

Though patients with T1D have been identified as a population at risk for CD who would benefit from routine screening, the optimal timing, frequency, and provider to spearhead this effort remain the object of debate. Implementation of our screening program in this known high-risk population and their FDRs revealed that a large proportion of individuals with undiagnosed CDA. Indeed, our active screening uncovered a two-fold increase in CDA in participants with T1D and a 2.8-fold increase in CDA in their FDRs. Furthermore, participants with T1D who screened positive for CDA were more frequently asymptomatic than participants with T1D only suggesting that routine screening is necessary to identify these patients.

Our study aimed at identifying clinical and laboratory characteristics to predict which individuals among this high-risk subgroup (T1D patients and their FDRs) may benefit most from screening for CD. While we did not find any significant clinical predictors of developing CD in our cohort, patients with T1D who screened positive for CDA had a trend towards a younger age of T1D onset and were less likely to report extra-intestinal or gastrointestinal symptoms than participants with T1D alone. Previous work has shown that up to 85% of patients with T1D who screen positive for CD are asymptomatic^[4]. While data is mixed, some studies suggest gastrointestinal symptoms are more frequent in patients with long-term T1D compared to control patients³¹. Given that patients with T1D and CDA had a lower median age at the time of screening and had a narrower age range than patients with T1D alone, it is possible that patients with both T1D and CDA were diagnosed with T1D for a shorter period of time. Additionally, studies suggest that patients with T1D and poor glycemic control have more frequent GI symptoms^[31,32]. Since we did not perform additional testing to assess glycemic control it is possible that more patients with T1D alone had poor glycemic control and a greater frequency of GI symptoms. Finally, our analysis of patients with T1D and CDA included eight patients who were diagnosed with CD prior to our screening program and thus were already on treatment for CD. Therefore, the low frequency of symptoms in patients with T1D and CDA may be explained by the inclusion of these patients already on a gluten free diet.

We also sought to describe the distribution of HLA and haptoglobin genotypes in our cohort and for the first time utilize the haptoglobin genotypes in a translational approach to identify predictors that may help to establish which patients among this unique cohort are more likely to develop CD. Our findings that participants with T1D are more likely to carry HLA DQ8 and participants with CD are more likely to carry HLA DQ2 compared to those without these conditions are in agreement with the published literature^[33-35]. Moreover, our findings that *HP2-1* and *HP2-2* are more frequent in this cohort compared to the general population is expected and in agreement with previous work due to the association of *HP2* with autoimmune conditions^[23]. These findings, along with those from our univariate analysis showing that lack of HLA DQ2/8 is associated with a lower risk of CDA, further establish that our cohort is well defined and that HLA DQ typing and analysis is robust.

The HLA genetics DR7-DQ2/DR4-DQ8 was significantly associated with screening positive for CDA in participants already diagnosed with T1D in our cohort. This is particularly interesting given that, while DR3-DQ2 is known to have a strong association with CD, DR7-DQ2 for some time had been overlooked as a risk allele for CD, with commercial clinical labs often not evaluating for this allele or mistakenly interpreting it as not increasing the risk of CD. While DR3-DQ2 is more frequent in patients with CD, 4.4% of patients carry DR7-DQ2^[36]. Furthermore, studies suggest

Table 3 Univariate and multiple regression models: Factors related to celiac disease autoimmunity in participants with type 1 diabetes

	Estimate	Se	Lower limit	Upper limit	Z stat	P-value
Univariate Model (exchangeable correlation)						
Female	0.6562	0.5151	-0.3534	1.6659	1.27	0.2027
Age	-0.0314	0.0152	-0.0611	-0.0017	-2.07	0.0383 ^A
Onset of T1D	-0.0658	0.0342	-0.1329	0.0013	-1.92	0.0546
Gastrointestinal (GI) symptoms	-0.5812	0.491	-1.5435	0.3811	-1.18	0.2365
Extraintestinal (EX) symptoms	-0.9204	0.6391	-2.1731	0.3323	-1.44	0.1498
Both GI and EX symptoms	-0.4724	0.6385	-1.7239	0.7791	-0.74	0.4594
No GI or EX symptoms	0.9074	0.4931	-0.059	1.8737	1.84	0.0657
Human Leukocyte Antigen (HLA) DQ2-DR3 Heterozygous	-0.7402	0.7571	-2.2241	0.7437	-0.98	0.3282
HLA DQ2-DR3 Homozygous	0.7843	0.67	-0.5332	2.1019	1.17	0.2433
HLA DQ2-DR7 Heterozygous	0.028	1.0649	-2.0592	2.1152	0.03	0.979
HLA DQ8	-0.3975	0.5234	-1.4233	0.6283	-0.76	0.4475
HLA DQ2-DR3/DQ8	0.2767	0.4814	-0.6668	1.2203	0.57	0.5654
HLA DQ2-DR7/DQ8	2.4851	0.6507	1.2098	3.7604	3.82	0.0001 ^B
HLA DQ2 Heterozygous	-0.575	0.6367	-1.823	0.6729	-0.9	0.3665
HLA DQ2 Homozygous	0.6192	0.666	-0.6862	1.9246	0.93	0.3525
Haptoglobin genotype (HP) 1-1	-0.6932	0.7671	-2.1967	0.8104	-0.9	0.3662
HP 2-1	0.1539	0.4414	-0.7112	1.0191	0.35	0.7273
HP 2-2	0.1525	0.4422	-0.7141	1.0192	0.34	0.7301
Any HP2	0.693	0.767	-0.81	2.197	0.9	0.366
First degree relative (FDR) with celiac disease (CD)	1.4091	0.6188	0.1962	2.622	2.28	0.0228 ^C
FDR with Type 1 diabetes (T1D)	0.0724	0.557	-1.0193	1.1641	0.13	0.8966
FDR with thyroid disease	0.7839	0.4619	-0.1215	1.6892	1.7	0.0897
FDR with other autoimmune disease	1.1634	0.4432	0.2947	2.0321	2.62	0.0087 ^D
CD in another relative	0.4136	0.6598	-0.8796	1.7068	0.63	0.5307
Multiple Regression Model (independence working correlation)						
Intercept	-3.3213	0.927	-5.1383	-1.5043	-3.58	0.0003
Age	-0.0121	0.019	-0.0494	0.0252	-0.64	0.5246
Onset of T1D	-0.0396	0.0425	-0.1229	0.0436	-0.93	0.3509
DQ2-DR7/DQ8	2.4131	0.8401	0.7666	4.0596	2.87	0.0041 ^E
No GI or EX symptoms	1.1905	0.6566	-0.0963	2.4774	1.81	0.0698
FDR with CD	0.6927	1.3443	-1.9421	3.3274	0.52	0.6064
FDR with thyroid disease	-0.8976	1.6214	-4.0756	2.2804	-0.55	0.5799
FDR with other autoimmune disease	1.9765	1.7861	-1.5241	5.4771	1.11	0.2685

Significant findings indicated by superscripts. T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity; GI sx: Gastrointestinal symptoms; Ex sx: Extraintestinal symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

that, in patients at-risk for CD, the presence of DR7-DQ2 with DR3-DQ2 is associated with an increased frequency of developing CD^[37]. Our findings are similar given that, despite a low frequency of participants with T1D carrying DR7-DQ2, those that do in combination with DR4-DQ8 have a high frequency of screening positive for CDA.

Our analysis did not demonstrate an association between carrying *HP2* and an increased risk of developing CD in participants with T1D or their family members. While we did not identify significant differences in *HP* genotype in this cohort, *HP2* was highly represented in our cohort. Additionally, there was a trend towards an underrepresentation of *HP1* in patients with both T1D and CD. Interestingly in FDRs there was a trend towards increased representation of *HP2-1*. These trends require further investigation with larger cohorts and should be compared to a group of individuals without a risk of autoimmune disease. Considering this, true trends may be somewhat masked by the nature of our study population; our cohort is made up of patients with a personal or family history of autoimmune disease, and thus a higher overall frequency of *HP2*. However, the purpose of our study was to identify predictors from a high-risk group. In our study, HLA type and having a family history of CD were the strongest predictors of developing CD. While we found that

Table 4 Univariate and multiple regression models: Factors related to celiac disease autoimmunity in first degree relatives of participants with type 1 diabetes

	Estimate	Se	Lower limit	Upper limit	Z stat	P-value
Univariate model (exchangeable correlation)						
White	1.26	0.88	-0.47	2.98	1.43	0.154
Female	0.5433	0.3674	-0.1768	1.2635	1.48	0.1392
Age	-0.0046	0.0097	-0.0236	0.0144	-0.47	0.6355
Gastrointestinal (GI) symptoms	0.2551	0.3395	-0.4103	0.9206	0.75	0.4524
Extraintestinal (EX) symptoms	-0.3748	0.4596	-1.2756	0.526	-0.82	0.4148
Both GI and EX symptoms	-0.2715	0.5305	-1.3113	0.7682	-0.51	0.6087
No GI or EX symptoms	-0.085	0.3419	-0.7551	0.5851	-0.25	0.8036
Human Leukocyte Antigen (HLA) DQ2-DR3 Heterozygous	0.4255	0.383	-0.3251	1.1761	1.11	0.2666
HLA DQ2-DR3 Homozygous	0.8042	0.7654	-0.696	2.304	1.05	0.2934
HLA DQ2-DR7 Heterozygous	-0.0376	0.6636	-1.3382	1.263	-0.06	0.9548
HLA DQ2-DR3/DR7 Homozygous	1.02	0.715	-0.381	2.42	1.43	0.153
HLA DQ8	-0.0981	0.4058	-0.8934	0.6972	-0.24	0.809
HLA DQ2-DR3/DQ8	0.9272	0.472	0.0021	1.8523	1.96	0.0495 ^A
HLA DQ2/DQ8 Negative	-2.1836	1.0232	-4.1891	-0.1781	-2.13	0.0328 ^B
HLA DQ2 Heterozygous	0.3561	0.388	-0.4043	1.1166	0.92	0.3586
HLA DQ2 Homozygous	0.6842	0.5435	-0.3811	1.7495	1.26	0.2081
Haptoglobin genotype (HP) 1-1	0.2901	0.4341	-0.5607	1.1409	0.67	0.5039
HP 2-1	0.6823	0.3429	0.0103	1.3544	1.99	0.0466 ^C
HP 2-2	-1.0763	0.4252	-1.9096	-0.2429	-2.53	0.0114 ^D
Any HP2	-0.29	0.434	-1.141	0.561	-0.67	0.503
First degree relative (FDR) with CDceliac disease (CD)	1.6768	0.3809	0.9303	2.4233	4.4	<.0001 ^E
FDR with thyroid disease	-0.503	0.4849	-1.4533	0.4473	-1.04	0.2995
FDR with other autoimmune disease	0.6817	0.3606	-0.025	1.3884	1.89	0.0587
CD in other relative	0.5217	0.7115	-0.8728	1.9161	0.73	0.4634
Multiple regression model (independence working correlation)						
Intercept	-2.8829	0.404	-3.6746	-2.0912	-7.14	< 0.0001
HLA DQ2-DR3/DQ8	0.9407	0.5381	-0.1139	1.9953	1.75	0.0804
HLA DQ2/DQ8 Negative	-1.9348	1.0275	-3.9487	0.0791	-1.88	0.0597
HP 2-1	0.0804	0.4492	-0.8001	0.9609	0.18	0.858
HP 2-2	-1.0149	0.563	-2.1185	0.0886	-1.8	0.0715
FDR with CD	2.3635	0.7576	0.8787	3.8483	3.12	0.0018 ^F
FDR with other autoimmune disease	-0.8269	0.7245	-2.2468	0.5932	-1.14	0.2538

Significant findings indicated by superscripts. T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity; GI sx: Gastrointestinal symptoms; Ex sx: Extraintestinal symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

carrying *HP2* is not a predictor of developing autoimmune disease in this already high-risk population there was a trend towards an increased representation of *HP2* in patients with T1D and CDA. Further, it is unclear why FDR have a lower risk of developing CDA if they carry *HP2* in homozygosity and future work evaluating this finding in a larger cohort is needed.

Limitations of our study include utilization of self-reported family history of CD, T1D, and other autoimmune diseases, and lack of endoscopy to confirm CD in patients found to have CDA at screening. While this was not feasible in this screening study, all patients that had a positive serologic test for CD were advised to undergo further confirmatory testing with repeat blood work and an endoscopy. All patients with a positive IgA tTG had a second confirmatory test with IgA EMA. The majority of patients in our cohort with elevated IgA tTG additionally tested positive for IgA EMA making a diagnosis of CD likely. However, for the purposes of analysis, we combined participants with a positive IgA tTG alone and those with a positive IgA tTG and EMA in our CDA estimate. The possibility for falsely elevated or transiently elevated IgA tTG in patients with T1D and other autoimmune disorders is well known, thus our CDA estimate may be an overestimation. However, our prevalence estimates are in line with previously published work^[4,5,7]. Finally, to assess follow-up

care in our cohort, a questionnaire was sent to participants during three years of the five-year study. Approximately 40% ($n = 24$) of participants with CDA responded to the questionnaire. Of those 38% ($n = 9$) of participants sought follow-up of their positive serology with a physician and the majority underwent endoscopy ($n = 7$). This highlights an additional limitation of screening studies in that appropriate follow-up is not ensured despite our efforts to provide educational materials and guidance.

In conclusion, implementation of a screening program increased identification of CDA in participants with T1D and their FDRs by 2 and 2.8-fold respectively. Participants with T1D carrying DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA. Haptoglobin genotype did not predict the development of CDA in this high-risk population. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population, and given the often asymptomatic nature of disease; physicians should have a low threshold for screening.

ARTICLE HIGHLIGHTS

Research background

Patients with type 1 diabetes (T1D) and their first-degree relatives (FDRs) are at increased risk of developing celiac disease (CD). The majority of patients with T1D and CD are asymptomatic at diagnosis and there are no universally accepted screening guidelines to evaluate for CD in patients with T1D or their FDRs. We employed a prospective program to serologically screen patients with T1D and their FDRs for CD. We then retrospectively aimed to identify clinical and genetic predictors that may increase the risk of developing CD in this cohort of individuals at high-risk of developing CD.

Research motivation

Patients with T1D are up to eight times more likely to develop CD, and their FDR's are up to six times more likely to develop CD. Given that many may be asymptomatic, there is a need to identify predictors of CDA development in this high-risk cohort. The main topics, the key problems to be solved, and the significance of solving these problems for future research in this field should be described in detail.

Research objectives

Our objective was to identify clinical and genetic predictors that may increase the risk of developing CD in patients with T1D. In addition, we aimed to understand which FDRs of the patients with T1D, who are already at an increased risk of developing autoimmune disease, were more likely to develop CD. Our ultimate goal was to identify which subjects may benefit most from screening to help guide future screening recommendations.

Research methods

Participants included patients diagnosed with T1D or FDR of a patient with T1D attending the annual Children with Diabetes (CWD) conference over a 5 year time period. Participants answered clinical questionnaires and had blood drawn for CD serological testing and genotyping. Prevalence of celiac disease autoimmunity (CDA) was described. We then retrospectively fit univariate and multiple logistic regression models for CDA, separately for subjects with T1D and for FDRs of subjects with T1D accounting for the correlation within families when indicated in order to identify predictors of developing CDA.

Research results

Implementation of a prospective screening program in patients with T1D and their FDRs increased identification of CDA by 2 and 2.8-fold respectively. Participants with T1D carrying DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA. In FDRs of patients with T1D, screening positive for CDA was significantly increased in those who reported having a family member diagnosed with CD. Haptoglobin genotype did not predict the development of CDA in this high-risk population.

Research conclusions

CDA is under recognized in patients with T1D and their FDR's and that prospective screening in this high-risk cohort increased the identification of CDA by at least 2 fold. Clinical symptoms were not helpful in distinguishing patients with CDA, as the majority of patients reported no symptoms. Haptoglobin genotype was not found to be a predictor of CDA in this cohort. In our cohort, FDRs of patients with T1D were more likely to screen positive for CDA if they had a family history of CD, while patients with T1D who carried the HLA genotype DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA.

Research perspectives

Given the high frequency of CDA in patients with T1D and their FDRs, physicians should have a low threshold to screen for CDA even in the absence of symptoms.

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Observational Study

Burden of diabetic foot ulcer in Nigeria: Current evidence from the multicenter evaluation of diabetic foot ulcer in Nigeria

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Institutional review board

statement: Approval for the study was given by the local Research and Ethics committee of each of the participating centers.

Informed consent statement:

Participation in this study was voluntary. Verbally granted informed consent was obtained from each patient prior to enrollment into the study. Confidentiality was ensured at all stages by means of unique coding system consisting of patients' initials and assigned numbers.

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Abstract

BACKGROUND

Nigeria bears the greatest burden of diabetes prevalence in Sub-Saharan Africa. Diabetic foot ulcer (DFU) is a serious and potentially life-threatening complication of diabetes. Significant improvements in diabetic foot incidence and outcomes have been recorded in many Western countries in the past decade. However, the current burden of DFU in Nigeria is largely unknown.

AIM

To evaluate the patients' profile, ulcer characteristics, associated co-morbidities and outcome of patients with DFU in Nigeria.

METHODS

Multicenter evaluation of diabetic foot ulcer in Nigeria was a one year multicenter observational study of patients hospitalized for DFU in six tertiary health institutions in Nigeria from March 2016 to March 2017. Demographic and diabetes information, ulcer characteristics and associated co-morbidities were assessed. Relevant laboratory and imaging studies were performed. All patients received appropriate multi-disciplinary care and were followed up until discharge or death. Outcome variables of interest were ulcer healing, lower

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extremity amputation (LEA), duration of hospitalization and mortality.

RESULTS

A total of 336 patients (55.1% male) with mean age of 55.9 ± 12.5 years were enrolled into this study. Majority (96.1%) had type 2 diabetes. Only 25.9% of the subjects had prior foot care knowledge. Most of the subjects presented late to the hospital and median (IQR) duration of ulcer at presentation was 39 (28-54) d. Ulcers were already advanced (Wagner grades ≥ 3) in 79.2% of the subjects while 76.8% of the ulcers were infected at the time of admission. The commonest co-morbidities were systemic hypertension, anemia and hyperglycemic emergencies. One hundred and nineteen subjects (35.4%) suffered LEA while 10.4% left against medical advice. The median (IQR) duration of hospitalization was 52.0 (29-66) d with case fatality rate of 20.5%.

CONCLUSION

The burden of DFU in Nigeria is very high. The major gaps include low level of foot care knowledge among diabetic patients, overdependence on self-medication and unorthodox medicine following development of foot ulceration, late hospital presentation, and high amputation and mortality rates. Extensive foot care education within the framework of a multi-disciplinary foot care team is highly desirable.

Key words: Burden; Diabetes; Epidemiology; Foot ulcer; Amputation; Mortality; Multicenter evaluation of diabetic foot ulcer in Nigeria; Nigeria; Africa

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Core tip: The multicenter evaluation of diabetic foot ulcer in Nigeria was a one year observational study of 336 adults who were hospitalized for diabetic foot ulcer in six tertiary hospitals in Nigeria. The subjects were managed by multi-disciplinary diabetic foot care teams and were followed up until discharge or death. This study demonstrated a high burden of diabetic foot ulcer in Nigeria which accounted for about a quarter of diabetes related hospital admissions over the study period. The study recorded high amputation and mortality rates of 35.4% and 20.5% respectively. Major challenges in diabetic foot care identified in this study include low level of foot care knowledge among the patients, poor health-seeking behavior and late hospital presentation.

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INTRODUCTION

Although diabetes mellitus (DM) prevalence is rising globally, Sub-Saharan Africa appears to be the worst-hit^[1]. In the last two decades, Nigeria, for instance has witnessed more than a 100% increase in the prevalence of the disease, from 2.2% in 1997 to nearly 6% in 2015^[2]. It is generally reported that Nigeria which is the most populous country in Africa has the greatest burden of diabetes within the Sub-Saharan sub-continent^[3]. This disproportionate increase in diabetes prevalence has largely been blamed on changing demographic dynamics including increasing urbanization and adoption of unhealthy lifestyles. Paralleling this increase in disease burden is also an upsurge in the prevalence of diabetes-related complications and death. One of the most devastating of these complications is diabetic foot ulcer (DFU), a costly, disabling but preventable complication of diabetes that is associated with significant morbidity and mortality.

DFU refers to a breach in the continuity of the skin epithelium involving its full thickness or beyond, distal to the ankle joints, in a person living with DM^[4]. Foot ulceration is common in patients with DM with current global prevalence of about 6.3%^[5]. It is estimated that a person with diabetes has a 25% lifetime risk of developing

DFU^[6]. In Africa with constrained resources and fractured health systems, the prevalence of DFU is higher at about 7.2%^[5]. The burden of DFU in Africa is substantial, constituting a major source of hospitalization and mortality^[7]. With the rapidly rising diabetes prevalence in Africa, the burden of DFU in this region is expected to be on the increase.

Many of the predisposing factors for DFU are well established and include advancing age, long duration of diabetes, poor glycemic control, presence of neuropathy and peripheral vascular disease^[8,9]. However, majority of DFUs are usually as a result of interplay among an at-risk foot, repeated micro-trauma and super-imposed infection^[4]. Managing DFUs is usually very challenging especially in resource-constrained settings. The cost of managing DFU is substantial, and DFUs account for up to 40% of diabetes-related expenditures, making it one of the most expensive diabetes complications to deal with^[10]. DFUs often heal very slowly resulting in prolonged hospitalization, or may fail to heal completely. They are also very prone to infection with resultant tissue necrosis and gangrene. Consequently, foot ulcerations are the commonest cause of lower extremity amputation (LEA) in persons with diabetes, accounting for up to 85% of LEAs in this population^[4]. The International Diabetes Federation estimates that at least one limb are lost to DFU somewhere in the world every 30 s^[11]. Lower limb amputation is associated with significant disabilities including loss of productivity and reduced quality of life^[11]. Furthermore, it has been observed that 5-year survival after an LEA is worse than many cancers^[12]. Foot ulceration in diabetic patients is therefore a medical, economic and psychosocial issue requiring serious attention.

The burden of DFU in Nigeria has been reportedly high, with prevalence rates ranging from 11%-32% among hospitalized patients^[13,14]. At about half a decade ago, amputation rate from DFU in Nigeria was as high as 52%^[15]. Furthermore, DFU is the commonest cause of diabetes-related mortality in Nigeria after hyperglycemic emergencies^[2]. Diabetic foot ulceration is therefore a matter of serious public health concern in Nigeria. Contemporary data on the actual burden of DFU in Nigeria are however very scanty, and available studies on this subject were single-centered and mostly retrospective. In order to fill this gap, we sought to evaluate the current burden of DFU in a larger population across multiple centers in Nigeria.

MATERIALS AND METHODS

Study areas and design

The multicenter evaluation of diabetic foot ulcer in Nigeria (MEDFUN) was an observational study conducted in six tertiary healthcare institutions in Nigeria, between March 2016 and April 2017. These centers include Enugu State University Teaching Hospital located in South-Eastern Nigeria, Lagos State University Teaching Hospital in the South-West, Aminu Kano Teaching Hospital in the North-West, Ahmadu Bello University Teaching Hospital Zaria also in the North-West, Federal Medical Center Keffi in the North-Central and Federal Medical Center Umuahia also located in the South-East. The locations and geographic spread of these study sites are indicated in a map of Nigeria hereby presented (Supplementary). All the centers render specialized tertiary health care and serve as referral centers for primary and secondary health facilities within and outside their geopolitical zone. The Research and Ethics committee of each of the participating centers approved the study protocol while verbal informed consent was obtained from each patient prior to recruitment.

Subjects and recruitment

In the present study, subjects with type 1 DM (T1DM) or type 2 DM (T2DM) hospitalized for DFU in any of the participating centers were consecutively enrolled after obtaining verbal consent. Distinction between T1DM and T2DM was made clinically as follows: Subjects who reported dependence on insulin for diabetes control since the time of diagnosis were classified as having T1DM while those who had been controlled on oral anti-diabetic drugs with or without insulin were adjudged to have T2DM. Pregnant women, subjects with diabetes other than types 1 and 2, and those with wounds limited to above the ankle joints were excluded.

Data collection and clinical measurements

Using a specially designed structured proforma, relevant socio-demographic and diabetes-related information such as gender, age, occupation, cigarette smoking status, diabetes type and duration, as well as the type of healthcare facility where the patient was receiving diabetes care prior to development of foot ulcer were obtained and documented. Knowledge of foot care was assessed and patients were interviewed

on whether they had received foot care education prior to foot ulceration. History of development and progression of ulcer including mechanism of ulceration, site of ulcer, duration of ulcer and prior ulcer treatment methods were also assessed. Clinical wound infection was determined according to the International Working Group on Diabetes Foot (IWGDF) guideline by the presence of purulent exudates or any two or more of the following: Periwound edema, periwound redness, local warmth, foul smell, pain or tenderness on palpation and fever^[4]. Commonly known risk factors for DFU were also evaluated, including history of previous DFU, barefoot walking, improper foot wear, visual impairment, foot deformity, peripheral neuropathy and peripheral artery disease (PAD). Peripheral neuropathy was diagnosed by loss of pressure perception to Semmes-Weinstein 10 g monofilament test or diminished vibration sense using the 128 Hz tuning fork. PAD was diagnosed based on impalpable dorsalis pedis and/or posterior tibial artery pulsations on manual palpation or significant arterial narrowing (> 50%) on Doppler ultrasonography of the lower limbs. The severity of ulcer was graded using two different ulcer classification systems, namely, the Wagner's grading system and the University of Texas wound classification system^[16,17].

Relevant laboratory and imaging studies were performed for each subject including urine protein using dipstick detection, full blood count, erythrocyte sedimentation rate, glycated hemoglobin (HbA1c), blood culture, ulcer specimen culture, lipid profile, plain radiograph of the foot and Doppler ultrasonography of both lower limbs. Co-morbid complications including hypertension, anemia, shock, hyperglycemic emergency, hypoglycemia, stroke, kidney disease and cardiac failure were explored and documented.

Patient management and outcome indicators

Every patient received appropriate multi-disciplinary care including bed rest, wound debridement, daily wound dressing, antibiotic therapy, skin grafting and limited amputation in addition to control of blood glucose and treatment of associated co-morbidities. All the primary investigators who led the multidisciplinary team were endocrinologists. Other relevant specialists including nutritionists, plastic surgeons, orthopedic surgeons and vascular surgeons were co-opted based on need and availability. None of the centers had a podiatrist, an important foot care specialist that is grossly in short supply in Nigeria. The decision to amputate or not was an exclusive prerogative of the multi-disciplinary footcare team at each study center. All the enrollees were followed up until discharge or death. Outcome variables of interest included ulcer healing, amputation, duration of hospitalization and mortality. We defined amputation above the mid-tarsal bone or involving the big toe as major amputation, otherwise it was minor. At the stoppage of data collection, records of medical admissions over the study period were reviewed retrospectively in all the centers to determine the total number of medical admissions and diabetes-related admissions.

Statistical analysis

Data were collated in all the six participating centers and analyzed using the Statistical Package for Social Sciences (IBM version 23.0; SPSS Inc., Chicago, IL, United States). Categorical variables were presented as numbers and percentages while continuous variables were presented as means and standard deviations or medians and interquartile ranges as appropriate. Analysis at this stage was mainly descriptive. Data were presented in frequency tables, bar charts, pie charts and line graphs as deemed appropriate. The Chi-Square test was used to test differences in categorical proportions while continuous variables were compared between two or more groups of interest using the Student's *t*-test. Statistical significance was established at $P < 0.05$.

RESULTS

There were 9778 total and 1350 (13.8%) diabetes related admissions in the medical wards over the study period. Out of this number, 336 patients with a male: female ratio of 1:0.8 had DFU, and this number accounted for 24.9% of DM-related admissions. Majority of the DFU subjects (96.1%) had type 2 diabetes. The mean \pm SD age and mean \pm SD duration of DM were 55.9 ± 12.5 years and 8.5 ± 5.7 years respectively. Most of the patients (71.7%) were not accessing diabetes care at the study centers but were referred because of the foot ulcer. Glycemic control was generally poor with mean HbA1c of $9.6 \pm 1.9\%$. Only 87 subjects (20.4%) had received foot care education prior to development of ulcer. Neuropathic and neuro-ischemic ulcers predominated in 37.2% and 40.2% of the subjects respectively. Ulcers were adjudged advanced (Wagner grade ≥ 3) in 79.2% of the subjects and majority were already

infected. The commonest co-morbidities were systemic hypertension (56.8%), anemia (53.6%) and hyperglycemic emergencies (36.6%). **Table 1** shows the clinical profile of the study participants while the ulcer grades are shown in **Figure 1**.

Identifiable factors that probably predisposed the patients to developing DFU are presented in **Figure 2**. Diabetic peripheral neuropathy (DPN) and PAD were present in 78.0% and 52.4% of the participants respectively. About 48.2% of the subjects admitted to barefoot walking while 28.6% have had previous foot ulceration. As shown in **Figure 3**, majority of the subjects have multiple risk factors such that up to 44.9% have four or more risk factors operating simultaneously.

Only 21.1% of our subjects sought treatment in hospital as their first option following development of foot ulcer. The most preferred initial treatment option was self-medication which was practiced by 42.0% of the patients. 19.6% of the subjects patronized traditional healers/herbalists while 9.2% relied on prayer houses. These are summarized in **Figure 4**.

Figure 5 shows the admission outcomes of the patients studied. Of the 336 subjects hospitalized for DFU, satisfactory wound healing occurred in 147 subjects (43.8%). One hundred and nineteen subjects (35.4%) underwent LEA of which 75.6% were major amputations. Thirty-five subjects (10.4%) left against medical advice, mainly due to refusal of amputation (48.6%) and financial constraint (42.9%). Sixty-nine deaths (20.5%) were recorded, including 34 deaths post LEA. The median time between admission and death was 16 d (interquartile range 10-33 d). The median (IQR) duration of hospitalization for the study population excluding those who discharged against medical advice was 52.0 (29-66) d. Both amputation and mortality rates significantly increased with higher ulcer grades (**Figure 6**).

DISCUSSION

With a population estimated at about 200 million people, Nigeria is the most populous black nation. And with diabetes prevalence of nearly 6% in adults, representing about 5-7 million adults, Nigeria currently harbors the largest number of people living with diabetes in the West African sub-region^[1]. Understanding the burden of diabetes and its complications in Nigeria is therefore a reliable sneak peek into the rest of Africa. Diabetic foot ulceration is one of the most challenging complications of DM. Due to absence of national data, prevalence rates of DFU in Nigeria from several single center studies vary widely from 11.7%-32%^[13,14]. Similar wide variations have also been reported for DFU outcomes with amputation rates ranging from 12.6%-52%^[14,15] and mortality rates ranging from as low as 8.7% to above 40%^[13,14,18]. The need to have a current and more representative national data on the outlook of DFU in Nigeria therefore became the driving force that birthed the MEDFUN study.

Our data shows that DFU constitutes about a quarter of diabetes related hospital admissions in Nigeria. This represents a much higher burden than what is obtainable in developed nations where DFU generally accounts for less than 10% of medical admissions^[19,20]. Our findings closely mirror the scenarios in some other African settings where the burden of DFU is also reportedly high^[21,22]. Worrisome too is the fact that the bulk of our patients (73.8%) belonged to the young and middle-age categories and nearly three-quarter have had diabetes for less than 10 years duration. This finding is supported by previous local studies and suggests that in Nigeria, DFU affects predominantly the actively-working segment of the population who are often their family bread winners^[14,15,18]. In contrast, majority of patients with DFU in Netherlands and Thailand for instance are above the age of 60 years and have had diabetes for longer duration^[20,23]. The socio-economic consequences of this scenario on a people already groaning under poverty and many communicable diseases could be better imagined.

The prevalence of diabetic foot disease largely reflects the quality of diabetes care as this complication of DM is largely preventable through proper diabetes management^[24]. Approximately 83% of our study subjects had HbA1c above 7%, a reflection of the poor quality of diabetes care in our locality especially at primary and secondary healthcare levels where the bulk of our patients came from. Poor glucose control has been widely reported in Nigeria even among subjects attending tertiary health institutions^[25,26]. Factors that may be responsible for this include poverty, poor drug compliance, poor access to healthcare, shortage of trained diabetes care manpower and diabetes status unawareness. It is noteworthy that 14.6% of the subjects in this study were unaware of their diabetes status until they presented with foot ulcer. Chronic hyperglycemia is generally known to predispose to many diabetes-related complications including peripheral neuropathy, and the latter is a potent risk

Table 1 Clinical profile of the patients with diabetic foot ulcers

Variable	n (%)	mean \pm SD
Age (yr)		55.9 \pm 12.5
< 45 yr	48 (14.3)	
45-64 yr	200 (59.5)	
\geq 65 yr	88 (26.2)	
Gender (male)	185 (55.1)	
Occupation		
Civil servants	61 (18.2)	
Traders	137 (40.8)	
Artisans	12 (3.6)	
Farmers	40 (11.9)	
Unemployed	86 (25.6)	
Cigarette smoking (current smokers)	17 (5.1)	
Diabetes type (type 2)	323 (96.1)	
Diabetes duration (yr)		8.5 \pm 5.7
\leq 10 yr	250 (74.4)	
11-20 yr	79 (23.5)	
> 20 yr	7 (2.1)	
Newly diagnosed diabetes	49 (14.6)	
Glycated hemoglobin (%) ($n = 296$)		9.6 \pm 1.9
HbA1c < 7%	17 (5.7)	
Referred from outside the study centers	241 (71.7)	
Ever had foot care education	87 (25.9)	
Type of Ulcer		
Neuropathic	125 (37.2)	
Ischemic	42 (12.5)	
Neuro-ischemic	135 (40.2)	
Non-neuropathic, non-ischemic	34 (10.1)	
Duration of ulcer before admission (d)		39 (28-54) ¹
Ulcer > 30 d duration	237 (70.5)	
Previous history of ulcer	96 (28.6)	
Advanced ulcer (Wagner grade \geq 3)	266 (79.2)	
Presence of wound infection	258 (76.8)	
Co-morbid complications		
Hypertension	191 (56.8)	
Shock	40 (11.9)	
Anemia	180 (53.6)	
Hyperglycemic emergency	123 (36.6)	
Hypoglycemia	33 (9.8)	
Cardiac failure	23 (6.8)	
Renal impairment	66 (19.6)	
Stroke	32 (9.5)	

¹Data presented as median (interquartile range).

factor for development of DFU^[8,9,27]. Up to 78% of our study subjects presented with DPN which we also identified as a major risk factor for DFU. DPN predisposes to DFU by causing loss of protective sensation in the feet as well as foot deformities, resulting in abnormal weight bearing, recurrent micro-trauma, callous formation and eventual ulceration^[8,9].

Our study uncovered very low levels of foot care knowledge among the participants. We observed that nearly three-quarter of the patients had never received foot care education since diagnosis of diabetes. This finding is of great concern owing to the strategic importance of proper foot care knowledge in the prevention of DFU and amputation. It has been demonstrated that diabetic patients who are

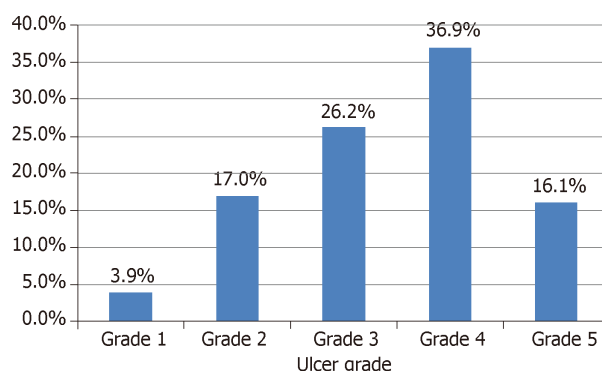


Figure 1 Distribution of diabetic foot ulcer severity by Wagner grading system.

knowledgeable about foot care are 3 times less likely to develop DFU and to suffer LEA^[28,29]. Patients who have adequate foot care knowledge are less likely to engage in harmful foot practices that could predispose to ulceration. They are also more likely to present earlier to the hospital following ulceration thereby reducing the likelihood of amputation. Other authors in Nigeria have observed low level of foot care knowledge both among the general diabetic population and those with DFU^[14,30]. In a recent multi-center study, 78.4% of the 352 diabetic patients surveyed had poor knowledge of foot care and the authors lamented that high risk behaviors such as bare foot walking and improper foot wear were rampant among the patients^[30]. Anumah *et al*^[14] recently reported that 84.7% of patients who were hospitalized for DFU at a tertiary hospital had no prior foot care education. Certified diabetes educators are grossly in short supply in Nigeria and almost non-existent in rural and semi-urban areas. This manpower shortage may largely explain this serious gap in diabetes care in our locality.

The poor health-seeking behavior of patients with DFU in Nigeria was also brought to bear in this study. Our data show that the practice of self-medications and patronage of unorthodox treatment outlets including native/herbal homes and prayer houses were common initial treatment options among the patients. Although this attitude may be partly attributable to poverty and poor access to healthcare, it may not be totally unconnected with the negative illness perceptions that are pervasive in Africa. In many traditional African cultures, diseases are often ascribed to diabolism and spiritual etiologies^[31-33]. In Lagos, Nigeria, as many as 46% of diabetic patients take alternative herbal medicines^[34]. The presence of a non-healing wound may therefore be misinterpreted as the outcome of “stepping on poison” or “spiritual attack” and orthodox care is usually not sought until the disease is advanced. This may partly account for the late hospital presentation which was observed among our study subjects. The ulcer had lasted more than 1 mo in over 70% of our subjects prior to hospitalization, with 79.3% of patients presenting with at least Wagner grade 3 ulcers. Delayed hospital presentation is also a common denominator in many previous studies of DFU in Nigeria^[13-15].

Amputation and mortality rates of 35.4% and 20.5% respectively that were observed in this study are unacceptably high and not in tandem with the trends in the civilized world. In Australia for instance, LEA rate from DFU is less than 2% with over 70% being minor amputation^[19]. Very low amputation and mortality rates were also reported in Netherlands, Thailand, and Scotland^[20,23,35]. Differences in the quality of healthcare systems are likely to be responsible for these discrepancies. We hypothesize that the poor glucose control and delay in hospital presentation following development of DFU contributed significantly to these unpleasant outcomes. Lavery *et al*^[36] demonstrated that duration of ulcer more than 30 d increased the probability of wound infection by nearly 5 times and that amputation was 154 times more likely in infected wounds. This is probably due to the higher propensity of accelerated tissue necrosis and gangrene in such wounds especially in a limb with compromised vascular supply. Wound infection was present in 76.8% of our patients while over half had developed some form of gangrene. Such patients are expected to suffer more amputation and death from overwhelming sepsis. Not surprisingly therefore, we observed significant associations between ulcer severity as measured by Wagner grading, and amputation as well as mortality ($P < 0.001$ respectively). Our data agree with many other previous studies in Nigeria that also reported high amputation and mortality rates among patients with DFU^[13,18,37]. Nigeria is therefore in dire need of total overhaul of diabetes care to stem this ugly tide. Importantly, appropriate multi-

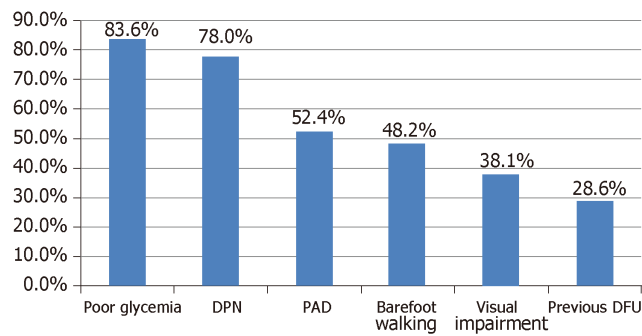


Figure 2 Prevalence of risk factors for diabetic foot ulcers in the study population. DFU: Diabetic foot ulcer; DPN: Diabetic peripheral neuropathy; PAD: Peripheral artery disease.

disciplinary care team approach led by an endocrinologist has been found to drastically improve diabetic foot outcomes and is hereby advocated^[38].

Conclusion

The results from this study revealed that the burden of DFU in Nigeria is still alarming even in this 21st century. This study has exposed several treatment gaps including poor knowledge of foot care among patients, high patronage of self-medications and unorthodox treatment, and delayed hospital presentation with advanced foot ulcers, resulting in prolonged hospitalization, high LEA rate and high mortality. Bridging these gaps through intensive public enlightenment programmes, foot care education of diabetic patients and establishment of well-trained diabetic foot care team may go a long way in reversing this ugly trend.

Strengths and limitations

To our knowledge, MEDFUN is the largest, most extensive and the only multi-center study on DFU both in Nigeria and the West-African sub-region. The limitations of this study however need to be highlighted. Firstly, the study centers covered only 4 out of the six geo-political zones of Nigeria. However, the 2 geo-political zones that were not included in this study share common characteristics with one or more of the other 4 zones. It is therefore arguable that our results are largely generalizable as a true reflection of the burden of DFU in Nigeria. Secondly, each of the participating centers adopted its own DFU management protocol based on availability of manpower. Clinical decisions were therefore dependent on the clinicians at each center. It is not unlikely that this lack of uniformity might have affected the outcome of this study. This is also applicable to the clinical measurements which are prone to inter-observer bias and laboratory tests which might have been influenced by performance variations of diagnostic equipments at the different study centers. However, this lack of uniformity is common in studies of this nature, including the widely cited Eurodiale study which was the largest multi-center DFU study in Europe^[30].

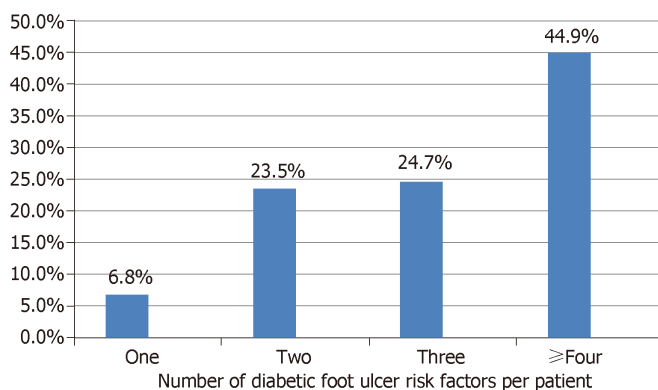


Figure 3 Per patient burden of diabetic foot ulcer risk factors.

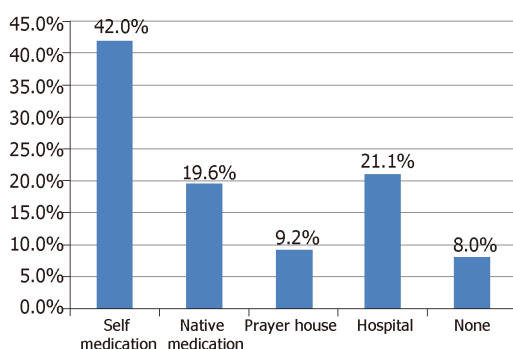


Figure 4 Preferred initial treatment options for patients with diabetic foot ulcers in Nigeria.

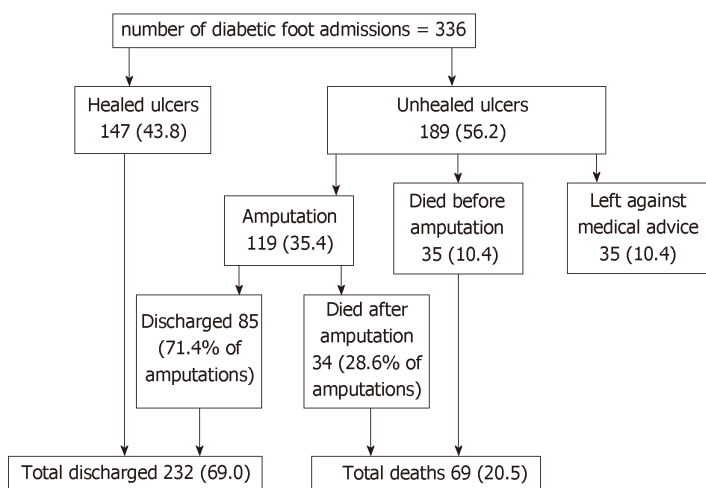


Figure 5 Outcomes of diabetic foot ulcer admissions in Nigeria.

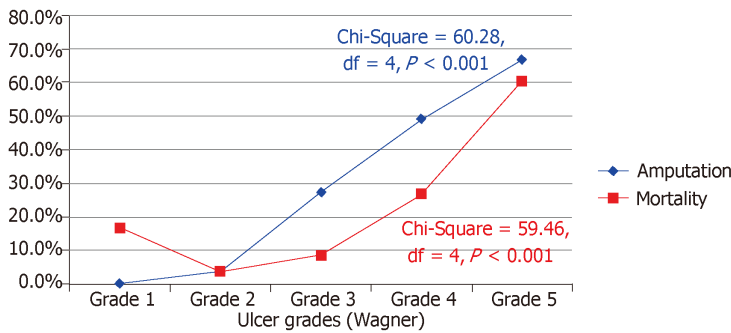


Figure 6 Amputation and mortality rates by ulcer grades.

ARTICLE HIGHLIGHTS

Research background

Diabetic foot ulcer (DFU) is a serious and costly complication of diabetes that is associated with high morbidity and mortality. However, DFU-related lower extremity amputation (LEA) and death are both preventable through appropriate healthcare measures.

Research motivation

The prevalence of diabetes in Nigeria is steadily rising with the country currently harboring the largest burden of diabetes in Sub-Saharan Africa. Evaluation of a disease burden helps in identifying healthcare gaps that need to be addressed. However, the current burden of DFU in Nigeria is largely unknown.

Research objectives

We evaluated the patient and ulcer characteristics as well as the outcomes of patients hospitalized for DFU in six tertiary healthcare centers in Nigeria over a one year period.

Research methods

In an observational study design, we followed up a total of 336 type 1 and type 2 diabetic patients who were hospitalized for DFU until they exited the hospital. Then we documented their baseline profile, clinical progress, disease outcomes and mode of exit.

Research results

The study revealed that DFU accounted for about a quarter of diabetes related hospitalization in Nigeria. It further showed that most of the affected patients lacked knowledge of foot care and resorted to self-medications or alternative medicine approaches following development of foot ulcer. Consequently, over three-quarter of the patients presented late to the hospital with advanced ulcer. The study revealed a high LEA and mortality rates of 35.4% and 20.5% respectively.

Research conclusions

We concluded that the burden of DFU in Nigeria is still substantial and decried the high degree of foot care ignorance and poor health-seeking behavior among patients with DFU in our country.

Research perspectives

We advocate for massive public enlightenment programmes about diabetic foot with emphasis on its prevention and timely treatment. Massive training of diabetes educators and podiatrists in Nigeria to improve foot care knowledge and foot care practice is strongly recommended.

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Observational Study

Prevalence and associated factors of hospitalization for dysglycemia among elderly type 2 diabetes patients: A nationwide study

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Abstract

BACKGROUND

The prevalence of older individuals with type 2 diabetes mellitus (T2DM) is increasing due to the aging population and improved medical care. These patients are very susceptible to disease and treatment-related hospitalizations, resulting in higher health care costs, morbidity, and decreased quality of life. However, data of treatment-related complications, especially dysglycemia-related hospitalizations, are lacking.

AIM

To assess the prevalence and associated factors for dysglycemia-related hospitalizations among elderly diabetic patients in Thailand using nationwide patient sample.

METHODS

statement: This study was approved by both the Institutional Review Board of the Royal Thai Army Medical Department and the Ethical Review Committee for Research in Human Subjects, the Ministry of Public Health of Thailand (IRB# S007h/54). Well-trained research nurses reviewed medical records and collected data into a case record form. Data entry into the case record form was then transferred to the central data management of the Medical Research Network of the Consortium of Thai Medical Schools to adjudicate that the process of data collection was compiled according to study protocol. The data management team was responsible for inquiries to study sites to verify data. Site monitoring was randomly performed in approximately 10% of study sites.

Informed consent statement: Patients were all patients were recruited from the outpatient clinic. Written informed consent was obtained from patients before enrolment.

Conflict-of-interest statement: The authors deny any conflict of interest.

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T2DM patients aged ≥ 65 years who received medical care at public hospitals in Thailand in the year 2014 were included. The prevalence of hospitalization due to dysglycemia within one year was examined. Multivariable logistic regression was performed to assess the independent factors associated with hospitalization due to hypoglycemia and hyperglycemia

RESULTS

A total of 11404 elderly T2DM patients were enrolled in this study. The mean age was 72.9 ± 5.5 years. The prevalence of hospital admissions due to diabetic ketoacidosis, hyperosmolar hyperglycemic state, hyperglycemic dehydration syndrome, and hypoglycemia among elderly T2DM patients in the year 2014 was 0.1%, 0.1%, 1.7% and 3.1%, respectively. Increased hospitalization due to hypoglycemia was associated with older age, female sex, had hypertension, dementia, lower body mass index, elevated hemoglobin A1C (HbA1C), decreased kidney function, insulin use. Increased hospitalization due to hyperglycemia was associated with dementia, depression, lower body mass index, elevated HbA1C, and insulin use.

CONCLUSION

The prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand was 4.9%. Close monitoring of blood glucose should be provided in high-risk patients for prevention and early detection for these complications.

Key words: Type 2 diabetes mellitus; Hospitalization; Diabetes in elderly; Dysglycemia; Hypoglycemia; Hyperglycemia

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Core tip: Currently, the numbers of older individuals over 65 years of age with type 2 diabetes mellitus (T2DM) are rising. However, data of treatment-related complication especially, dysglycemia-related hospitalization is lack. In this study, we conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. We demonstrated that the prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand was 4.9%. The close monitoring of blood glucose should be provided in high-risk patients for prevention and early detection for these complications.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common chronic disease worldwide that poses a significant crisis in the global health system^[1,2]. The associated morbidity and mortality can be caused by the disease itself or its associated multisystem complications that can increase in incidence and severity with prolonged disease duration. This disease, which has a higher prevalence in the older population, is also increasing in the elderly population likely due to the aging population^[3,4]. The prevalence of individuals over 65 years of age with T2DM in Thailand reached 17.2% as reported by the InterASIA study in 2003^[5]. Furthermore, increased age unsurprisingly has been found to be a significant predictor of higher health-care costs among diabetic patients^[6,7].

Older patients with T2DM are more susceptible to dysglycemia-related complications requiring hospitalizations and associated morbidity and mortality^[8]. Several recent studies showed that intensive glucose control strategies may derive less benefit and have demonstrated increased harms^[3,9]. Increasingly, the importance of specialized care and management for the geriatric population on clinical outcomes has

been recognized, and as such the treatment approach used T2DM in the elderly population should differ from those in the younger patients^[8]. However, DM care quality metrics established more than a decade ago have primarily focused on prevention of hyperglycemia and its complications^[9]. The current state of clinical practice in relation to established quality metrics and its impact on dysglycemic-related hospitalizations in elderly T2DM in Thailand is unknown. Thus, the aim of this study sought to determine whether prevalence of dysglycemia-related hospitalization in elderly T2DM in Thailand and the associated factors.

MATERIALS AND METHODS

Study design and population

This was an analysis on the DM/HT dataset in 2014^[10]. This was a nationwide survey conducted annually in Thailand to evaluate the status of medical care in T2DM patients who visited the public hospitals of the Thai Ministry of Public Health and the clinics in the Thailand National Health Security Office's program. The Inclusion criteria of this DM/HT survey consisted of T2DM patients aged ≥ 35 years who received regular medical care in the targeted hospital for at least 12 mo. Patients who received care at primary care units outside Bangkok and University hospitals were excluded from the study. A two-stage stratified cluster sampling method was used to select a nationally and provincially representative sample of T2DM patients in Thailand. The first stage of sample collection consisted of the provinces that constituted 77 strata. The second stage of sample collection was the hospitals' levels in each province, which were stratified into five strata according to the size of the hospital. These five strata were regional (> 500 beds), provincial (200-500 beds), large community (80-120 beds), medium community (60 beds), and small community (10-30 beds) hospitals. All regional ($n = 25$) and provincial ($n = 70$) hospitals were enrolled, but only 456 (62% out of 736) community hospitals were included. Of 456 community hospitals, 10%, 20%, and 70% were large, medium and small community hospitals, respectively (Figure 1).

All patients were recruited from the outpatient clinic. Written informed consent was obtained from patients before enrollment. This study was approved by both the Institutional Review Board of the Royal Thai Army Medical Department and the Ethical Review Committee for Research in Human Subjects, the Ministry of Public Health of Thailand due to the regulations of bureaucratic systems in Thailand. Well-trained research nurses reviewed medical records and collected data into a case record form. Data entry into the case record form was then transferred to the central data management of the Medical Research Network of the Consortium of Thai Medical Schools to adjudicate that the process of data collection was compiled according to study protocol. The data management team was responsible for inquiries to study sites to verify data. Site monitoring was randomly performed in approximately 10% of study sites. To focus on the hospital admission due to dysglycemia in elderly T2DM patients during 2014, we selected only patients aged ≥ 65 years for analysis in this study.

Data collection

Clinical characteristics, demographic information, medication, and laboratory data were collected using manual data retrieval from the medical record as described above. Body mass index (BMI) was stratified by using criteria for an Asian population^[11]. GFR was estimated based on age, sex, race and the most recent creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation^[12]. We examined the prevalence of hospitalization due to hypoglycemia and hyperglycemia in the year 2014. Hyperglycemia complication included diabetic ketoacidosis, hyperosmolar hyperglycemic state, and hyperglycemic dehydration syndrome.

Statistical analysis

Continuous variables were presented as mean \pm SD. Categorical variables were presented as count with percentage. Backward stepwise multivariable logistic regression analysis was performed to identify factors associated with hospital admission due to hypoglycemic and hyperglycemic complications. Odds ratio (OR) with 95%CI was reported. Possible interactions and collinearities were also tested. A P -value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL, United States).

A two-stage stratified cluster sampling method

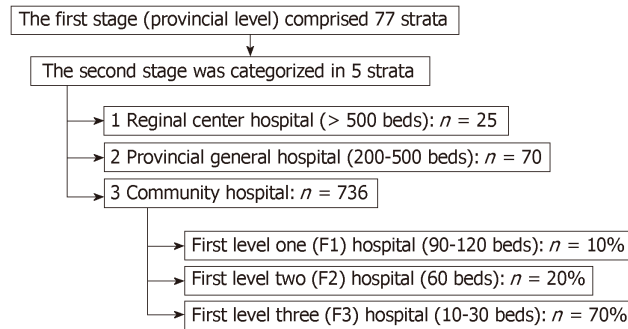


Figure 1 Flow chart of the participant selection.

RESULTS

Clinical characteristics

A total of 11404 elderly T2DM patients were included in the analysis. The clinical characteristics are summarized in Table 1. The mean age was 72.9 ± 5.5 years. Twenty-nine point four percent of the patients were aged > 75 years. Thirty-one point five percent were male. The mean diabetic duration was 7.5 ± 4.4 years. Twenty point nine percent of the patients used insulin. The mean BMI was 24.3 ± 4.3 kg/m². The mean hemoglobin A1C (HbA1C) was $7.5\% \pm 1.9\%$. The mean estimated glomerular filtration rate (eGFR) was 54.7 ± 21.5 mL/min per 1.73 m² (Table 1).

Prevalence of dysglycemia-related hospitalizations among elderly T2DM patients

The prevalence of dysglycemia-related hospitalizations among elderly T2DM patients during the year 2014 was 4.9% ($n = 558$). Among elderly T2DM patients, 11 (0.1%), 16 (0.1%), and 192 (1.7%) were admitted in hospital due to diabetic ketoacidosis, hyperosmolar hyperglycemic state, and hyperglycemic dehydration syndrome, respectively, whereas 356 (3.1%) were admitted in hospital due to hypoglycemia.

Associated factors associated with hypoglycemia-related and hyperglycemia-related hospitalizations

In multivariable analysis, older age, female sex, had hypertension, dementia, lower BMI, insulin use, elevated HbA1c, and decreased eGFR were associated with increased risk of hospital admission due to hypoglycemia, whereas overweight and obesity were associated with decreased risk of hospital admission (Table 2).

In multivariable analysis, dementia, depression, insulin use, and HbA1C 8.5% and above were associated with increased risk of hospital admission due to hyperglycemia, whereas overweight was associated with decreased risk of hospital admission (Table 3).

DISCUSSION

This was a large nationwide, multicenter, one-year period cross-sectional study that examined prevalence and associated factors for hospitalization due to dysglycemia among elderly T2DM patients during the year 2014. Despite a prior study that showed an overall decreasing trend in dysglycemia-related hospital admissions among elderly Thai T2DM patients^[13], our data describe that the prevalence of hypoglycemia-related hospitalization is higher than previously reported from United States^[7,14], England^[15], Canada^[16], Italy^[17], Denmark^[18] and South Korea^[19] (Table 4). This might be due to several reasons. First, the United States report consisted of combined data from both diabetic and non-diabetic patients. Second, our study included different sized hospitals, and the smaller sized hospital may have had limited availability of specialists. This may have negatively affected the quality of care for these special populations. Moreover, our study found that hypoglycemia-related hospitalization is higher than hyperglycemia in old diabetic patients. It could translate that elderly T2DM patients were likely to treat with rigorous glycemic control.

Our study revealed that age, female sex, hypertension, dementia, insulin use, low BMI, elevated HbA1C and low eGFR are associated with hypoglycemia-related hospitalizations. Older age and its association with severe hypoglycemia is consistent

Table 1 Baseline characteristics

Characteristics	All
N	11404
Age (yr)	72.9 ± 5.5
65-75	8055 (70.6)
> 75	3349 (29.4)
Male	3594 (31.5)
Duration of diabetes (yr)	7.5 ± 4.4
Hypertension	9831 (86.2)
Dyslipidemia	8048 (70.6)
Cancer	96 (0.8)
Dementia	24 (0.2)
Depression	106 (0.9)
Cerebrovascular disease	451 (4.0)
Cardiovascular disease	1129 (9.9)
Peripheral artery disease	82 (0.7)
Peripheral neuropathy	448 (3.9)
Diabetic retinopathy	688 (6.0)
Smoking	326 (2.9)
Insulin	2385 (20.9)
BMI (kg/m ²)	24.3 ± 4.3
< 17.5	391 (3.6)
17.5-22.9	3992 (36.6)
23.0-27.9	4655 (42.6)
≥ 28.0	1882 (17.2)
HbA1C (%)	7.5 ± 1.9
< 7.0	3760 (43.7)
7.0-8.5	2892 (33.6)
> 8.5	1947 (22.6)
eGFR (mL/min per 1.73 m ²)	54.7 ± 21.5
≥ 60	4034 (38.5)
< 60	6448 (61.5)
Prevalence (%)	
Dysglycemia-related hospitalization	558 (4.9)
Hypoglycemia-related hospitalization	356 (3.1)
Diabetic ketoacidosis related hospitalization	11 (0.1)
Hyperosmolar hyperglycemic state-related hospitalization	16 (0.1)
Hyperglycemic dehydration syndrome-related hospitalization	192 (1.7)

BMI: Body mass index; HbA1C: Hemoglobin A1C; eGFR: Estimated glomerular filtration rate.

with several reports^[19,20]. The Korean cohort demonstrated that older patients, females, several comorbidities such as chronic kidney disease and dementia, and insulin use were associated with a high risk of hypoglycemia^[19]. Another previous study found that in patients who are aged ≥ 80 years, severe hypoglycemia accounted for up to one in six hospital admissions^[20]. Older patients may have multiple factors that can predispose them to develop hypoglycemia such as polypharmacy, age-related changes in pharmacokinetics and pharmacodynamics, decreased hormonal regulation and counter-regulation, suboptimal intake of water and/or food, decreased intestinal absorption, and cognitive impairment^[21,22]. They may also be burdened with diseases that affect their ability to effectively metabolize hypoglycemic agents or respond to hypoglycemia, such as heart failure, liver disease, sarcopenia^[23] and kidney dysfunction^[24]. Female sex is associated with hypoglycemia related hospitalizations, consistent with a Korean cohort^[19]. Females are hypothesized to develop hypoglycemia more readily due to lower muscle mass, less tolerability of hypoglycemic symptoms, stricter diet control, and less access to medications than males^[25,26]. Our study found that elderly T2DM patients with hypertension are

Table 2 Crude and adjusted odds ratios of factors that were independently associated with hospital admission due to hypoglycemic complication, using multivariate analysis

Variables	Crude OR (95%CI)	P-value	Adjusted OR (95%CI) ¹	P-value
Age (yr)				
65-75	Reference		Reference	
> 75	1.87 (1.51-2.32)	< 0.001	1.79 (1.37-2.35)	< 0.001
Gender				
Male	Reference		Reference	
Female	1.64 (1.27-2.11)	< 0.001	1.63 (1.20-2.21)	< 0.01
Duration of diabetes (yr)	1.02 (1.01-1.03)	0.04		
Hypertension	1.48 (1.04-2.11)	0.03	1.63 (1.04-2.56)	0.03
Dyslipidemia	1.10 (0.87-1.39)	0.42		
Cancer	0.66 (0.16-2.68)	0.56		
Dementia	6.27 (2.13-18.43)	0.001	6.98 (1.80-26.98)	< 0.01
Depression	0.29 (0.04-2.11)	0.22		
Cerebrovascular disease	1.31 (0.81-2.12)	0.28		
Cardiovascular disease	1.33 (0.97-1.83)	0.08		
Peripheral artery disease	2.48 (1.07-5.72)	0.03		
Peripheral neuropathy	1.24 (0.75-2.03)	0.40		
Diabetic retinopathy	1.51 (1.04-2.20)	0.03	1.45 (0.93-2.24)	0.10
Smoking	0.58 (0.25-1.30)	0.18		
Insulin	3.36 (2.71-4.15)	< 0.001	3.47 (2.61-4.60)	< 0.001
BMI (kg/m ²)				
< 17.5	1.80 (1.20-2.71)	<0.01	1.80 (1.12-2.90)	0.02
17.5-22.9	Reference		Reference	
23.0-27.9	0.54 (0.43-0.69)	< 0.001	0.44 (0.33-0.60)	< 0.001
> 28.0	0.39 (0.27-0.57)	< 0.001	0.30 (0.19-0.48)	< 0.001
HbA1C (%)				
< 7.0	Reference		Reference	
7.0-8.5	1.42 (1.05-1.92)	0.02	1.41 (1.03-1.93)	0.01
≥ 8.5	1.97 (1.45-2.68)	< 0.001	1.49 (1.06-2.09)	0.02
eGFR (mL/min per 1.73 m ²)				
≥ 60	Reference		Reference	
< 60	2.59 (1.97-3.39)	< 0.001	1.53 (1.12-2.11)	0.01

¹Adjusted for age, gender, duration of diabetes mellitus, hypertension, dyslipidemia, cancer, dementia, depression, cerebrovascular disease, coronary artery disease, peripheral artery disease, peripheral neuropathy, diabetic retinopathy, smoking, insulin, body mass index, hemoglobin A1C and estimated glomerular filtration rate using backward (Wald) method. BMI: Body mass index; HbA1C: Hemoglobin A1C; eGFR: Estimated glomerular filtration rate; OR: Odds ratio; 95%CI: 95% confidence interval.

associated with hypoglycemic admissions. This finding provides novel insights. A possible cause might be due to polypharmacy since these elderly T2DM patients with hypertension had more comorbidities and more diabetic complications that affect hypoglycemic drugs metabolism.

A previous community-based study in Sweden study^[27] found that glycemic control is worse in patients with T2DM alone than in those with T2DM combined with hypertension. It is related to different degrees of insulin resistance and insulin secretion. Normotensive T2DM patients had reduced insulin secretion which accounts for the higher HbA1C and lower risk for developing hypoglycemia^[27]. Dementia in elderly T2DM patients has previously been described as correlating with a higher risk for hypoglycemia^[28,29]. The possible contributing factors in dementia patients included low compliance, medication errors and increased susceptibility to medication overdoses^[30]. Furthermore, dementia patients generally have lower dietary intake (*e.g.*, dysphagia), loss of functional capacity^[31], increased difficulty preparing food, and higher likelihood of hypoglycemic unawareness^[32,33]. Consequently, these patients may not be diagnosed with hypoglycemia until they develop severe life-threatening symptoms or signs that require hospitalization.

Exogenous insulin use as an associated risk factor for hypoglycemia makes inherent

Table 3 Crude and adjusted odds ratios of factors that were independently associated with hospital admission due to hyperglycemic complication, using multivariate analysis

Variables	Crude OR (95%CI)	P value	Adjusted OR (95%CI) ¹	P value
Age (yr)				
65-75	Reference			
> 75	0.81 (0.59-1.10)	0.18		
Gender				
Male	Reference			
Female	0.95 (0.72-1.27)	0.74		
Duration of diabetes (yr)	0.98 (0.97-1.01)	0.09		
Hypertension	0.63 (0.45-0.88)	< 0.01		
Dyslipidemia	0.86 (0.65-1.15)	0.31		
Cancer	1.09 (0.27-4.46)	0.90		
Dementia	10.44 (3.54-30.79)	< 0.001	19.08 (4.42-82.45)	< 0.001
Depression	2.58 (1.04-6.39)	0.04	3.98 (1.48-10.71)	< 0.01
Cerebrovascular disease	1.69 (0.97-2.93)	0.06		
Cardiovascular disease	1.70 (1.18-2.47)	< 0.01		
Peripheral artery disease	0.63 (0.09-4.56)	0.65		
Peripheral neuropathy	1.70 (0.98-2.95)	0.06		
Diabetic retinopathy	1.68 (1.07-2.65)	0.03		
Smoking	0.80 (0.33-1.94)	0.61		
Insulin	7.77 (5.85-10.32)	< 0.001	6.37 (4.30-9.45)	< 0.001
BMI (kg/m ²)				
< 17.5	1.79 (1.03-3.13)	0.04	1.59 (0.75-3.36)	0.23
17.5-22.9	Reference		Reference	
23.0-27.9	0.73 (0.53-0.99)	0.04	0.64 (0.43-0.95)	0.03
> 28.0	0.75 (0.50-1.14)	0.18	0.62 (0.37-1.05)	0.07
HbA1C (%)				
< 7.0	Reference		Reference	
7.0-8.5	1.93 (1.07-3.45)	0.03	1.83 (0.99-3.40)	0.05
≥ 8.5	9.88 (6.01-16.23)	< 0.001	5.97 (3.46-10.28)	< 0.001
eGFR (mL/min per 1.73 m ²)				
≥ 60	Reference			
< 60	2.04 (1.48-2.82)	< 0.001		

¹Adjusted for age, gender, duration of diabetes mellitus, hypertension, dyslipidemia, cancer, dementia, depression, cerebrovascular disease, coronary artery disease, peripheral artery disease, peripheral neuropathy, diabetic retinopathy, smoking, insulin, body mass index, hemoglobin A1C and estimated glomerular filtration rate using backward (Wald) method. BMI: Body mass index; HbA1C: Hemoglobin A1C; eGFR: Estimated glomerular filtration rate; OR: Odds ratio; 95%CI: 95% confidence interval.

sense^[34], especially when compared with medications that work primarily via other mechanisms, such as decreasing hepatic gluconeogenesis, decreasing intestinal absorption of glucose, increasing endogenous insulin sensitivity or increasing gluconeogenesis in the muscles. Elderly T2DM patients may be at higher risk for hypoglycemia as they may require insulin due to a longer chronicity of T2DM resulting in subsequent decreased endogenous insulin production, may require multiple hypoglycemic drugs, and often may have hepatic and/or renal impairments^[35]. Insulin should be used with caution in older adults. The administration of insulin therapy requires good visual acuity, motor skills and cognitive ability in the patient, especially for regimens that require multiple daily injections. This may be too complex for patients with several comorbidities and limited functional status. Our study also demonstrated that low BMI is associated with hypoglycemia related hospitalizations as well. A low BMI in elderly patients may cause higher risk for hypoglycemia due to lower muscle mass, suboptimal nutrition status and low glycogen storage^[36,37]. Our study additionally revealed that an elevated HbA1C is associated with hypoglycemic admissions. An elevated HbA1C may increase hypoglycemic risk due to more aggressive blood glucose control resulting in labile blood sugars, or a higher association with polypharmacy.

Table 4 Rate of dysglycemia related hospitalization stratified by country

Ref.	Outcome	Populations	Country	Rate of admission (per year)
Kaewput <i>et al</i> ^[13] , 2019	Dysglycemia	Type 2 diabetes, age ≥ 65 years old	Thailand	3.7%
Lombardo <i>et al</i> ^[17] , 2013	Dysglycemia	Any type of diabetes, subgroup; age ≥ 65 years old	Italy	6.7 per 1000 person-years
Lipska <i>et al</i> ^[7] , 2014	Hypoglycemia	General population, subgroup; age ≥ 65 years old	United States	612 per 100000 person-years
Fu <i>et al</i> ^[14] , 2014	Hypoglycemia	Type 2 diabetes, age ≥ 65 years old	United States	0.59 per 1000 person-years
Zhong <i>et al</i> ^[15] , 2017	Hypoglycemia	Any type of diabetes, age ≥ 65 years old	England	3.52 per 1000 person-years
Clemens <i>et al</i> ^[16] , 2015	Hypoglycemia	Patients with treated diabetes, age ≥ 65 years old	Canada	0.4%
Lombardo <i>et al</i> ^[17] , 2013	Hypoglycemia	Any type of diabetes, all age groups	Italy	0.4 per 1000 person-years
Kim <i>et al</i> ^[19] , 2016	Hypoglycemia	Type 2 diabetes, age ≥ 65 years old	Korea	9.3 per 1000 person-years
Lipska <i>et al</i> ^[7] , 2014	Hyperglycemia	General population, subgroup; age ≥ 65 years old	United States	367 per 100000 person-years
Lombardo <i>et al</i> ^[17] , 2013	Hyperglycemia	Any type of diabetes, all age groups	Italy	6.7 per 1000 person-years
Henriksen <i>et al</i> ^[18] , 2007	Diabetic ketoacidosis	General population, all age groups	Denmark	12.9 per 100000 person-years

Our study also supported that a low eGFR is associated with severe hypoglycemia in elderly T2DM patients, consistent with a prior report^[19]. Impaired kidney function has been shown to significantly increase the risk of hypoglycemia^[24]. The kidney may play an essential role in glucose metabolism and serve as a defense mechanism against hypoglycemia. Furthermore, impaired renal function limits insulin and other antidiabetic drugs clearance. Patients with advanced kidney disease may also have chronic inflammation and anorexia that leads to suboptimal nutrition and a reduction in glycogen stores^[24]. Hence, in addition to their risk of polypharmacy, cognitive decline, dementia, sarcopenia, and frailty, older adults with diabetes and impaired renal function are at risk of hypoglycemia^[24,38].

There were certain factors that appeared to protect against hypoglycemia-related hospitalizations. An elevated BMI is associated with decreased risk of hypoglycemia-related hospitalization, consistent with a prior Korean report^[19]. Overweight and obese patients may have protective factors against hypoglycemia such as a higher dietary intake and more glucose reserves in the form of higher glycogen storage, muscle mass, and fat mass.

Our report describes a higher prevalence of hyperglycemia-related hospitalizations than a previous report from the United States^[7] (Table 4). Similar to hypoglycemia-related hospitalizations, the difference in prevalence reported may be due to differences in study design, where other study included both diabetic and nondiabetic patients and our study included patients from varying hospital sizes.

This study showed that dementia, depression, insulin use, and HbA1C were associated with an increased risk of hyperglycemia-related hospitalization. Conversely, overweight patients had a decreased risk of hyperglycemia-related hospitalization. Dementia and depression as risk factors for hyperglycemia are supported by other reports^[39]. It may be due to poor compliance or inability to access medications^[31]. Insulin use and elevated HbA1C were also risk factors for hyperglycemic hospitalizations. This could be due to an association with more advanced diabetic diseases, higher patient prevalence of comorbidities and their associated complications and poor compliance. Conversely, overweight was found to be inversely associated with hyperglycemia-related hospitalizations. This can probably be explained by the fact that improved glycemic control is associated with weight gain.

The strengths of this study included the large representative patient cohort and the adjustment for multiple clinical variables. This report is a large nationwide cross-sectional study that included varying hospital sizes in Thailand. The multivariable logistical regression statistical analysis performed for assessing associated hypoglycemia and hyperglycemia-related hospitalization factors included several possible confounders such as age, gender, smoking, BMI, duration of diabetes, comorbidities, insulin use and laboratory parameter. The comorbidities included hypertension, dyslipidemia, cancer, dementia, depression, cerebrovascular disease, coronary artery disease, peripheral artery disease, peripheral neuropathy, and diabetic retinopathy. Laboratory parameters included HbA1C and eGFR for its final model adjustment.

There are several limitations of this study. First, data collection was performed using retrospective medical record review; therefore, incomplete data records with missing diagnoses cannot be verified. Second, the study population does not include patients from university hospitals. Consequently, the prevalence of hypoglycemia and

hyperglycemia-related hospitalization may be significantly underestimated. Third, we only measured hospitalization rates for dysglycemia. Dysglycemic events resulting in death prior to hospital admission were not captured. Fourth, we did not adjust the final statistical models for several possible confounders such as alcohol consumption and former tobacco use as this data was unavailable. One previous study had demonstrated an associated risk of hypoglycemia in T1DM patients with alcohol use^[40]. Conversely, another study did not show an association between alcohol consumption and risk of hypoglycemia in T2DM patients. Pietraszek *et al*^[41] had instead reported that acute intake of alcohol does not increase hypoglycemic risk in diet-controlled T2DM subjects; an alcohol-related hypoglycemic effect only occurred when sulfonylurea was co-administered. Furthermore, long-term alcohol use seems to be associated with improved glycemic control in T2DM probably due to improved insulin sensitivity. Former tobacco use may have also affected our outcomes if patients had decided to quit smoking due to health conditions related to the dysglycemic admission. Last, this was a cross-sectional study which had duration of only one year.

Serial blood glucose monitoring is already recommended for prevention of glycemic complications in elderly T2DM patients. However, the importance of timely and more frequent blood glucose monitoring in elderly T2DM patients with associated risk factors for dysglycemic hospitalization should be emphasized. Increased awareness, dedication of resources, and early intervention to prevent complications related to the management of diabetes is key to improving both the care of elderly patients and healthcare expenditure. The American Diabetes Association guidelines recognizes the need to incorporate geriatrics components into the assessment and management of diabetes^[9]. By recognizing the multiple risk factors in elderly T2DM patients identified in our study, a multidisciplinary approach^[9,42,43] to the individualization of a patient's optimal glucose level and its medical management can be performed. Future clinical practice improvements can then be compared to the prevalence rates identified in this study.

In conclusion, prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand was higher than developed countries at 4.9%. Elderly T2DM patients should be evaluated for risk factors of dysglycemia and may benefit from more frequent blood glucose monitoring and individualization of care in order to prevent dysglycemia-associated hospitalizations.

ARTICLE HIGHLIGHTS

Research background

The prevalence of older individuals with type 2 diabetes mellitus (T2DM) is increasing due to the aging population and improved medical care. These patients are very susceptible to disease and treatment-related hospitalizations, resulting in higher health care costs, morbidity, and decreased quality of life. However, data of treatment-related complications, especially dysglycemia-related hospitalizations, are lacking

Research motivation

This study would provide further support to the importance of regular monitoring of blood glucose, and glucose control should be individualized for the elderly T2DM patients. To further investigate the association between each variable and dysglycemia were assessed using multivariate logistic regression. Furthermore, it would motivate future research on whether more intensive monitoring of T2DM patients may allow earlier detection and prevention of dysglycemia-related hospitalization. The authors conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools.

Research objectives

We conducted this study to determine the prevalence and associated factors for hospitalizations due to dysglycemia among elderly T2DM patients in Thailand using nationwide patient sample.

Research methods

We conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. This study evaluated adult T2DM patients from 831 public hospitals in Thailand in the year 2014. We examined the prevalence of hospitalization due to hypoglycemia and hyperglycemia. Hyperglycemia complication included diabetic ketoacidosis, hyperosmolar hyperglycemic state, and hyperglycemic dehydration syndrome. The association factors between dysglycemia-related hospitalizations were assessed using multivariate logistic regression.

Research results

In this study, a total of 11404 elderly T2DM patients were enrolled in this study. The mean age

was 72.9 ± 5.5 years. The prevalence of hospital admission due to diabetic ketoacidosis, hyperosmolar hyperglycemic state, hyperglycemic dehydration syndrome, and hypoglycemia among elderly T2DM patients in the year 2014 was 0.1%, 0.1%, 1.7% and 3.1%, respectively. Increased hospitalization due to hypoglycemia was associated with older age, female sex, had hypertension, dementia, lower body mass index, elevated hemoglobin A1C (HbA1C), decreased kidney function, insulin use. Increased hospitalization due to hyperglycemia was associated with dementia, depression, lower body mass index, elevated HbA1C, and insulin use.

Research conclusions

We found that prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand had higher than developed countries. Elderly T2DM patients, especially in patients with associated factors, should be closely monitored blood glucose.

Research perspectives

Serial blood glucose monitoring should be recommended to prevent glycemic complications especially, in the elderly T2DM patients with associated factors cannot be over-emphasized. The early intervention to prevent further complications and adequate control of diabetes is a key to the reduction of complications of diabetes itself and treatment-related complications. Among elderly T2DM patients with multiple morbidities, the glucose control should be individualized.

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Prospective Study

Optimized health care for subjects with type 1 diabetes in a resource constraint society: A three-year follow-up study from Pakistan

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Institutional review board

statement: Ethical approval was obtained by the Institutional Review Board (IRB) of BIDE with approval/reference number: BIDE/IRB/Prof.Yakoob-IML/02/11/10/025.

Informed consent statement:

Informed consent was obtained from patients above 19 years of age and below 19 years were enrolled after obtaining informed consent from their parents.

Conflict-of-interest statement: The authors declare that they have no

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Abstract

BACKGROUND

Inadequate health infrastructure and poverty especially in rural areas are the main hindrance in the optimal management of subjects with type 1 diabetes (T1D) in Pakistan.

AIM

To observe effectiveness of diabetes care through development of model clinics for subjects with T1D in the province of Sindh Pakistan.

METHODS

A welfare project with name of "Insulin My Life", was started in province of Sindh, Pakistan. This was collaborative work of Baqai Institute of Diabetology and Endocrinology, World Diabetes Foundation and Baqai Medical University between February 2010 to February 2013. Under this project thirty-four T1D clinics were established. Electronic database was designed for demographic, biochemical, anthropometric and medical examination. Monthly consultation was part of the standardized diabetes care. All the recruited subjects with T1D were provided free insulins and related materials.

RESULTS

Out of 1428 subjects, 795 (55.7%) were males and 633 (44.3%) were females. Subjects were categorized into ≤ 5 years of age 103 (7.2%), between 6-12 years 323 (22.6%), between 13-18 years 428 (29.7%) and ≥ 19 years of age 574 (40.2%) groups. Glycemic control as assessed by HbA1c was significantly improved ($P <$

conflict of interest.

CONSORT 2010 statement: The guidelines of the CONSORT 2010 Statement have been adopted.

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0.0001) at three years follow up as compared to baseline in all age groups. Decreasing trends of mean self-monitoring blood glucose were observed at different meal timings in all age groups. No significant change was found in the frequency of neuropathy, nephropathy and retinopathy during the study period ($P > 0.05$).

CONCLUSION

This study gives us long-term longitudinal data of people with T1D in a resource constraint society. With provision of standardized and comprehensive care significant improvement in glycemic control without any change in the frequency of microvascular complications was observed over 3 years.

Key words: Insulin My Life; Type 1 diabetes; Insulin; Care; Pakistan

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Core tip: This study adds the three years follow up of subjects with type 1 diabetes (recent) by providing all healthcare related facilities. This study will highlight the impact of integrated and comprehensive care on the glycemic control and complications of diabetes.

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INTRODUCTION

Annually, more than 132600 subjects under 19 years of age have been diagnosed with type 1 diabetes (T1D) globally^[1]. It is also estimated that currently around 1106500 subjects (0-19 years) are living with T1D worldwide^[1]. Although, there are clear geographic differences in trends but the estimated annual increase in T1D is around 3%^[2]. In 2015, according to IDF, more than 7 million cases of diabetes are reported in Pakistan out of which 2% are suffering from T1D^[3]. The incidence of T1D in Pakistan has been reported as 1.02 per 100000 per year^[4].

Uncontrolled T1D can lead to microvascular and macrovascular complications mostly in young age group posing a challenge for health care professionals^[2,5]. Majority of subjects with T1D living in developing countries have minimum or no access to optimal care^[2,6]. As a result, these subjects are prone to acute and chronic complications of T1D affecting their quality of life^[7].

Limited studies are available on acute and chronic complications in people with T1D from Pakistan^[8]. A study conducted in the province of Sindh, showed higher rate of complication in subjects with T1D. Authors have reported that every fourth person with T1D is suffering from any one of the chronic complication while 2% subjects with T1D had diabetic ketoacidosis (DKA) and 21% had history of DKA^[9]. Similar trend was noted in smaller scale studies from this region^[8,10].

Inadequate health infrastructure and poverty especially in rural areas are the main hindrance in the optimal management of subjects with T1D in Pakistan^[11-13]. In Pakistan, 33% people lives with poverty and most of the populations (40%) does not receive basic health services^[14]. Health expenses are 0.7%-0.8% of gross domestic product of Pakistan, while 3.5% of total governmental budget. Overall health care system in Pakistan also offers the support for diabetes but subjects with T1D needs specific attention and optimal care^[7,14].

The study aims to observe effectiveness of optimal care for subjects with T1D including (free periodic consultations, education, dietary advice, provision of insulin and syringes, glucometers, and assessment of glycemic control through HbA1c 6 monthly) by establishing model clinics throughout the province of Sindh, Pakistan.

MATERIALS AND METHODS

A welfare project with name of “Insulin My Life (IML)”, was started in the province of Sindh in between February 2010 to February 2013. This was a collaborative work of Baqai Institute of Diabetology and Endocrinology (BIDE), World Diabetes Foundation and Baqai Medical University. Ethical approval was obtained by the Institutional Review Board (IRB) of BIDE with approval/reference number: BIDE/IRB/Prof.Yakoob-IML/02/11/10/025. Subjects with only T1D were included in this study. Informed consent was obtained from above 19 years of age and below 19 years were enrolled after obtaining informed consent from their parents by diabetes educators and physicians.

Three days’ workshop for doctors and educators

A total of 34 physicians with post graduate diploma in diabetes and 30 diabetes educators were identified from each district of Sindh. A three days structured training program as per the standard guidelines^[15,16] for the management of T1D and prevention of complications was designed for the physicians and for the educators separately.

Community based awareness and education sessions through camps and media coverage

More than 0.3 million teachers were sensitized about T1D specifically for the identification and management of emergencies in subjects with T1D. A total of 654 community based awareness camps and group sessions were held in the vicinity of identified clinics. In these awareness camps knowledge of self-monitoring blood glucose (SMBG), insulin using techniques, dose regime, optimal targets for glycemic control, adequate diet, physical activity, sick day rule, signs and symptoms of hypoglycemia and hyperglycemia were provided to subjects with T1D and their family members.

Printed educational material in English, Urdu and regional language (Sindhi) was also provided to subjects with T1DM, their parents and community. Eighteen televisions and 30 radio programmes in local and regional languages were also telecasted as a part of awareness campaign. A dedicated website www.insulinmylife.com was also launched to disseminate relevant information regarding T1D^[17].

Establishment of model Type 1 diabetic clinics and 24-h helpline service

Thirty-four model type 1 diabetic clinics were established at least one in each district of Sindh during the initial phase of the project (Figure 1)^[17]. A 24 h telephonic helpline service was made available to all project registrants. Through 24 h helpline service trained diabetes educators in consultation with primary consultant gave advises and sort out day to day problems including dose adjustments, hypo and hyperglycemic management. In case of emergency these registered subjects with diabetes were advised to contact emergency services.

Diagnosis of T1D

Biochemical parameters include glucose level in fasting, after 2-h of postprandial glucose, HbA1c, proteinuria and urinary ketones. Polyphagia, polyuria, polydipsia, weight loss history, and DKA history which are confirmed if previous records are present were recorded. In suspected cases of DKA, blood pH, HCO₃ was done^[9].

Provision of optimal care for T1D

No single subject had free insulin and blood sugar testing equipment at the start of the study. All registered subjects with T1D were asked to have free of cost consultation with physician, diabetes educators, free coverage for insulin and glucose testing equipment after every six months.

Subjects with other than T1D were excluded from the study. HbA1c, microalbuminuria test and consultation with a dietitian were offered after every 6 months. Free medical supplies including insulin, glucometers, glucose strips, lancets and insulin syringes, SMBG recording booklets were provided to the participants and they were asked to monitor their glucose readings with a record of these readings to be maintained in diaries. All children (less than and equal to 12 years of age) with T1D were referred to pediatrician as and when needed. The Growth chart with growth velocity was also followed throughout the study period.

Glycemic control assessment

Glycemic control was assessed by checking FBS and RBS at baseline and end of the study along with fasting HbA1c at baseline and after every 6 mo during 3 years

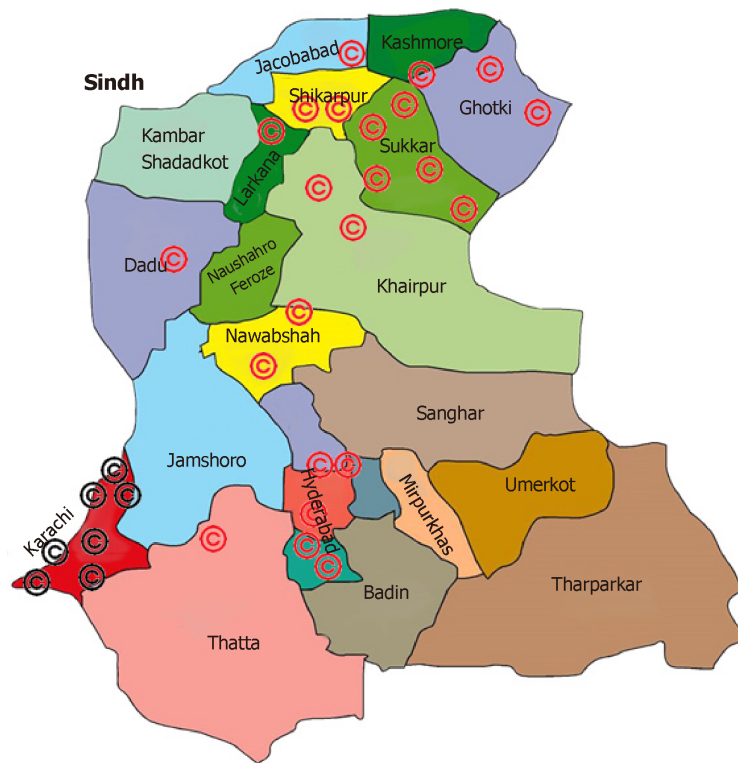


Figure 1 Type 1 diabetic model clinics in the province of Sindh.

follow up. Glycemic control was also assessed by using SMBG level at different meal timings in all age groups. Those who have HbA1c between 6.5%-8%, 9%-10% and $\geq 10\%$ were considered good, fair and poor control, respectively^[15,18].

Screening of micro vascular complications

Vista 20 direct ophthalmoscope was used for fundus examine. Retinopathy was confirmed by normal, microdots, hard exudates, pre-proliferative and proliferative or maculopathy. Protein $> 1+$ on dipstick (Combur 10, Roche Diagnostics) show nephropathy. Twenty-four hours quantitative analyses of urine for protein and creatinine were done. Neuropathy was known as absent touch or vibratory sensations of the feet. The 10-g monofilament and vibration sensation by 128 Hz tuning fork was used for touch sensation.

Data collection

Electronic and centralized database was designed to records demographic, biochemical, anthropometric and medical examination.

Statistical analysis

Statistical Package for Social Sciences (SPSS, version 20) was used for demographic and biochemical data. Continuous data was presented as mean, standard deviation and categorical data as numbers and percentages. Chi-square test was used for comparison of percentages and *t* test was performed for the mean difference comparison. Statistically significant was considered as *P*-value < 0.05 .

RESULTS

T1D model clinics in the province of Sindh-Pakistan are shown in Figure 1. Out of 1428 subjects 790 (55.3%) were males and 638 (44.7%) were females. Subjects were categorized into four groups according to age as ≤ 5 years of age ($n = 103$, 7.2%), between 6-12 years ($n = 323$, 22.6%), between 13-18 years ($n = 428$, 29.7%) and ≥ 19 years of age ($n = 574$, 40.2%) groups. Mean age (years) at the time of diagnosis in ≤ 5 years of age was $3.2 (\pm 1.5)$ and at the time of recruitment $3.5 (\pm 1.5)$, between 6-12 years was $8.3 (\pm 2.5)$ and $9.5 (\pm 1.9)$, between 13-18 years $13.7 (\pm 3.6)$ and $15.6 (\pm 1.7)$ and in ≥ 19 years of age groups $22 (\pm 6.3)$ and $25.7 (\pm 5.5)$, respectively. Duration of diabetes, family history of diabetes, weight, systolic and diastolic blood pressure were

noted in all age groups along with serum creatinine at baseline (Table 1).

Mean HbA1c at baseline *vs* end of study in ≤ 5 years of age subjects was (11.5 ± 2.04 *vs* 10.2 ± 2.12 , $P = 0.026$), between 6–12 years was (10.7 ± 2.28 *vs* 8.9 ± 2.24 , $P \leq 0.0001$), between 13–18 years was (10.5 ± 2.76 *vs* 8.7 ± 2.49 , $P \leq 0.0001$) and (9.6 ± 2.52 *vs* 8.5 ± 2.17 , $P < 0.0001$) in ≥ 19 years of age. A significant decrease in HbA1c was observed in all age categories ($P < 0.05$) (Table 2). The comparison of systolic, diastolic blood pressure along with fasting and random blood glucose were also presented in Table 2. Glycemic control as retrieved by HbA1c was significantly improved at final visit as compared to the baseline in all age groups. At baseline visit good glycemic control was observed in 3.6% subjects which increased to 25.9% at the end of study for ≤ 5 years of age. Similar trend can be seen in age 6–12 years (baseline 13.5% *vs* end line 36.3%, $P < 0.0001$), for age 13–18 years (14.7% *vs* 37.7%, $P < 0.00001$) and (26.8% *vs* 62.1%, $P < 0.0001$) for ≥ 19 years of age group (Table 3).

During three years follow up decreasing trends of mean SMBG were also observed at different meal timings in all age groups (Table 3). Comparatively lower mean SMBG values were observed compared to first month during the study period (Table 4). Graphical representation of microvascular complications was shown in Figure 2. The frequency of retinopathy shows a slight increasing (non-significant) trend, while the frequency of nephropathy and neuropathy almost remained the same during the study period. Significant improvement in HbA1c levels was observed in all age groups at end of study period (at 3 years) (Figure 3).

DISCUSSION

In this observational study, a three year follow up of people with T1D registered under project of IML in the province of Sindh Pakistan. Significant improvement in the glycemic control was noted with provision of comprehensive care, awareness and treatment free of cost.

Though it is difficult to achieve optimum glycemic control among adolescents, regardless the type of diabetes^[19], what we have observed that with proper care fewer people remained in the poor glycemic category and many people achieved fair to good control (Table 2). This has been shown by Diabetes Patient Verlaufs-dokumentation (DPV) registry also that healthy outcomes can be achieved in individuals with T1D when provided with optimized and personalized care^[20]. Good glycemic control not only important for decreasing the morbidity, but it can decrease diabetes related mortality rate as well as shown by Nordwall M related DM registry^[21,22]. On the other hand, without proper access to standardized care people with T1D suffer from adverse results even at an earlier age^[23]. In our study, over 3 years, people with T1D in each age category showed downward trend of HbA1c and this decline was statistically significant.

With provision of free glucostrips and glucometers it was made possible for study registered participants to check blood glucose at least 2 times/d. However, the annual cost per participant which include consultation fee, lab diagnosis, glucometers, insulins, strips, lancets and syringes, *etc.* was 61000pkr (436USD), per month 5083pkr (36USD) and per day 169pkr (1.2USD). SMBG profile of our cohort also showed downward trend at different mealtimes and this proves that by continuous education and pursuing its effectiveness enhances the motivation of subjects and their families to achieve better glycemic control. Study from Bulgarian suggests that due to families' devotion to diabetes control, children under six years achieved good glycemic control^[24]. Glycemic control with chronic complications was clearly shown by landmark study that is in Diabetes Control and Complication Trial (DCCT)^[25,26]. On the contrary association between poor glycemic control and increase risk of chronic complication was shown by several studies^[26].

In study from Southeast Sweden, prolonged uncontrolled HbA1c was closely associated with the development of severe complications in individuals with T1D^[22]. Another observational, population based study from DPV registry indicates that poor HbA1c was found to be a powerful biomarker for the development of retinopathy, nephropathy and neuropathy in patients with T1D^[27]. Time to onset of complications was also influenced by HbA1c as in the primary prevention cohort of DCCT^[22]. However, in our study rate of complication including nephropathy, and neuropathy remained the same throughout the study period through there was non-significant rise in frequency of retinopathy.

This study with best of our knowledge, concludes that it is first of its kind from Pakistan, giving us long-term longitudinal data of patients with T1D in a resource constraint society. With provision of standardized and comprehensive care significant improvement in glycemic control without any change in the frequency of

Table 1 Baseline demographic and clinical characteristic of study subjects

Variables	0-5 yr	6-12 yr	13-18 yr	19 and above
<i>n</i> (%)	103 (7.21)	323 (22.62)	428 (29.97)	574 (40.2)
Male	50 (48.5)	155 (48)	251 (58.6)	334 (58.2)
Female	53 (51.5)	168 (52)	177 (41.4)	240 (41.8)
Age at diagnosis (yr)	3.2 ± 1.5	8.3 ± 2.5	13.7 ± 3.6	22 ± 6.3
Age at recruitment (yr)	3.5 ± 1.5	9.5 ± 1.9	15.6 ± 1.7	25.7 ± 5.5
Duration of diabetes (yr)	0.3 ± 0.7	1.1 ± 2	1.9 ± 3.3	3.7 ± 5.4
Family history of diabetes	37 (35.9)	131 (40.6)	199 (46.5)	276 (48.1)
Weight (kg)	13.90 ± 2.61	28.31 ± 13.01	42.86 ± 10.64	53.11 ± 10.34
Serum Creatinine (mg/dL)	0.64 ± 0.18	0.80 ± 0.19	0.94 ± 0.43	0.92 ± 0.31
Cholesterol (mg/dL)	---	153.67 ± 33.06	155.63 ± 40.01	163.18 ± 28.58
Triglyceride (mg/dL)	---	83.25 ± 42.96	103.47 ± 73.66	94.46 ± 60.24
High density lipoproteins (mg/dL)	---	39.48 ± 12.21	42.46 ± 11.73	41.52 ± 10.45
Low density lipoproteins (mg/dL)	---	79.70 ± 26.07	86.34 ± 30.67	98.94 ± 30.44

Data presented as mean ± SD and *n* (%).

microvascular complications was observed over 3 years.

Limitations

In a resource constraint society like Pakistan, there is lack of an infrastructure for current study to provide health care system in a proper way. But, with available resources such kind of data was considered as the best available option. All the study participants during the study duration were coming to their respective medical centers for the required care. However, in remote areas the follow-up HbA1c was not completely available. This study helps us to know more about T1D in Pakistan than ever before, but much is still to be learned. This study need to be replicated at Nationwide level.

Table 2 Comparison of clinical measures from baseline to last follow up

Variables		Age (yr)			
		0-5 yr	6-12 yr	13-18 yr	19 and above
HbA1c (%)	Baseline (<i>n</i> = 1428)	11.5 ± 2.04	10.7 ± 2.28	10.5 ± 2.76	9.6 ± 2.52
	End line (<i>n</i> = 516)	10.2 ± 2.12	8.9 ± 2.24	8.7 ± 2.49	8.5 ± 2.17
	<i>P</i> -value	0.026	< 0.0001	< 0.0001	< 0.0001
Systolic blood pressure (mmHg)	Baseline (<i>n</i> = 1428)	108.3 ± 20.8	105.4 ± 14.7	106.3 ± 14.7	108.2 ± 14.9
	End line (<i>n</i> = 1428)	92.4 ± 10.3	99.1 ± 13.3	105.3 ± 13.3	112.7 ± 14.2
	<i>P</i> -value	0.001	0.0002	0.500	0.001
Diastolic blood pressure (mmHg)	Baseline (<i>n</i> = 1428)	74.6 ± 11.2	72.9 ± 10.6	73.7 ± 10.5	74.3 ± 10
	End line (<i>n</i> = 1428)	65 ± 6.6	66.2 ± 7.7	71.3 ± 8.3	74.6 ± 8.5
	<i>P</i> -value	0.001	0.0004	0.002	0.747
Fasting blood sugar (mg/dL)	Baseline (<i>n</i> = 1428)	199.2 ± 109.4	266.4 ± 118.9	282.4 ± 107	266.20 ± 112.6
	End line (<i>n</i> = 1428)	77.0 ± 2.64	293.86 ± 105.57	270.96 ± 104.83	252.43 ± 117.13
	<i>P</i> -value	0.059	0.292	0.550	0.415
Random Blood Sugar (mg/dl)	Baseline (<i>n</i> = 1428)	326.2 ± 161.1	342.4 ± 149.7	360.3 ± 135.4	338.9 ± 131
	End line (<i>n</i> = 1428)	522.33 ± 267.54	479.24 ± 189.99	394.63 ± 171.36	354.86 ± 157.43
	<i>P</i> -value	0.002	<0.0001	0.138	0.476

Data presented as mean ± SD.

Table 3 Age distributed glycemic status on first and last visit of the study period

Glycemic category	Age (yr)							
	0-5 yr		6-12 yr		13-18 yr		19 and above	
	Baselinevisit	Lastvisit	Baselinevisit	Last visit	Baselinevisit	Last visit	Baselinevisit	Last visit
Good glycemic control (HbA1c < 6.5%-8%)	3.6	25.9	13.5	36.3	14.7	37.7	26.8	62.1
Fair glycemic control (HbA1c 9%-10%)	14.3	40.7	16.2	19.1	14.7	14	26.8	12.4
Poor glycemic control (HbA1c ≥ 10%)	82.1	33.3	70.3	44.5	70.7	48	46.5	25.5

Data presented as percentages (%).

Table 4 Trends of mean self-monitoring blood glucose readings during the study period

Timing	Month 1	Year 1 (month 2-12)	Year 2(month 13-24)	Year 3(month 25-36)
Before breakfast				
0-5 yr	213.5	202.6	184.1	150.9
6-12 yr	197.3	173.6	193.1	146.2
13-18 yr	180.3	169.9	168.3	137.8
19 and above	163.2	151.5	139.6	138
After 2 h of breakfast				
0-5 yr	328.5	246.9	167.7	194
6-12 yr	215.5	209.5	203.5	190.3
13-18 yr	232.5	203.5	215.5	173.8
19 and above	200.3	178.6	166.2	184.6
Before lunch				
0-5 yr	248.2	265.8	132.3	140.2
6-12 yr	180.1	191.2	194.3	184
13-18 yr	200.3	189.7	192.8	148.9
19 and above	164	160.2	147.4	151.7
After 2 h of lunch				

0-5 yr	281.6	252.2	204.7	257.5
6-12 yr	269.8	226.8	237.3	207.6
13-18 yr	246.6	230.6	237.4	194.8
19 and above	223.1	211.8	202.8	203.8
Before dinner				
0-5 yr	299.2	276	216.2	239.9
6-12 yr	262	261.1	242.6	218.1
13-18 yr	255	228.4	234	205.5
19 and above	223.6	191.8	211.4	182.1
After 2 h of dinner				
0-5 yr	297.3	254.3	196.8	239.8
6-12 yr	239.3	247.8	244.7	192.5
13-18 yr	240.1	219.2	213.7	179.8
19 and above	217.4	195.9	212.1	180.7
Before sleeping				
0-5 yr	273.3	254.9	212.5	214.7
6-12 yr	230.7	230.6	181.1	211.3
13-18 yr	195	223.3	218.8	165.7
19 and above	167.3	167.9	196.4	195

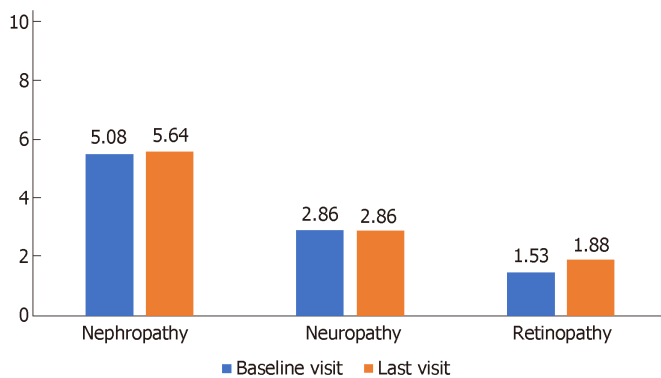


Figure 2 Complications rate of patients with ≥ 10 years diabetes duration.

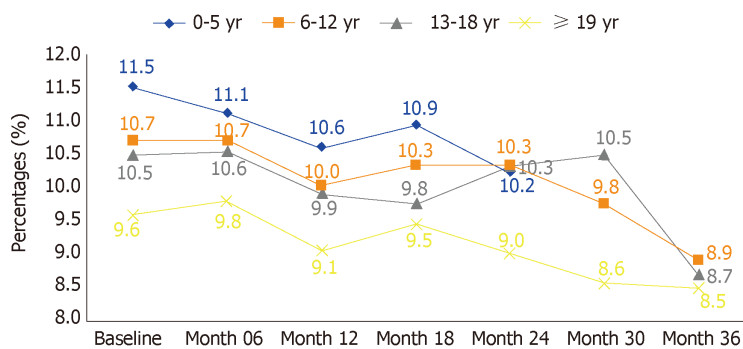


Figure 3 Trends of glycosylated hemoglobin (HbA1c levels).

ARTICLE HIGHLIGHTS

Research background

Inadequate health infrastructure and poverty especially in rural areas are the main hindrance in the optimal management of subjects with type 1 diabetes (T1D) in Pakistan.

Research motivation

The current study with lack of an infrastructure provides health care system in a proper way with available resources, to evaluate patient centered outcomes in the measurement of progression and treatment. Such kind of data was considered as the best available option.

Research objectives

The objective of this study is to observe the effectiveness of diabetes care through development of model clinics for subjects with T1D in the province of Sindh Pakistan.

Research methods

In this welfare project “Insulin My Life (IML)”, subjects with only T1D were included. Thirty-four model T1D clinic were established and total of 654 community based awareness camps and group sessions were held. All registered subjects with T1D were asked to have free of cost consultation with physician, diabetes educators, free coverage for insulin and glucose testing equipment after every six months. Glycemic control was assessed by checking FBS and RBS at baseline and end of the study along with fasting HbA1c at baseline and after every 6 mo during 3 years follow up. Glycemic control was also assessed by using self-monitoring blood glucose level (SMBG) at different meal timings in all age groups.

Research results

Out of 1428 subjects 790 (55.3%) were males and 638 (44.7%) were females. Glycemic control as retrieved by HbA1c was significantly improved at final visit as compared to the baseline in all age groups. At baseline visit good glycemic control was observed in 3.6% subjects which increased to 25.9% at the end of study for ≤ 5 years of age. Similar trend can be seen in age 6-12 years, 13-18 years, and ≥ 19 years of age group. Comparatively lower mean SMBG values were observed compared to first month during the study period.

Research conclusions

With provision of standardized and comprehensive care significant improvement in glycemic control without any change in the frequency of microvascular complications was observed over 3 years.

Research perspectives

This study helps us to know more about T1D in Pakistan than ever before, but much is still to be learned. This study need to be replicated at Nationwide level.

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Pharmacologic adjunctive to insulin therapies in type 1 diabetes: The journey has just begun

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Abstract

Treatment of type 1 diabetes (T1D) is currently based exclusively on insulin replacement therapy. However, there is a need for better glycemic control, lower hypoglycemia rates, more effective weight management, and further reduction of cardiovascular risk in people with T1D. In this context, agents from the pharmaceutical quiver of type 2 diabetes are being tested in clinical trials, as adjunctive to insulin therapies for T1D patients. Despite the limited amount of relevant evidence and the inter-class variability, it can be said that these agents have a role in optimizing metabolic control, assisting weight management and reducing glycemic variability in people with T1D. Specific safety issues, including the increased risk of hypoglycemia and diabetic ketoacidosis, as well as the effects of these treatments on major cardiovascular outcomes should be further assessed by future studies, before these therapeutic choices become widely available for T1D management.

Key words: Type 1 diabetes; Insulin; Adjunctive therapies; Cardiovascular risk

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Core tip: Adjunctive to insulin therapies in type 1 diabetes (T1D) may have a role in optimizing metabolic control, assisting weight management and reducing glycemic variability. Specific safety issues should be further assessed by future studies, before these therapeutic choices become widely available for T1D management.

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INTRODUCTION

Treatment of type 1 diabetes (T1D) is currently based exclusively on insulin replacement therapy, either by multiple daily injections (MDI) or *via* continuous subcutaneous insulin infusion ("insulin pumps") and closed-loop (also known as "artificial pancreas") insulin delivery systems. Whole pancreas and islet cell transplantations are alternative therapeutic options for carefully selected patients meeting specific eligibility criteria; still, these procedures are available only in a few number of specialized centers around the world, thus, being unavailable for the vast majority of people living with T1D^[1].

The idea of using agents from the pharmaceutical quiver of type 2 diabetes (T2D) as adjunctive to insulin therapies in T1D is not recent; back in 1985, Gin *et al*^[2], published their research on the effects of metformin on insulin sensitivity in patients with T1D and since then, a number of agents from different therapeutic classes have been tested in clinical trials. In the present article, we aim to discuss the rationale behind the use of adjunctive therapies in T1D, strengths and limitations of such an approach, as well as gaps in existing knowledge that deserve further evaluation by future research.

WHY IS THERE A NEED FOR ADJUNCTIVE THERAPIES IN T1D?

We live in the era of long- and short-acting insulin analogues (and the very recently introduced ultra-fast acting insulin analogues), which mimic physiological insulin release in a more effective way than human insulin, resulting in better metabolic control and lower hypoglycemia rates, as compared to the latter^[3]. Hence, what would adjunctive to insulin treatments contribute more to T1D management in everyday, clinical practice?

First, despite the progress been made during the past years, there is still an imperative need for better glycemic control in people with T1D. Results from a multi-centre, observational, cross-sectional study from Central and Eastern Europe (DEPAC Survey), involving more than 10000 individuals, proved that only 13.1% of T1D patients had glycated hemoglobin A_{1c} (HbA_{1c}) levels within target (< 6.5% / 47.5 mmol/mol)^[4]. Mean HbA_{1c} concentration among participants was 8.2% (66.1 mmol/mol), ranging from 7.7% (60.7 mmol/mol) to 9.8% (83.6 mmol/mol) among different countries.

Secondly, it is well established that people with T1D are in a greater risk of developing atherosclerotic disease, compared to the general population^[5]. Data from the United Kingdom General Practice Research Database (UK GPRD), indicate a hazard ratio for major cardiovascular disease (CVD) event (myocardial infarction, acute coronary heart disease death, coronary revascularizations, or stroke) of 3.6 (95%CI: 2.9-4.5) in men with T1D and of 7.7 (95%CI: 5.5-10.7) in women with T1D, compared to people without diabetes^[6]. Considering the impressive cardioprotective effects that specific agents used in T2D management have demonstrated in recent, randomized clinical trials^[7], it is reasonable to consider that these outcomes could be also applicable in T1D populations; however, this is something that remains to be proven by future research.

Thirdly, insulin resistance and adipose tissue inflammation as a result of increased body weight, are key components of T2D pathogenesis^[8]. A number of novel agents for T2D management, including glucagon-like peptide-1 (GLP-1) agonists and sodium-glucose co-transporter 2 (SGLT-2) inhibitors, exert optimal effects on body weight, through a variety of acting mechanisms^[9]. However, obesity is being increasingly recognized as a major health problem among people with T1D, as well. Results from a prospective study from the United States, where participants with T1D were being followed for a median of 18 years, demonstrated that overweight increased by 47% and the prevalence of obesity increased 7-fold during the above period, with 22.7% of people with T1D having body mass index (BMI) equal or greater to 30 kg/m²^[10], at the end of the study. In the same study, only seven percent of patients were on intensive insulin therapy (three or more daily insulin injections) at baseline (1986-1988), in contrast with the end of the follow-up period (2004-2007),

when this percentage reached 82%. Therefore, the aforementioned results could be attributed to the increasing rate of the adoption of a “Western” dietary model combined with poor physical activity by a significant proportion of the population worldwide, along with the intensification of insulin therapy during the last decades, which is known to positively correlate with weight gain^[11]. It is also known that weight, insulin resistance and CVD risk significantly interplay in people with diabetes. In a prospective cohort study following 603 patients with T1D for 10 years, classic insulin resistance-related factors, including dyslipidemia and waist-to-hip ratio, were found to predict future coronary artery disease events^[12], suggesting a strong need for effective management of traditional CVD risk factors, apart from T2D, in T1D as well.

There is data suggesting limitations in insulin availability and affordability in specific areas of the world, particularly for low-income patients^[13]. Reduction of insulin dose as a result of adjunctive therapies may prove helpful for those who consider insulin cost as a significant barrier to treatment adherence. Finally, there is no doubt that intensive compared to conventional glycemic control results in lower rates of both micro- and macro-vascular complications in individuals with T1D^[14]. However, this can be only achieved at a cost of increased incidence of hypoglycemia^[15], which is known to be related with cardiac dysrhythmias, CVD events and death^[16]. As a result, clinicians are often required to navigate “through stormy waters” and balance their clinical practice between intensive metabolic control and hypoglycemia, in a way that is not always easy.

AN OVERVIEW OF AVAILABLE EVIDENCE

Considering the above, there is an increasing amount of evidence suggesting that adjunctive to insulin treatments may assist glycemic control and weight management in T1D. Metformin has been shown to manifest optimal effects on BMI, total and low-density lipoprotein cholesterol concentrations, and total daily insulin dose (TDD), still not on HbA_{1c} which following a transient reduction during the first months of therapy, returns to its baseline values^[17]. The REMOVAL trial aimed to explore the effects of metformin on carotid intima media thickness (cIMT) in a sample of 428 T1D patients with multiple cardiovascular risk factors, aged over 40 years^[18]. Progression of mean cIMT was not significantly reduced with metformin, although maximal cIMT was significantly lower in the metformin group, as compared to placebo. Furthermore, metformin use has been linked to an increasing trend of the incidence of hypoglycemia^[19], a clue that requires further assessment by additional studies, particularly with the use of Continuous Glucose Monitoring systems. Overall, existing data do not support that metformin may improve glycemic control, though it might have a wider role in reducing CVD risk in people with T1D.

Dipeptidyl peptidase-4 (DPP-4) inhibitors have been tested in a very small numbers of trials and safe conclusions regarding these agents cannot be drawn. Their impact on glycemic control, seems to be non-significant^[20]; nevertheless, there is preliminary data indicating that sitagliptin might lower postprandial glucose levels in patients treated with a closed-loop system^[21] and preserve beta-cell function in individuals with slowly progressive T1D^[22]. In addition, DPP-4 inhibitors probably exert some important immunoregulatory actions^[23], thus, deserving further evaluation as adjunctive treatments in T1D or other autoimmune types of diabetes [Latent Autoimmune Diabetes in Adults (LADA), for example].

GLP-1 agonists have been demonstrated to significantly reduce HbA_{1c}, body weight and TDD (particularly bolus doses), when used in people with T1D^[24]. However, some studies raised concerns regarding their safety. In ADJUNCT ONE trial, 1398 patients with T1D were randomized to receive either liraglutide at varying doses or placebo, on top of insulin whose dose was adjusted according to a treat-to-target protocol over 52 wk^[25]. Symptomatic hypoglycemia was increased in all liraglutide groups as compared to placebo. Hyperglycemia with ketosis was more frequent in the group of patients receiving liraglutide at 1.8 mg, probably due to nausea related to its use and concomitant reduction of insulin dose. Similar reductions in HbA_{1c}, BMI and insulin dose have been observed with pramlintide, an injectable synthetic amylin analogue, being the only drug approved by the United States Food and Drug Administration, as an adjunctive to insulin therapy in T1D^[26]. Its use in everyday practice is limited by the fact that it should be subcutaneously administered three to four times a day before meals, being nonpractical for patients already on MDI regimens.

Probably, the most promising results in the field are coming from studies conducted with SGLT-2 inhibitors. These agents seem to contribute to better glycemic

control, lower body weight and insulin dose and most importantly, without increasing hypoglycemia rates^[27]. In addition, preliminary evidence suggests that they reduce glycemic variability^[28], a parameter that is being increasingly recognized to be related to the development of diabetic complications^[29]. On the other hand, a systematic review and meta-analysis of ten studies using SGLT-2 inhibitors on top of insulin in T1D, pointed towards an increased risk of diabetic ketoacidosis (DKA) in patients treated with these agents versus placebo^[27]. The review identified 16 incidents of both hyperglycemic and normoglycemic DKA in a total of 581 patients. Similar to the clinical experience from the use of SGLT-2 inhibitors in people with T2D, a consistent increase in the incidence of genital tract infections, particularly among females, has been documented in individuals with T1D, as well^[30]. As a result, gains and risks should be carefully balanced prior to the use of these drugs in everyday practice. **Table 1** summarizes the main advantages and pitfalls of the use of various therapeutic classes as adjunctive treatments in T1D.

A CRITICAL APPRAISAL OF RELEVANT STUDIES

The aforementioned results should be interpreted with caution, given that relevant data manifest specific weaknesses. First, the number of studies and patients involved is limited, rendering the extraction of definite conclusions challenging. Secondly, most of relevant studies have been designed to explore “conventional” outcomes, such as changes in HbA_{1c}, body weight and insulin dose. Data on glycemic variability, insulin resistance and oxidative stress markers are scarce, being inversely proportional to the significance that these parameters are gradually gaining, regarding their contribution to the development of diabetes complications.

Moreover, all of these studies are considering people with T1D as an homogenous group of patients, who will overall get - or not get - benefit from adjunctive therapies^[31]. It is well established that some people with autoimmune diabetes (either long-term T1D or LADA) share common pathophysiological and phenotypic features with T2D, thus, being difficult to draw the borderline between distinct diabetes types, in these cases^[32]. The need for individualized treatment approaches is emphatically highlighted by the paradigm of thiazolidinedione use in T1D; when pioglitazone was added on insulin in lean adolescents with T1D, it had no remarkable effect on glycemic control. In contrast, it resulted in a significant weight gain (+ 3.8 kg), as compared to placebo^[33]. Differently, rosiglitazone significantly decreased both HbA_{1c} and TDD, when it was administered in overweight subjects with T1D, where insulin resistance had an apparently important pathogenetic role in the development of metabolic disarrangement^[34].

Finally, trials with “hard” CVD end points in T1D populations are currently lacking, being necessary to clarify whether the remarkable effects of specific agents on CVD morbidity and mortality in people with T2D, can be translated to respective CVD benefits in people with T1D. **Table 2** summarizes the main limitations of available evidence on the use of various drugs as adjunctive treatments in T1D.

FUTURE CLINICAL RESEARCH STUDIES

Despite the initial enthusiasm for potential clinical implications of immunotherapy in T1D, research in the field has so far failed to prevent the onset or to reverse autoimmune diabetes^[35]. Stem cell therapies, immune ablation and standard immunosuppressants have been tested in several studies, nevertheless not being able to confirm the expectations derived from animal models, at least for the moment. Immune prevention strategies have tested low insulin doses and alternative administration routes (*e.g.*, oral insulin) to prevent diabetes in individuals at high risk of T1D, still showed no remarkable benefit^[36]. Studies using non-antigen specific immunosuppressive drugs demonstrated encouraging results in prolonging remission of T1D; however, at a cost of toxicity and side effects^[37]. Leptin might prove useful in suppressing glucagon concentrations^[38], but clinical benefits of its use in T1D should be further evaluated by clinical trials. As a result, safety and efficacy of these treatments in T1D remain an area for forthcoming studies.

CONCLUSION

In conclusion, despite the limitations of available evidence and the inter-class variability, adjunctive to insulin therapies may have a role in optimizing metabolic

Table 1 Advantages and pitfalls of the use of various therapeutic classes as adjunctive treatments in type 1 diabetes

Therapeutic class	Advantages	Pitfalls
Biguanides (metformin)	Optimal effects on body weight, lipid concentrations and insulin dose	Effect on HbA _{1c} not sustainable over time. Potentially greater risk of hypoglycemia
DPP-4 inhibitors	Immunoregulatory actions. Potential role in preserving beta-cell function. Good safety profile	Non-significant effect on HbA _{1c}
GLP-1 agonists	Significant reductions in HbA _{1c} , body weight and insulin dose (particularly bolus doses)	Greater risk of hypoglycemia and DKA
Amylin analogues (pramlintide)	FDA approved. Significant reductions in HbA _{1c} , body weight and insulin dose (particularly bolus doses)	It should be subcutaneously administered 3-4 times/d
SGLT-2 inhibitors	Optimal effects on HbA _{1c} , body weight, insulin dose and glycemic variability. They do not increase risk of hypoglycemia	Increased risk of DKA and genital tract infections
Thiazolidinediones	Reduction in HbA _{1c} and insulin dose in insulin-resistant T1D patients	Weight gain. Not effective in lean patients

HbA_{1c}: Glycated hemoglobin A_{1c}; DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; FDA: United States Food and Drug Administration; SGLT-2: Sodium-glucose co-transporter 2; T1D: Type 1 diabetes; DKA: Diabetic ketoacidosis.

control, assisting weight management and reducing glycemic variability in people with T1D. Specific safety issues, including the increased risk of hypoglycemia and DKA, as well as the effects of these treatments on major cardiovascular outcomes should be further assessed by future studies, before these therapeutic choices become widely available for T1D management. It seems that for both physicians and people with T1D, a fascinating journey to the land of pharmacologic adjunctive to insulin therapies has just begun.

Table 2 Main limitations of available evidence on the use of various drugs as adjunctive treatments in type 1 diabetes

Limitations of clinical trials	Small number of studies and patients involved
	Heterogeneity in study designs and explored outcomes
	“Conventional” outcomes explored: changes in HbA _{1c} , body weight and insulin dose. Data on glycemic variability, IR and OS markers are scarce
	Not taking into account the clinical heterogeneity of patients with T1D
	Trials exploring the effects of adjunctive treatments on “hard” CVD end points in T1D patients are currently unavailable

HbA_{1c}: Glycated hemoglobin A_{1c}; T1D: Type 1 diabetes; CVD: Cardiovascular disease; IR: Insulin resistance; OS: Oxidative stress.

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Retrospective Study

Risk factors in patients with type 2 diabetes in Bengaluru: A retrospective study

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Abstract

BACKGROUND

Risk factors such as hereditary, ecological, and metabolic are interrelated and contribute to the development of type 2 diabetes mellitus. Family history (FH) of diabetes mellitus, age, obesity, and physical inactivity are some of the risk factors for the development of type 2 diabetes.

AIM

To study various aetiological determinants and risk factors for type 2 diabetes in Bangalore, India. This retrospective study examined questionnaire from patients attending the Diabetes Clinic.

METHODS

Data on various parameters were obtained through a questionnaire from 533 patients on the first visit to the diabetes clinic. Data regarding various aetiological determinants and risk factors *viz.*: Genetic risk factor and few modifiable risk factors were collected. Chi-squared test was used for statistical analysis.

RESULTS

A higher incidence of type 2 diabetes in males and younger population was observed in Bangalore, India. Obesity and FH were significant risk factors for not only type 2 diabetes but also early onset of diabetes. In addition, maternal history of type 2 diabetes and consanguinity increased incidence of early onset type 2 diabetes.

CONCLUSION

Risk factors such as obesity and FH (maternal history of type 2 diabetes) and consanguinity may play an important role in screening of family members of type 2 diabetes patients which may lead to early intervention and reduced risk of subsequent complications. Moreover, susceptible population can be counselled for the management of the type 2 diabetes including periodic investigation of blood glucose levels and lifestyle changes.

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Key words: Type 2 diabetes mellitus; Young onset diabetes; Family history; Consanguinity; Diabetes risk factors; Obesity

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Core tip: Obesity, family history, maternal history of type 2 diabetes, and consanguinity play an important role in increasing incidence of early onset type 2 diabetes and should be used as parameters in screening of patients for type 2 diabetes. This may aid in initiating early life style changes to delay the onset of disease and/or reduce its severity. It may also lead to early diagnosis in high risk patients.

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INTRODUCTION

According to International Diabetes Federation (IDF) Diabetes Atlas eighth edition 2017, the IDF South-East Asia region is at the epicentre of the diabetes crisis which includes India at the second position behind China with a diabetes population of 82 million. In 2017, there were more than 72946400 cases of diabetes in India. As per the IDF estimates India would gallop to the first position with a diabetes population of 151 million by 2045^[1].

Hereditary, ecological, and metabolic risk factors contribute to the development of type 2 diabetes mellitus (T2DM) and are interrelated. Higher risk of diabetes with a family history (FH) of diabetes mellitus, age, obesity, and physical inactivity has been identified. Influence of dietary habits and lifestyle are critical and are responsible for higher occurrence and prevalence of obesity and diabetes in the urban population^[2,3]. In addition, individuals with T2DM are often accompanied with co-morbid conditions such as cardiovascular diseases, diabetic neuropathy, nephropathy, and retinopathy^[4]. This imminent crisis warrants study of aetiology and risk factors in the “real world” medical practice. “Real world” practice environment is an invaluable source of information and may reveal important trends in the aetiology, management, and treatment of diseases in “real world” medical practice.

Bearing in mind the need to preserve the naturalistic environment and manage with available staff resources for compiling data in the busy medical practice setting and yet generate meaningful conclusions. We at our diabetes centre initiated a data collection drive in form of a questionnaire. Our objective for the retrospective analysis was to gain insights into the patient profile and associated risk factors.

MATERIALS AND METHODS

Patients

This is a retrospective analysis of information obtained from patients with T2DM diagnosis attending the outpatient department (OPD) from July 2016 to July 2017. The patients who visited our OPD for the first time were required to complete a questionnaire. Our staff assists the incapable patients in completing the questionnaire. With the help of the questionnaire, information regarding various aetiological determinants and risk factors were sought namely: genetic risk factor-FH, demographic characteristics- age, gender, and ethnicity and among modifiable risk factors- obesity and physical inactivity.

The bases of categorising patients in different groups are described as follow: (1) FH: It includes information on history of T2DM in either or both parents or a first degree relative, accordingly they were classified as either positive with FH (FH+) or no FH (FH-) of diabetes mellitus; (2) Proposed classification of weight by body mass index (BMI) in adult Asians^[5]: < 18.5 kg/m²: Underweight; 18.5–22.9 kg/m²: Normal BMI; ≥ 23 kg/m²: Overweight; At risk 23–24.9 kg/m² increased; Obese I 25–29.9 kg/m² moderate; Obese II ≥ 30 kg/m² severe; (3) Physical activity: “Sedentary” was defined as patients who neither exercised nor walked at all. Among these, patients who were

working had a sedentary job profile. “Strenuous” was defined as people who did some form of exercise like walking, jogging, were trained for marathons or whose job involved significant physical activity like labourers, sales personnel, etc.

Statistical analysis

Chi-squared test as recommended by Campbell and Richardson was used. The confidence interval was calculated^[6].

RESULTS

Information on various parameters described above was obtained from 533 patients. Among these type 1 diabetes ($n = 2$), gestational diabetes mellitus ($n = 1$), chronic pancreatitis ($n = 1$), prediabetes ($n = 6$) those with no diagnosis of T2DM ($n = 2$), incomplete information ($n = 2$) were excluded. Thus, of the 533 questionnaires obtained, 519 were considered evaluable based on the information provided. The overall characteristics of patients are listed in Table 1.

Gender

Compared to females the proportion of male patients diagnosed with T2DM was significantly higher (55.68% vs 44.12%; $P = 0.0002$).

Obesity

Among 519 patients the information on BMI was available for 479 patients. It was noted that the patient population diagnosed with T2DM was significantly overweight or obese (88.30% vs 11.69%; $P < 0.0001$). In patients in the age group up to 40 years, the prevalence of obesity and diagnosis of T2DM was higher in males than females (80.76% vs 77.27%); whereas in patients 41-50 years the proportion was reverse (females vs males; 85.39 vs 79.31).

Physical activity

Among the patients who were diagnosed with T2DM, significantly higher proportion of patients followed a sedentary lifestyle compared to a strenuous one (74.89% vs 25.10%; $P < 0.0001$). Across the age groups the proportion of patients with a sedentary lifestyle or occupation was significantly higher compared to strenuous. However, even in patients in the latter group, obesity was prevalent; probably due to a diet conducive to weight gain (Table 2).

FH

Among 519 patients, 308 (59.34%) had a FH+ of diabetes. Compared to paternal, the maternal positive FH was higher in patients diagnosed with T2DM (59.68% vs 49.52%).

Risk factors for early onset type 2 diabetes

The patients were categorised into five age groups according to the age of onset of type 2 diabetes. It was noted that the proportion of patients with onset of diabetes at younger age groups (≤ 40 years and 41-50 years) was significantly higher, almost twice compared to older age groups (51 to ≥ 70 years) (Table 3).

Further subgroup analysis demonstrated that in the 125 patients with new onset or recent (< 3 mo) onset of diabetes in the one year study period (July 2016-2017), the proportion patients with young onset diabetes [YOD (aged ≤ 40 years)] was numerically the highest (Table 4).

FH

Among 519 patients, 308 had a FH+ of diabetes. Of these 39.93% patients were ≤ 40 years whereas those with FH- the percent patients with YOD were almost half (39.93% vs 20.85%, $P < 0.0001$) (Table 5). In non-obese, T2DM patients diagnosed early, about 80% had a FH+. On exclusion of consanguinity cases, 28.57% demonstrated FH+ as a risk factor. However, consanguinity was not a significant independent risk factor in non-obese patients since all consanguineous cases had positive FH (Table 6).

Effect of consanguinity

Among 506 patients for whom the consanguinity data was available, 141 patients reported consanguineous marriages of first-degree cousins (CG+). When these patients were grouped according to age of onset of diabetes, YOD was noted in approximately 35% patients. Also between age group comparison in CG+ patients indicated that, age group 1 (age ≤ 40 years) had almost twice as patients with T2DM than (CG+) age group 3 (age 51-60 years). After adjusting for obesity as a risk factor,

Table 1 Demographic characteristics of outpatient department patients diagnosed with type 2 diabetes mellitus included in the retrospective analysis

Characteristics	n (%)
Total patients	519
Male	289 (55.68)
Female	229 (44.12)
Transgender	1 (0.19)
Average age (yr)	53.28
Ethnicity	Indian

the consanguinity parameter was still a significant risk parameter for developing early onset diabetes (age ≤ 40 years) (CI: 1.7293 to 23.3104; $P = 0.0178$) (Table 7). For the remaining age groups, there was no significant difference between consanguinity and obesity as a risk factor for onset of T2DM.

Effect of hypothyroidism

Among female patients, hypothyroidism did not demonstrate any significant impact on age of onset of diabetes.

DISCUSSION

Gender roles and gender identity are influenced by a complex relationship between genetic, endocrine, and social factors^[7]. Gender is a vital genetic factor in regulation of homeostasis and affects susceptibility to cardio-metabolic risk factors. It also influences management of T2DM. Previous studies have demonstrated inconsistent gender distribution among patients diagnosed with T2DM. In 2013, IDF reported that there were 14 million times more men affected with diabetes than women^[8]. Studies in Northern India show female predominance whereas data from Southern India have reported higher prevalence in males. Few others have found no gender inclination in prevalence of T2DM^[9]. Data from our retrospective analysis reaffirm the higher prevalence of T2DM in males in Southern India. Men apparently are more disposed than women to the consequences of inactivity and obesity, conceivably due to variances in insulin sensitivity and regional fat deposition^[10].

Several studies have shown a high prevalence of abdominal obesity and generalized obesity as evaluated by body fat percentage in type 2 diabetic individuals^[11,12]. Approximately 44% of the diabetes burden, is attributable to overweight or obesity^[13]. In the current study, obesity was a major risk factor for T2DM similar to the findings in previous studies. The data showed that the proportion patients with T2DM being obese or overweight patients was eight times higher than patients who were non-obese/non-overweight. The proposed mechanisms linking the two are increased production of adipokines/cytokines, which may lead to insulin resistance and decrease in levels of adiponectin, ectopic fat deposition, mitochondrial dysfunction which not only decreases insulin sensitivity but also affects β -cell function^[14].

Apart from genetics, obesity is rooted primarily in improper diet or physical inactivity, however in the current study we observed that even in patients who had an active or strenuous lifestyle the prevalence of obesity was comparable to the sedentary group. This may imply that the nutritional transition, to highly-saturated fats, sugar, and refined foods and the transport facilities and increased stress, particularly in the urban populations may play an important role^[15].

A FH of diabetes is related with a range of metabolic abnormalities and is a strong risk factor for the development of T2DM. The elevated risk of T2DM is mediated, at least in part, by both genetic and common environmental components amongst family members^[15]. In our study more than half of the patients diagnosed with T2DM indicated FH+ of diabetes. Also the risk is greater with maternal than paternal FH, the findings in our study substantiate the same since approximately 10% higher risk was noted in patients with positive maternal FH.

Further subgroup analysis according to the influence of factors discussed previously on early onset T2DM showed that the proportion of patients diagnosed with T2DM in the younger age group (≤ 40 years -50 years) was twice as high than the older patient group (> 50 years), $P < 0.0001$.

Subgroup analysis of our data demonstrated that in the patients with new onset or

Table 2 Proportion of patients based on physical activity: Sub grouped based on body mass index

Groups	Age range (Y)	Sedentary (%)	Obese/OW (%)	Strenuous (%)	Obese/OW (%)	Sedentary vs strenuous P
Group 1	≤ 40	68.00	75	31.90	84.61	< 0.0001
Group 2	41-50	75.60	80.64	24.39	87.50	< 0.0001
Group 3	51-60	77.35	78.04	22.64	91.66	< 0.0001
Group 4	61-70	87.50	100	12.50	100.00	< 0.0001
Group 5	> 70	83.33	50	16.66	50.00	< 0.0001

OW: Overweight.

recent (< 3 mo) onset of diabetes in the one year study period (July 2016-2017), the proportion patients with YOD (aged ≤ 40 years) was numerically the highest compared to other age groups and significantly higher in patients in the age groups < 40-50 years compared to patients in the age group of > 50 years. Like it was pointed out in the discussion regarding FH+ the proportion of patients with FH+ were twice at higher risk of YOD than FH-, which reconfirms that FH+ could be an important factor increasing susceptibility to YOD.

Even in absence of obesity as a risk factor, FH+ had a significant influence on YOD with more than 80% with documented FH+. However, consanguinity was not a significant independent risk factor in non-obese patients. After adjusting for obesity as a risk factor, the consanguinity parameter was still a greater risk parameter for developing early onset diabetes (age ≤ 40 years).

Type 2 diabetes and its related complications enforce heavy health burdens worldwide and there have been not effective measures to fully manage with the diseases. T2DM affecting almost all populations in both developed and developing countries with high rates of diabetes-related morbidity and mortality. Multiple risk factors mainly obesity, FH specifically maternal history of type 2 diabetes and consanguinity play an important role to development of T2DM. To overcome these risk factors, screening of patient's family members is essential to identify in early stage and conquer this disease and improve the quality of life with increases in overall life span of individuals.

Strengths and limitations

We have used subjects of verified incident diabetes mellitus cases within south region of India (Bengaluru). The diversity of the cohort in terms of lifestyle and social characteristics due to metropolis city allows a robust assessment of the risk factors for diabetes mellitus. However, there are some limitations in this study. We used retrospective data that lacked detailed patient's information in detail on lifestyle as well as physical, hereditary, and some laboratory parameters. We have tried to use all possible parameters that define the risk factors most accurately. However, more detailed information on large set of population in future studies can help understand the risk of diabetes.

Table 3 Distribution of patients according to age of onset of type 2 diabetes mellitus

Age at onset of diabetes (yr)		%	P value
Group 1	≤ 40	32.30	0.0001 Group 1 vs 3 < 0.0001 Group 1 vs 4 < 0.0001 Group 1 vs 5 < 0.0001 Group 2 vs 3 < 0.0001 Group 2 vs 4 < 0.0001 Group 2 vs 5
Group 2	41-50	34.04	
Group 3	51-60	21.27	
Group 4	61-70	12.18	
Group 5	> 70	0.21	

Table 4 Age at new onset (< 3 mo) of diabetes (years)

Age at new onset (< 3 mo) of diabetes (yr)		n (%)
Total new T2DM diagnosis		97
Group 1	≤ 40	32 (32.98)
Group 2	41-50	27 (27.83)
Group 3	51-60	25 (25.77)
Group 4	61-70	7 (7.21)
Group 5	> 70	6 (6.18)

T2DM: Type 2 diabetes mellitus.

Table 5 Association of family history and age of young onset diabetes

Parameter	FH+	FH-	P value
FH n (%)	308 (59.34)	211 (40.65)	< 0.0001
Age of onset ≤ 40 yr (%)	39.93	20.85	< 0.0001

FH+: Positive family history; FH-: No family history.

Table 6 Association of Family history, consanguinity and young onset diabetes

Risk factor	Non obese T2DM patients (%)		P value
FH+	80	20	P < 0.0001
CG+	51.42	48.57	NS

T2DM: Type 2 diabetes mellitus; FH+: Positive family history; CG+: First-degree cousins.

Table 7 Association of consanguinity and young onset diabetes

Age group	T2DM patients (%)	T2DM obese patients (%)	P value
≤ 40 yr	34.50	21.83	P = 0.0178
51-60 yr	17.60	14.78	
	P = 0.0012	NS	

T2DM: Type 2 diabetes mellitus; NS: Not significant.

ARTICLE HIGHLIGHTS

Research background

The highest risk of diabetes with a family history (FH) of diabetes mellitus, age, obesity, and physical inactivity were identified. Influence of dietary practices and lifestyle factors are critical, making occurrence and prevalence of obesity and diabetes significantly more in the urban

population. As per the International Diabetes Federation estimates India would gallop to the first position with a diabetes population of 151 million by 2045.

Research motivation

“Real world” practice environment is an invaluable source of information and reveals important trends in the “real world” medical practice.

Research objectives

Our diabetes centre initiated a data collection drive in form of a questionnaire. Our objective for the retrospective analysis was to gain insights into the patient profile and associated risk factors.

Research methods

Information was obtained through a questionnaire from patients on their first visit to our diabetes clinic. Information regarding various aetiological determinants and risk factors *viz.*: Genetic risk factor and few modifiable risk factors was sought. Chi-squared test is used for statistical analysis.

Research results

Statistical analysis of the organized information obtained indicated a higher incidence of type 2 diabetes in males and younger population. Obesity, FH was significant risk factors for not only type 2 diabetes but also early onset of diabetes. In addition, maternal history of type 2 diabetes and consanguinity were found to play an important role in increasing incidence of early onset type 2 diabetes.

Research conclusions

Particular attention to risk factors like obesity, FH specifically maternal history of type 2 diabetes and consanguinity may be important for screening of patient's family members to initiate early intervention and reduce risk of subsequent complications. Moreover, susceptible population can be counselled regarding the risk, periodic investigation of blood glucose levels and lifestyle changes.

Research perspectives

Multiple risk factors mainly obesity, FH specifically maternal history of type 2 diabetes and consanguinity play an important role to development of type 2 diabetes mellitus. To overcome this risk factors, screening of patient's family members is essential to identify in early stage and conquer this disease and improve the quality of life with increases in overall life span of individuals.

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Observational Study

Management and control of type 2 diabetes mellitus in Lebanon: Results from the International Diabetes Management Practices Study Wave 6

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Institutional review board statement: Ethics committee's approval was obtained from participating centers where such committees are in place.

Informed consent statement: A signed written informed consent was obtained from all the participating patients before the application of any study-related procedures. This was available in Arabic and English.

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Abstract

BACKGROUND

Diabetes mellitus is a worldwide public health problem associated with significant complications. There is lack of data on the quality of care of patients with diabetes, specifically among the non-Western countries. Efforts have been made in Lebanon to better study the characteristics of patients with diabetes mellitus in order to improve glycemic control and prevent late-term complications.

AIM

To investigate control and therapeutic management of patients with diabetes mellitus in the current medical practice in Lebanon.

METHODS

Wave 6 of the International Diabetes Management Practice Study in Lebanon is an international and multicenter study involving selected countries.

RESULTS

Only 1 patient with type 1 diabetes and 595 patients with type 2 diabetes were included in Wave 6. Average age was around 60 years, with a mean body mass index of 30. The mean fasting serum glucose was 159.42 mg/dL, and the mean glycosylated hemoglobin (HbA1c) level was 7.98 with around 30% achieving an HbA1c target of < 7%. More patients were on oral anti-diabetic medications. Screening of diabetic complications has improved over the years. A large percentage is diagnosed with hypertension and dyslipidemia, the majority of

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whom were treated but only a small percentage were controlled.

CONCLUSION

Diabetes, with its associated dyslipidemia and hypertension, is still not very well controlled. Screening for diabetes complications has improved over the years. Patients need to have more proper care, and physicians need to follow diabetes guidelines, and to have a larger number of patients who have appropriate treatment of diabetes, hypertension and lipids.

Key words: Diabetes complications; Dyslipidemia; Hypertension; Blood pressure control; Glycemic control

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Core tip: This paper assessed the therapeutic management and control of patients with diabetes mellitus in the current medical practice in Lebanon. It also identified the proportion of subjects with target glycosylated hemoglobin, good blood pressure and lipid control, showing that it was suboptimal. Screening of diabetes-related complications is improving. Treating physicians and caregivers are becoming more aware of the importance of screening, but despite all their efforts, glycemic and metabolic control of the Lebanese type 2 diabetes mellitus population is still suboptimal.

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INTRODUCTION

Diabetes mellitus is a major public health issue worldwide that is associated with significant complications. The International Diabetes Federation (IDF) estimated that there would be an increase in the number of patients with diabetes worldwide, from 592 million persons in 2015 to 642 million in 2040^[1]. This rise is expected to be greatest in developing countries^[2]. In Lebanon, the prevalence of diabetes was found to be 7.8% for the population aged 20-79 years^[3]. A strong positive correlation was found between type 2 diabetes and both higher body mass index (BMI) and sedentary lifestyle^[4]. There is a lack of data on the quality of care of patients with diabetes, specifically among non-Western countries.

In Lebanon, a study demonstrated that glycosylated hemoglobin (HbA1c) was only reported in 40% out of the 204 diabetic patients assessed, with controlled diabetes (HbA1c < 7%) in 28.4%, and an HbA1c ≥ 8.5% in 38.3%^[5]. In addition, a high prevalence of micro-vascular diabetic complications was found among Lebanese with diabetes, with at least one third having neuropathy or retinopathy, and almost 50% having albuminuria. As for macro-vascular complications, 20% were found to have coronary artery disease and peripheral vascular disease (PVD), and 4.1% had cerebrovascular disease^[6]. Therefore, there is a need to better assess the current practices in diabetes management, and put some action into place in order to improve the quality of care for these patients. This is especially important for patients from both the United Kingdom Prospective Diabetes Study^[7] carried out on type 2 diabetes mellitus patients, as well as the Diabetes Control and Complications Trial^[8] carried out on type 1 diabetes patients, which showed that tight glucose control can prevent the onset and progression of microvascular complications to a significant extent. In the long-term, it can even prevent cardiovascular events, as was shown in their follow-up studies^[9,10].

In recognition of this, the International Diabetes Management Practices Study (IDMPS) was set to collect data in a standardized manner in order to primarily assess the therapeutic management and control of type 2 diabetes mellitus in the current medical practice in the Lebanese population, and reflect on the characteristics of Lebanese patients with type 2 diabetes. Previously, data were reported in Lebanon between the years 2006 and 2012^[11,12]. In this paper, data from Wave 2013-2014 of the

IDMPS were retrieved and analyzed. Secondary endpoints included the proportion of subjects with target HbA1c in compliance with the international recommendations' guidelines^[13,14], the frequency of hypoglycemia episodes, and the assessment of the health economic impact of type 2 diabetes and its complications.

MATERIALS AND METHODS

The IDMPS is an international, observational study conducted in multiple selected centers in different non-Western countries. This study included patients with type 1 and type 2 diabetes mellitus, who were selected in a random fashion from a representative pool of diabetic patients. The IDMPS consisted of six waves, beginning in the year 2006 and ending in 2014, with each wave being conducted yearly and consisting of a cross-sectional and longitudinal phase.

The cross-sectional phase was conducted through yearly surveys of 2 wk duration. The survey tried to assess the demographic characteristics of type 1 and type 2 patients with diabetes mellitus, along with their therapeutic management in the current medical practice. The longitudinal phase was conducted in the first two out of five waves, and consisted of a 9-mo follow-up period focused on different parameters. The sixth Wave of the study did not include a longitudinal phase.

The number of participating physicians and their profile was decided upon on a country basis, where the number of physicians chosen depended on the patient sample size, which was individualized in each country. Since each physician was asked to enroll 10 patients with diabetes mellitus, the number of physicians was determined by dividing the number of patients by 10. Physicians were selected randomly and were asked to recruit, during a 2-wk period, the first 10 patients with type 2 diabetes presenting to their clinics who were older than 18 years of age, as well as the first 5 patients with type 1 diabetes. In Lebanon, 80 sites were selected and the plan was to recruit 1,000 patients. A total of 77 physicians and 1,159 patients were included in 2011, and 60 physicians and 600 adult male or female patients were included into the sixth Wave in the year 2013. A signed written informed consent was obtained from all participating patients before the application of any study-related procedures. These consent forms were available in English as well as in Arabic for those participants who did not understand the English language. Ethics committee approval was obtained from participating centers where such committees are in place.

Exclusion criteria included concomitant enrollment in any other study, gestational diabetes, and cancer of the pancreas. Finally, 596 were included in the analysis population. Among these patients, one was a type 1 diabetic patient and the rest were type 2.

Data were collected on the demographic characteristics of the patients, their relevant medical history, the treatments prescribed for their diabetes, whether oral, insulin or others, the frequency of screening and testing for any observed diabetes complications, and cardiovascular risk factors. Data on metabolic control were also evaluated. The IMPDS 2006 and 2011 data were already published in separate papers^[11,12].

The SAP (version of 6 November 2014) used for this analysis aimed at describing the cross-sectional analysis of the sixth year (Wave 2013-2014). Proportions are reported as percentages of completely included populations, and means are reported as continuous variables \pm standard deviations.

RESULTS

Physician characteristics

In Lebanon, in Wave 2013-2014 of the cross-sectional IDMPS study, the total number of physicians enrolling at least 1 patient into the study was 60. Among these physicians, 47 were specialized and 13 non-specialized. Forty-three (71.7% were males) had a mean age of 52.18 ± 9.63 years. Forty-seven (78.3%) were endocrinologists and the remainder were either internists, general practitioners or cardiologists. The median length of duration of medical practice was 21.42 ± 9.47 . The mean total number of patients with diabetes seen per day among the included physicians was 16 ± 12 .

Patient characteristics

Six hundred patients with diabetes mellitus were recruited. Five hundred and ninety-six patients met the eligibility criteria for analysis. One patient with type 1 diabetes (representing 0.1% of all type 1 diabetes mellitus patients), and 595 with type 2

diabetes (representing 10.9% of all type 2 diabetes mellitus patients) were recruited. The analyses were done on patients with type 2 diabetes mellitus due to having only 1 patient with type 1 diabetes mellitus. Demographic characteristics of the type 2 diabetes patients are included in [Table 1](#).

The average population age was 59 years, with a higher percentage of the male population included. The average BMI of inclusion was 30, which is in the obese range, and this was higher than the one reported in 2011. As for female participants, 23.8 had a BMI ≥ 30 , and 47.9% had a BMI between 25-29. As for male participants, 25% had a BMI ≥ 30 , and 50% had a BMI between 25-29. The majority of patients were on oral glucose lowering therapy. In addition, the mean time of diabetes diagnosis was 8.8 years. Around 75% had a positive family history of diabetes. Around 70% of patients had dyslipidemia, the majority of which were treated, but metabolic control was not achieved, as only 40% of treated patients had low-density lipoprotein (LDL) less than 100 mg/dL, and 44% had Triglyceride levels less than 150 mg/dL. In [Table 2](#), data from 2013 was compared to that of 2011.

Glycemic control

Concerning patient management, the patient with type 1 diabetes mellitus was maintained on insulin therapy. As for the patients with type 2 diabetes, the majority were on oral anti-diabetic medications. The percentage of patients on insulin therapy was 3.7% in year 2013. Patients included had on average 8 years of diabetes. The percentage who had diabetes diagnosed more than 20 years ago was 6.4% and 70.1% of patients had health insurance (69% was public insurance, 19% was private insurance, and the rest had both forms of insurance).

The number of patients who had a glucose meter was found to be 439 (75.3%), where 406 (94.4%) did self-monitoring with their glucose meter. Regarding the glycemic control of the studied population, the mean fasting serum glucose was 159.42 mg/dL and the mean HbA1c level was 7.98, with around 30% achieving an HbA1c target of less than 7%. Eighty-eight (15.4%) patients experienced one hypoglycemic episode, among which 61.9% were on insulin treatment and 28.8% on combined oral hypoglycemic agents and insulin. As for severe hypoglycemia, it was more clearly found in the insulin-treated group, as expected. Out of all participants, 87.4% had metabolic syndrome based on the IDF definition. Among the included patients with diabetes, 318 (56.5%) mentioned that they do follow a healthy diet and exercise plan.

Screening for diabetes complications

The screening for diabetes complications, both micro-vascular and macro-vascular, by healthcare professionals appears to be improving. Patients were questioned about being screened at least once for diabetic complications during the last year before recruitment.

Concerning microvascular complications, it was found that in 2013, 65.7% of patients with type 2 diabetes were screened for retinopathy, 82.5% for nephropathy and 53.9% for peripheral neuropathy.

Foot examination screening occurred in 63.9%. Screening for cardiovascular disease occurred in 76.9% of patients. Screening for hyperlipidemia occurred in 95%, and screening for blood pressure control was in 86.1% ([Table 3](#)). When all late diabetes complications were combined, 38.6% of patients were found to have at least one complication ([Table 4](#)).

Blood pressure and lipid control

Three hundreds and fifty-seven (60.3%) patients reported having hypertension, among which 98% were being treated for it. The mean systolic blood pressure was 130, and the mean diastolic blood pressure was 77.9. Around 40% of patients had a systolic blood pressure of less than 130, and an equal number had a diastolic blood pressure less than 80. Concerning antihypertensive treatment, 33.3% of patients were treated with Angiotensin Converting Enzyme Inhibitors (ACEI) and 50% were on angiotensin II receptor blockers (ARB). The use of anti-platelets therapy was 42.4% in the year 2013. In addition, many patients (52.7%) were found to be smokers.

Dyslipidemia was found to be prevalent among our patients with diabetes mellitus (68.4%), and 94.8% were being treated ([Table 5](#)). Concerning the patients' fasting lipid profile, 39.2% of patients had an LDL < 100 mg/dL and their mean fasting LDL was 125.45 ± 135.12 mg/dL. In contrast, 44% of the type 2 diabetic population had triglyceride levels below 150 mg/dL in 2013, with a mean TG level of 178.53 ± 104.12 . In addition, 86.5% of treated patients were on statin therapy.

The Lebanese population of patients with type 2 diabetes has an increase in the mean BMI over the years, where the average BMI of inclusion was 30, which is in the obese range. This was higher than that reported in the years 2006 and 2011, when it

Table 1 Demographic characteristics of patients with diabetes mellitus

Characteristics of type 2 diabetes patients	
Age, mean \pm SD	59.37 \pm 10.85
Female, <i>n</i> (%)	307 (51.6)
Body mass index in kg/m ² , mean \pm SD	30.13 \pm 5.47
Waist circumference in cm, mean \pm SD	100.25 \pm 13.14
Residence, <i>n</i> (%)	
Urban	375 (63.0)
Rural	126 (21.2)
Sub-urban	94 (15.8)
Health insurance	
Yes	417 (70.1)
No	178 (29.9)
Time since diabetes diagnosis in yr, mean \pm SD	8.88 \pm 7.19
Family history of diabetes, <i>n</i> (%)	
Yes	436 (76)
No	138 (24)
Time since diabetes diagnosis in yr, <i>n</i> (%)	
≤ 1	85 (14.3)
1-5	162 (27.3)
5-10	137 (23.1)
10-20	171 (28.8)
> 20	38 (6.4)
Treatment of diabetes	
Diet and exercise alone	10 (1.7)
OGLD	421 (70.7)
Insulin treatment	22 (3.7)
OGLD treatment + insulin treatment	124 (20.8)
Other (No OGLD - no insulin - no diet)	18 (3)
Level of education, <i>n</i> (%)	
Illiterate	54 (9.1)
Primary	207 (34.9)
Secondary	228 (38.4)
University/higher education	104 (17.5)
Health insurance coverage, <i>n</i> (%)	
None	178 (29.9)
Public	288 (69.1)
Private	80 (19.2)
Public and private	49 (11.8)
Smoking habit, <i>n</i> (%)	
Never	150 (25.2)
Former	131 (22.0)
Current	314 (52.8)
Hypertension, <i>n</i> (%) [95%CI]	356 (60.2) [56.2%; 64.2%]
Patient receiving anti-platelet therapy, <i>n</i> (%)	236 (42.4)
Dyslipidemia, <i>n</i> (%) [95%CI]	401 (68.3) [64.4%; 72.1%]

OGLD: Oral glucose lowering drugs.

was 29.04 and 28.92, respectively. This could be due to a lack of compliance of patients to proper diet instructions, or the lack of sufficient education by physicians regarding the importance of losing weight and physical activity. BMI is known to be strongly and independently associated with type 2 diabetes mellitus, and this has been confirmed in Lebanese patients as well^[4,15].

Among the recruited cohort of patients with type 2 Diabetes in 2013, the mean

Table 2 Baseline characteristics of patients with diabetes mellitus in 2011 and 2013

	IDMPS 2013	IDMPS 2011
Total patients recruited	596	1157
Age in yr	59.37	56.42
Male/female, <i>n/n</i>	289/307	588/569
BMI, kg/m ²	30.13	28.92
BP control		
Mean BP	130.03/77.89	130.8/79.7
SBP < 130, %	40.2	43.7
DBP < 80, %	39.8	33
HTN treatment		
ACEI, %	33.3	36.2
ARB, %	50	50.1
Antiplatelet therapy, %	42.4	45.7
Dyslipidemia and management		
Diagnosed with dyslipidemia, %	68.3	68
LDL < 100 mg/dL, %	39.2	39.3
HDL ≥ 40 mg/dL, %	68.5	62.7
TG < 150 mg/dL, %	44.4	39
Statin therapy, %	86.5	82.7
Glycemic control		
Mean HbA1c	7.98	7.79
HbA1c < 7%, %	31.4	36.1

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HTN: Hypertension; ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin II receptor blockers; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides; HbA1c: Glycosylated hemoglobin.

duration of diabetes was 8.88 (\pm 7.19) years, which was similar to that found in 2011, which was 8.11 (\pm 7.23) years. As for the long-standing history of diabetes, being defined as more than 20 years, it was found to be 6.4% in the year 2013, compared to 30.3% (around one third) in 2011.

In addition, it was found that 30% were able to achieve an HbA1c target of less 7%, and this stresses the need for having more proper follow-ups of patients, as well as the need to follow international guidelines on diabetes^[9,10]. This would ensure that a higher percentage of patients have better control of their diabetes mellitus and are below the target required for HbA1c levels.

In our study, we also noted that insulin use as the sole treatment strategy has declined from 7.5% in 2006 to 3.7% in year 2013, and thus more patients were found to be on a combination of oral hypoglycemic agents and insulin. This could be interpreted by the fact that a lesser number of included patients in year 2013 had long-standing diabetes mellitus of more than 20 years duration. The other explanation could also be partly related to the vast new hypoglycemic agents, which were introduced to the Lebanese market during that period of time, namely the incretins and SGLT 2 inhibitor family.

Concerning the screening for microvascular complications, 65.7% of patients in 2013 with type 2 diabetes were screened for retinopathy, while this was 68.5% in 2011 and 53.2% in 2006. As for diabetic nephropathy and peripheral neuropathy, the screening proportions were 82.5% and 53.9%, respectively (as compared to being 63.4% and 47.9% in year 2006). Foot examination screening occurred in 63.9%. Screening for cardiovascular disease occurred in 76.9% of patients, which was 77.1% in the year 2011. It was noted that there were improved screening rates for diabetes complications in 2013 when compared to the year 2006, which probably shows that there is currently an increased awareness by physicians of the importance of screening to prevent late complications and poorer outcomes. However, it is important to note that screening methods have not increased when compared to the year 2011. However, upon checking other studies, it was noted that there was a worldwide failure to achieve glycemic targets. In the multinational, observational study that included 66,726 people with type 2 diabetes, who were enrolled from 28 countries across four continents (Asia, Africa, Europe and South America), 53.5% had

Table 3 Screening of diabetes-related complications in patients with diabetes mellitus

Screening for any diabetes-related complication, n (%)	
Yes	553 (97.9)
No	12 (2.1)
Screening for cardiovascular disease	
At least one time during the past year	376 (76.9)
Never	113 (23.1)
Eye screening	
At least one time during the past year	295 (65.7)
Never	154 (34.3)
Number of eye screening, mean (SD)	1.20 (0.57)
Screening for nerve damage	
At least one time during the past year	232 (53.8)
Never	199 (46.2)
Number of screening for nerve damage, mean \pm SD	1.75 \pm 1.01
Screening for kidney damage	
At least one time during the past year	425 (82.5)
Never	90 (17.5)
Number of screening for kidney damage, mean \pm SD	1.87 \pm 1.02
Screening for foot examination	
At least one time during the past year	297 (63.9)
Never	168 (36.1)
Number of screening for foot examination, mean \pm SD	1.90 \pm 1.08
Screening for blood lipid control	
At least one time during the past year	496 (94.8)
Never	27 (5.2)
Number of screening for blood lipid control, mean \pm SD	2.06 \pm 1.04
Screening for blood pressure control n (%)	
At least one time during the past year	286 (86.1)
Never	46 (13.9)
Number of screening for blood pressure control, mean \pm SD	2.57 \pm 1.17

microvascular complications and 27.2% had macrovascular complications^[16]. Similarly, in another study in India, a high prevalence of complications among patients with diabetes mellitus was reported, with 60% having neuropathies, 20% having cataracts, 15.4% having retinopathies, 32.3% having coronary heart disease, 11.5% having PVD, and 6.9% having a history of cerebrovascular accidents^[17].

With regards to hypertension, a large percentage (60%) were diagnosed with hypertension, the majority of which were treated. However, a small percentage had a systolic blood pressure of less than 130, and an equal number had a diastolic blood pressure less than 80. This again shows the need to have more proper control of hypertension in patients with diabetes mellitus. When comparing our results to another study, it was found that the frequency of WHO-defined hypertension was high in the non-insulin-dependent patients who were older than 55 years, with a percentage of 43% for males and 52% for females, in a sample of 5,842 patients attending ten diabetic clinics in the London area^[18]. The prevalence of hypertension was also looked at amongst 450 persons with diabetes mellitus in Benin city, and a prevalence rate of 54.2% was found^[19]. In addition, a study in Jordan was conducted, and the prevalence of hypertension defined as a BP > 130/80 or on medication for high blood pressure among type 2 diabetes patients was 72.4% (70.9% of males and 73.9% of females)^[20].

Concerning antihypertensive treatment, less patients were treated with ACEI (33.3%), as compared to an earlier report showing 46.2% usage in 2006 and 36.2% in 2011, respectively. As for ARB, 50% were on this medication, and this was the same in the year 2011. The pattern of antihypertensive treatment among type 2 diabetes patients were looked at in 9,975 patients obtained from an outpatient medical center of the Department of Veterans Affairs, and it was found that over 60% of patients were receiving ACEI or ARB^[21]. Another study was conducted in the rural

Table 4 Reported diabetes-related complications for patients with type 2 diabetes mellitus

Reported diabetes-related complications	n (%)
Any diabetes-related complication	264 (45.7)
Microvascular complications	
At least one microvascular complication	223 (38.6)
Retinopathy	77 (13.3)
Sensory neuropathy: abnormal sensation in distal limbs	125 (21.6)
Microalbuminuria: lab test	130 (22.5)
Proteinuria: dip stick	20 (3.5)
Dialysis	1 (0.2)
Amputation: below knee or above knee	2 (0.3)
Foot ulcer: active or past history	13 (2.2)
Macrovascular complications	
At least one macrovascular complication	122 (21.1)
Angina	31 (5.4)
Myocardial infarction/acute coronary syndrome	38 (6.6)
Heart failure	11 (1.9)
Stroke with partial recovery	4 (0.7)
Stroke with full recovery	8 (1.4)
Peripheral vascular disease	59 (10.2)
History of revascularization: <i>e.g.</i> , PTCA, CABG	41 (7.1)
Other complications	20 (3.5)

southeastern Australia, including a total of 449 patients with hypertension and diabetes, and 39% of those patients were taking ACEI, while another 39% were taking ARB^[22].

Despite clinical practice guidelines recommending the use of antiplatelet therapy in patients with diabetes, the use of anti-platelets therapy in our study was shown to have decreased from 2006 to 2013, from 60.6% to 42.4% in the year 2013. In another study, in a cohort of primary care patients with type 1 or type 2 diabetes, which was part of a larger project called the Vermont Diabetes Information System, the prevalence of antiplatelet use was found to be 54%^[23].

The same applies to lipid control, where 68% of our patients had dyslipidemia, and around 40% only had LDL < 100 mg/dL despite having 86.5% of patients on statin therapy. This shows that patients need more proper care, and physicians need to follow diabetes guidelines so as to have a larger number of patients who have appropriate treatment of their diabetes, hypertension and lipid control. Our results are better than another study done, which showed that diabetic dyslipidemia participants were being treated less often with lipid-lowering therapy^[24].

Conclusion

In conclusion, the Wave 6 results of the IDMPS study does reveal a promising improvement in the management of diabetes mellitus; however, not enough patients are actually achieving the target glycemic control. Thus, a national effort is needed in order to have a more appropriate control of diabetes, hypertension and lipids. Screening of diabetes-related complications is improving, but at a slow rate. Treating physicians are becoming more aware of the necessity of screening for complications, but despite all their efforts, the glycemic and metabolic control of the Lebanese type 2 diabetes population is still not sufficient. There should be more emphasis on educating the population about the importance of lifestyle modifications and obesity control, which will eventually help to improve type 2 diabetic patient outcomes.

Table 5 Dyslipidemia among patients with diabetes mellitus, n (%)

Dyslipidemia among patients with type 2 diabetes mellitus	
Patient diagnosed with dyslipidemia	402 (68.4)
Patient treated for lipids	380 (94.8)
Current treatment	
Statins	329 (86.6)
Fibrates	92 (24.2)
Nicotinic acid	0
Other treatment for dyslipidemia	3 (0.8)

ARTICLE HIGHLIGHTS

Research background

Diabetes mellitus is a common worldwide problem associated with significant morbidities and mortalities. This paper assesses the therapeutic management and control of patients with diabetes mellitus in the current medical practice in the Lebanese population. It identifies the proportion of subjects with target glycosylated hemoglobin (HbA1c) in compliance with the international recommendations' guidelines, and the factors that would be predictive of reaching target HbA1c. It also identifies the percentage of patients with diabetes who are screened for complications of diabetes. Furthermore, the percentage of patients who have hypertension or dyslipidemia, or who are taking antiplatelet treatment, is also tackled. In addition, the assessment of the health economic impact of patients with type 2 diabetes and its complications is tackled.

Research motivation

The results discuss our findings in relation to the treatment strategies and goals recommended by the American Diabetes Association and the European Association for the Study of Diabetes. This will also help physicians with better management and follow-up practices for patients with diabetes mellitus, and underscores the need for proper screening of complications and other risk factors commonly associated with diabetes mellitus.

Research objectives

In this paper, data from Wave 2013-2014 of the International Diabetes Management Practices Study (IDMPS) were retrieved and analyzed. Endpoints included the proportion of subjects with target HbA1c in compliance with the international recommendations' guidelines, the frequency of screening for diabetes complications and its risk factors, and the assessment of the health economic impact of type 2 diabetes and its complications.

Research methods

The IDMPS is an international study, observational in nature, conducted in multiple centers in non-Western countries, and included patients with diabetes mellitus who were randomly selected from a representative pool of diabetic patients. It involved six waves, beginning in 2006 and ending in 2014, with each wave being conducted yearly, and consisting of a cross-sectional and longitudinal phase. The cross-sectional phase was conducted through yearly surveys of 2 wk duration, and tried to assess the demographic characteristics of patients with diabetes mellitus, along with their therapeutic management in the current medical practice. The sixth Wave of the study did not include a longitudinal phase. A total of 60 physicians and 600 adult male or female patients were included into the sixth Wave in the year 2013. A signed written informed consent was obtained from all the participating patients before the application of any study-related procedures. Ethics committee approval was obtained from participating centers where such committees are in place. The SAP (version of 6 November 2014) used for this analysis aimed at describing the cross-sectional analysis of the sixth year (Wave 2013-2014). Proportions are reported as percentages of all the included populations, and means are reported as continuous variables \pm standard deviations.

Research results

Five hundred and ninety-five patients with type 2 diabetes were included in Wave 6, and only a single patient with type one diabetes mellitus was included. The average age was around 60 years, with a mean BMI of 30. The mean fasting serum glucose was 159.42 mg/dL and mean HbA1c level was 7.98, with around 30% achieving an HbA1c target of less than 7%. More patients were on oral anti-diabetic medications, probably related to the recent introduction of new oral medications. Screening of diabetic complications has improved over the years. A large percentage of patients were diagnosed with hypertension and dyslipidemia, the majority of which were treated, but a small percentage controlled.

Research conclusions

The results of the IDMPS study Wave 6 shows that there is a promising improvement in the

management of diabetes mellitus; however, it is still obvious that there are not enough patients achieving the target glycemic control. There is still not sufficient screening for diabetes-related complications and its risk factors. This is in concordance with the hypothesis we had prior to study initiation.

Research perspectives

This research offers new perspectives concerning the need for more awareness campaigns to both physicians and patients with diabetes mellitus. Thus, a national effort is needed in order to have a more appropriate control of diabetes, hypertension and lipids. Screening of diabetes-related complications is improving, but at a slow rate and not significantly compared to previous years. There should be more emphasis on educating the population about the importance of lifestyle modifications and obesity control, which will eventually help to improve type 2 diabetic patient outcomes.

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Are serum leptin levels predicted by lipoproteins, vitamin D and body composition?

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Abstract

BACKGROUND

Both obesity and vitamin D deficiency are important health issues in Pakistan. The connection between body composition, Vitamin D and leptin in young adults is important to be studied as body composition may affect bone health and therefore the possibility of osteoporosis in later life. Few studies have attempted to investigate the effect of body composition and leptin with vitamin D in adolescence.

AIM

To investigate the association of serum leptin with body composition, lipids and 25-hydroxyvitamin D (25OHD) in adults.

METHODS

This cross-sectional study was conducted on 167 apparently healthy adults. Demographics were recorded, bioelectrical impedance analysis was performed and clinical history noted. Serum leptin was measured using DIA source kit on ELISA and total 25OHD was measured on ADVIA-Centaur; Siemens. Total cholesterol and high density lipoprotein cholesterol were quantified using Enzymatic Endpoint Method and Cholesterol Oxidase-Phenol Aminophenazone method respectively. Biochemical analysis was done in the Departments of Pathology and Laboratory Medicine and Biological and Biomedical Sciences, Aga Khan University Hospital Karachi Pakistan.

RESULTS

Median age of the group ($n = 167$) was 20 years (IQR 27-20); 55.7% were females.

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Majority (89.2%, $n = 149$) of the study group was 25OHD deficient, 6% ($n = 10$) had insufficient serum 25OHD levels and 4.8% ($n = 8$) had sufficient D levels. Females, had higher median leptin levels [2.71 (IQR 4.76-1.66 ng/mL)] compared to their counterparts [1.3 (3.60-0.54 ng/mL), $P < 0.01$]. Multiple regression analysis suggested that basal metabolic rate, muscle mass, body fat percent, bone mass and serum 25OHD were the most contributing factors to serum leptin levels. Bone mass and serum 25OHD in fact bore a negative correlation with leptin.

CONCLUSION

The results indicate that basal metabolic rate, muscle mass, body fat percent, bone mass and serum 25OHD have an impact on serum leptin. Being a cross sectional study causal relationship between leptin and other variables could not be determined.

Key words: Leptin; Vitamin D; Obesity; Vitamin D deficiency; Body fat

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Core tip: The paper explored variables like anthropometric measurements, body composition, vitamin D and lipoproteins as predictors for serum leptin levels among representative population of healthy adults in Pakistan. The cross-sectional nature of this study could not elucidate causal relationships. However, it outlines important interplay between circulating leptin, vitamin D and body composition. The results indicate that basal metabolic rate, muscle mass, body fat percent, bone mass and serum vitamin D have an impact on serum leptin.

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INTRODUCTION

Quite a few cross-sectional studies have explored leptin and obesity in relation to Vitamin D deficiency (VDD) but the findings is inconsistent and conflicting^[1-4]. The limited available literature differs considerably in terms of study design, subjects studied and results reported, making it challenging to infer. The cause-effect relationship between leptin, VDD and obesity remains unclear. The link between vitamin D and obesity has been put forward by some genetic and secondary lines of evidences^[5-7]. Secondary factors common to both obesity and VDD are environmental aspects like dietary, racial, topographical, seasonal, and environmental pollution^[8-10]. Genetic studies have shown to link molecular variations of Vitamin D metabolism with obesity^[11-13]. The hereditary risk factors include polycystic ovary disease, cytochrome P450, locus 20q13, vitamin D-binding protein gene polymorphisms, and *aP2* gene. The VDR polymorphisms were described to be linked to adiposity phenotypes^[14,15].

Given the potential for positive associations of VDD and leptin concentration with obesity and the development of cardiovascular disease, the identification of modifiable lifestyle factors associated with leptin levels is vitally important to prevent obesity and its associated complications^[16]. Additionally, the connection between body composition, especially fat content in the body, and Vitamin D and leptin in young adults is clinically vital because management that modify body weight may affect bone health and therefore the possibility of osteoporosis in later life. Few studies have attempted to investigate the effect of body composition and leptin with vitamin D in adolescence^[17,18]. This study was conducted to analyze serum leptin concentrations in samples from adult volunteers and correlate it with body composition parameters, difference in gender, 25-hydroxy vitamin D (25OHD) and lipids.

MATERIALS AND METHODS

Study design and study group

This cross sectional analysis was done from June 2014 till January 2016, in the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine and Department of Biological and Biomedical Sciences, Aga Khan University Hospital Karachi Pakistan. Apparently healthy adult volunteers were invited to join in the research once informed consent was taken. Approval from the Aga Khan University Ethical Review Committee was taken for conducting this study (ERC number: 2810-Pat-ERC-13). A 4-d phlebotomy camp was set up in the Multidisciplinary Laboratory on the University campus for collection of blood samples. Subjects were excluded if they reported having had: intramuscular supplementation of Vitamin D in the last 6 mo, remarkable changes in weight or diet in the last 6 mo, any disease that may affect their Vitamin D levels or any known metabolic disorder. Subjects taking small dose of oral Vitamin D supplements, for example such that may be contained in a daily multivitamin pill (400 IU) since such a low amount would not significantly change serum 25OHD levels, were allowed to participate^[19].

Clinical history, anthropometric measurements and phlebotomy

An interviewer-administered history form tailored for the adult Pakistani urban population^[20], was used to assess the clinical history and health status of the study group. Waist circumference was measured in cm. Each subject's weight (kg) and height (m) were recorded, and the resultant body mass index (BMI) was calculated and reported in kg/m². BMI values were categorized as per the recommended reference ranges for the Pakistani population^[21]. A bioelectrical impedance analyzer (BIA) by Tanita was used to assess body composition variables of the study group. BIA is a validated method of estimating adiposity in both clinical and non-clinical settings^[22]. BIA measurements included total body fat percentage (reported as %), total body water percentage (reported as %), total muscle mass (reported in kg), basal metabolic rate (reported in kcal) and total bone mass (reported in kg). International body fat reference ranges for young adults were used to group subjects.

After informed consent blood was drawn in gel separation tubes and centrifuged at 3500 rpm for 5 min for separation of serum. Separated serum was transferred to aliquots, labeled with the participant's serial number and immediately stored at -30°C.

A BIA was used to assess body composition variables of the study group. BIA is a validated method of estimating adiposity in both clinical and non-clinical settings^[22]. BIA measurements included total body fat percentage, total body water percentage, total muscle mass, basal metabolic rate and metabolic age, total bone mass and visceral fat mass. International body fat reference ranges for young adults were used to group subjects.

Biochemical analysis and interpretation

Leptin was measured using manual ELISA on a kit from DIA source (Belgium). The microtiter wells were coated with a monoclonal anti-leptin antibody. 25OHD was quantified by chemiluminescence on ADVIA Centaur Immunoassay System (Siemens AG, Munich, Germany) in batch. The measuring range of the assay was 3.7-150 ng/mL. Total cholesterol was measured using an enzymatic endpoint method, while cholesterol oxidase-phenol aminophenazone method was used for analysis of high density lipoprotein (HDL) cholesterol by using kits from Randox Laboratories, United Kingdom on spectrophotometer. Non HDL was calculated by subtracting HDL from total cholesterol. To validate the biochemical results high and low levels of quality control material for leptin, 25OHD, cholesterol and HDL were run with the batches. Classification of VDD was based on the circulating 25OHD concentrations of less than 20 ng/mL. Levels between 20-30 ng/mL as insufficiency and > 30 ng/mL as sufficient D levels. Serum leptin was interpreted taking both BMI and gender into account.

Statistical analysis

All Statistical analysis was done on R-Software (R Version 3.5.3) and Statistical Package for Social Sciences (Version 22, SPSS Inc. Chicago, IL.). All parametric variables were reported as mean \pm SD and non-parametric as median with interquartile ranges 75th-25th. Differences between two groups of parametric variables were evaluated using independent samples *t*-test. To compare median values between two groups Mann Whitney *U* test was applied. Serum leptin and 25OHD were not normally distributed and hence were log transformed before correlation was conducted. As an initial step, we explored Pearson correlational relationships and applied Chi-square for categorical data between the measured independent variables taking serum leptin as dependent variable. Univariate analysis was conducted and variables that had significant association with leptin in univariate analysis at *P* < 0.2

were entered in multivariable analysis. Multivariate regression analyses were conducted for the dependent variable (serum leptin) and independent variables [BMI, basal metabolic rate (BMR), muscle mass, body water %, body fat %, bone mass, waist circumference, log 25OHD, male gender]. For working out the magnitude of associations between independent-dependent variables, beta weight of the variables was calculated. All tests were two-tailed, and the level of significance was set to P -value less than 0.05 as significant and < 0.01 was considered greatly significant.

RESULTS

Description of demographics and body composition of study group

A total of 167 subjects were enrolled; there was a near equal gender distribution with Female: Male 93:74. No statistically significant difference for age was found between the two genders. None of the subjects studied had any clinical signs of disease associated with obesity and were not on any medications. Of the total seven were cigarette smokers (4.1%); 2 smokers were females and the rest were males. Characteristics of the study population are summarized in [Table 1](#).

No statistically significant difference in BMI between males and females was noted however, the male adolescents had significantly higher body height and weight than their female counterparts ($P < 0.001$). According to the South-Asian Classification of weight status, 14.4% ($n = 24$) of the study-subjects were categorized as underweight (less than 18.5 kg/m²), 40.1% ($n = 67$) as normal (from 18.5 to 22.9 kg/m²), 22.2% ($n = 37$) as overweight (from 23 to 25 kg/m²) and 23.4% ($n = 39$) as obese (greater than 25 kg/m²).

Taking age and gender into account study subjects were additionally stratified according to body fat percent as under fat, healthy, over fat and obese. Upon stratification according to body fat percent 14.4% ($n = 24$) were obese while 12.6% ($n = 21$) were overweight. Body fat % was significantly higher amongst subjects who were overweight and obese as per BMI [mean body fat %: 27.5 (7.5)] as compared to non-obese [mean body fat %: 17.5 (6.8)] as depicted in [Table 2](#). Percent of body water in study subjects was ideal (*i.e.*, 45%-60% in females and between 50%-65% in males) in 80% subjects ($n = 142$), low in 6% ($n = 10$) and excessive in 9% ($n = 15$).

Biochemical specifics of study subjects

A Shapiro Wilk test ($P < 0.05$) and visual scrutiny of 25OHD and leptin histograms, Q-Q plots and box plots showed that both analytes were not normally distributed, with a skewness of 1.8 (S.E 0.18) and 3.5 (S.E 0.18) respectively and kurtosis of 5.9 (S.E 0.37) and 17.4 (S.E 0.37) respectively. Majority (89.2%, $n = 149$) of the study group was 25OHD deficient, 6% ($n = 10$) had insufficient serum 25OHD levels and 4.8% ($n = 8$) had sufficient D levels. Of a total of 167 sera analyzed, the lowermost and uppermost serum leptin levels noted were 0.02 and 45.3 ng/mL, respectively. Females had higher median leptin levels [2.71 (IQR: 4.76-1.66 ng/mL)] compared to males [1.3 (3.60-0.54 ng/mL), $P < 0.01$]. Overall 17 (10.1%) study subjects had raised serum leptin levels with 88.2% ($n = 15$) of these subjects being Vitamin D deficient. However, 89.3% ($n = 134$) of the subjects with normal serum leptin were also deficient in 25OHD.

Determinants of serum leptin

The [Table 2](#) describes the relevant Pearson correlation coefficients and their degrees of significance between leptin and other variables in the total study population and in both genders separately. A greatly significant, moderate positive linear relationship was seen between log of leptin and BMI, waist circumference and also with total body fat percent in females ($P < 0.01$). While a greatly significant negative association was prominent between log of serum leptin and total water percent and basic metabolic rate in the females ($P < 0.01$). In males log leptin exhibited positive relation with BMI ($P < 0.05$), waist circumference ($P < 0.05$) and body fat percent ($P < 0.01$) and inverse correlation with total water percent, muscle mass and log 25OHD ($P < 0.01$). Variables that had significant association with leptin in univariate analysis at $P < 0.2$ were entered in multivariable analysis.

Multiple regression analysis displayed that BMR, muscle and bone mass, body fat percent, 25OHD and gender were the utmost contributing factors to serum leptin levels. Bone and muscle mass and serum 25OHD bore an inverse relation with serum leptin. The value of R square and adjusted R square was 0.387 and 0.352 respectively specifying strong association between various independent and dependent variables. This showed a positive relationship of 38.7% between independent and dependent variable (leptin). Corrected R square indicated the fit of the model more closely in population. [Table 3](#) summarizes the results of multivariate regression analysis. It

Table 1 Demographics, body composition and biochemical parameters and comparison amongst genders and obese vs non obese

Description	Overall	Males	Females	P value	Overweight and obese (BMI > 23 kg/m ²)	Non obese (BMI < 23 kg/m ²)	P value
<i>n</i>	167	74	93		76 (50% Females)	91 (60.4% Females)	
Anthropometric parameters							
Median age (IQR) in yr	20 (27-20)	21(29-20)	20(23-20)	0.136	21 (35.5-20)	20 (21-20)	< 0.01
Mean height (SD) in cm	168 (9.2)	172 (8.2)	164.8 (8.7)	< 0.01	167.9 (9.7)	168.1 (8.8)	0.837
Mean weight (SD) in kg	65.0 (4.4)	70.2 (14.9)	60.9 (12.6)	< 0.01	75.1 (14.1)	56.6 (7.7)	< 0.01
Mean BMI (SD) in kg/m ²	23 (4.6)	23.7 (5.1)	23.7 (5.1)	0.056	26.6 (4.3)	19.9 (1.8)	< 0.01
Mean waist circumference (SD) in cm	79.1 (12.8)	83.3 (13.3)	75.7 (11.3)	< 0.01	86.9 (12.4)	72.5 (8.8)	< 0.01
Body composition							
Mean total body fat % (SD)	22.1 (8.7)	19.7 (9.0)	23.9 (8.0)	< 0.01	27.5 (7.5)	17.5 (6.8)	< 0.01
Under fat <i>n</i> (%)	44 (26.3)	5 (6.8)	39 (41.9)	< 0.01	3 (3.9)	41 (45)	< 0.01
Healthy <i>n</i> (%)	78 (46.7)	38 (51.4)	40 (43)	< 0.01	33 (43.4)	45 (49.4)	< 0.01
Over fat <i>n</i> (%)	21 (12.6)	11 (14.9)	10 (10.8)	< 0.01	19 (25)	2 (2.1)	< 0.01
Obese <i>n</i> (%)	24 (14.4)	20 (27)	4 (4.3)	< 0.01	21 (27.6)	3 (3.2)	< 0.01
Mean total body water %	55.7 (6.1)	57.8 (6.3)	54.1 (5.4)	< 0.01	52.3 (4.9)	58.6 (5.5)	0.255
Ideal body water <i>n</i> (%)	142 (85)	61 (82.4)	81 (87.1)	0.235	68 (89.4%)	74 (81.3%)	< 0.01
Mean muscle mass (SD) in kg	45.7 (7.9)	50.2 (8.4)	42 (5.1)	< 0.01	46.3 (8.7)	45.1 (7.2)	0.357
Median BMR (IQR) in kcal	1501 (1750-1336)	1732 (1875-1551)	1377 (1525-1300)	< 0.01	1690 (1892.5-1421.5)	1417 (1612-1308)	< 0.01
Median bone mass (IQR) in kg	2.5 (3-2.1)	2.9 (3.2-2.6)	2.2 (2.4-2)	< 0.01	2.9 (3.2-2.3)	2.2 (2.8-2.0)	< 0.01
Healthy bone mass <i>n</i> (%)	71 (42.5)	31 (41.9)	40 (43)	0.884	36 (47.3%)	35 (38.4%)	0.273
Biochemical parameters							
Median leptin (ng/mL)	2.2 (4.5-1.0)	1.3 (3.60-0.54)	2.71 (4.76-1.66)	< 0.01	3.0 (5.8-1.5)	1.7 (3.4- -0.7)	< 0.01
Elevated serum leptin <i>n</i> (%)	17 (10.2)	7 (9.5)	10 (10.8)	0.784	9 (11.8%)	8 (8.8%)	0.61
Median 25OHD (IQR) in ng/mL	12.1 (16.1-7.7)	12.5 (16-8.5)	11.5 (16.6-7.5)	0.877	12.1 (16.8-8.5)	12 (15.9-7.1)	0.479
25OHD status: Deficiency <i>n</i> (%)	149 (89.2)	69 (93.2)	80 (86)		67 (88.1%)	82 (90.1%)	
25OHD status: Insufficiency <i>n</i> (%)	10 (6)	5 (6.8)	5 (5.4)	0.03	4 (5.2%)	6 (6.5%)	0.585
Sufficiency <i>n</i> (%)	8 (4.8)	-	8 (8.6)		5 (6.5%)	3 (3.2%)	
Mean total cholesterol (SD) in mg/dL	175.8 (46.3)	172 (49.4)	178.8 (43.7)	0.345	180.8 (48.6)	171 ± 44	0.199
Mean HDL (SD) in mg/dL	41.1 (9.6)	40.4 (10)	41.6 (9.2)	0.44	40.5 (9.4)	41.540.5 ± 9.49.7	0.498
Mean non-HDL (SD) in mg/dL	134.7 (47.2)	131.5 (52.4)	137.2 (42.8)	0.446	14040.5 (9.450.1)	13040.5 ± 9.444.4	0.163

Parametric variables are reported as mean ± SD and non-parametric as median (interquartile ranges 75th-25th). Coefficients (*r*) and *P* values are calculated using Pearson's correlation analysis. *P*-value less than 0.05 was considered as significant and < 0.001 as highly significant. BMI: Body mass index; BMR: Basal metabolic rate; 25OHD: 25-hydroxy vitamin D; HDL: High density lipoprotein.

Table 2 Correlation of metabolic and biochemical factors with serum log leptin according to gender distribution

Gender	n	BMI (kg/m ²)	Waist circumference (cm)	Total body fat %	Total body water %	Muscle mass (kg)	BMR (kcal)	Bone mass (kg)	Total cholesterol (mg/dL)	Mean HDL(mg/dL)	Mean non-HDL(mg/dL)	Log 25OHD (ng/mL)
Male	74	0.256 ¹	0.256 ¹	0.47 ²	-0.331 ²	-0.499 ²	-0.018	-0.206	-0.053	-0.139	-0.023	-0.479 ²
Female	93	0.377 ²	0.362 ²	0.322 ²	-0.271 ²	0.02	-0.332 ^b	0.247 ^a	-0.169	-0.176	-0.135	-0.102
Overall	167	0.239 ²	0.181 ¹	0.445 ²	-0.364 ²	-0.426 ²	-0.047	-0.137	-0.72	-0.127	-0.045	-0.275 ²

¹Correlation is significant at the 0.05 level (2-tailed);

²Correlation is significant at the 0.01 level (2-tailed). Univariate analysis was conducted and variables that had significant association with leptin in univariate analysis at $P < 0.2$ were considered in multivariable analysis. Coefficients (r) and P values are calculated using Pearson's correlation analysis. P -value less than 0.05 was considered as significant and < 0.001 as highly significant. BMI: Body mass index; BMR: Basal metabolic rate; 25OHD: 25-hydroxy vitamin D; HDL: High density lipoprotein.

shows that among various parameters (like BMR, female gender and bone mass) which were contributing towards leptin levels body fat percent with standardized beta weight of 0.488 was the most influential factor in leptin values followed by muscle mass (beta of -0.265) and 25OHD (beta of -0.253). Body mass index, waist circumference and body water percent were not good predictors for serum leptin. Statistics from multi-collinearity displayed tolerance < 10 indicating good associations with leptin. Moreover, muscle mass, bone mass, 25OHD and male gender showed a negative influence on leptin levels.

DISCUSSION

This study explored variables like anthropometric measurements, body composition, 25OHD and lipoproteins as predictors for serum leptin levels among representative population of healthy adults in Pakistan. Majority of the subjects were D deficient (89.2%). This is not surprising as previous published papers have shown high prevalence of VDD both in Pakistanis living abroad or residing in Pakistan^[23,24]. Pakistan is among the sun-drenched countries and cutaneous production of vitamin D is possible throughout the year. However, despite this favorable climatic condition, research reports from our center showed widespread VDD^[25-28]. Serum 25OHD showed non-significant and poor association with BMI in this study; contradicting to reports from other part of the world which showed inverse relationship of vitamin D with BMI^[4]. The proposed hypothesis for could be attributed to Vitamin D lipophilic nature, that leads to 25OHD storage or sequestration in fat tissue. This volume-distribution effect could result in diminished vitamin D bioavailability and VDD in those with extra body weight. Reason for poor association of 25OHD with BMI could be that majority of our population was deficient in Vitamin D. Relationship could not be established as even non-obese subjects in this study were D deficient (88.7%). Similar trend was observed in previous study by our group, yet low vitamin D did not depict any change in BMD which may highlight the bone mineralization effects of raised leptin^[29].

Obesity has been associated with both leptin and VDD^[30,31]. Leptin regulates body fat mass and has a significant role in the control of body weight^[32]. Leptin is directly associated with fat mass, circulating leptin molecules carry information to the brain (hypothalamus) regarding the energy stored in adipose tissue, diminishing appetite and affecting energy expenditure^[33]. The receptors of leptin molecules are found in all places in the body indicating a general role^[34]. Obesity is considered a leptin resistant state resulting in excessive growth of adipose tissue and high serum leptin levels. An indirect effect is of UVB radiation exposure and the latitude gradients on VDD and obesity. Hoseinzadeh *et al*^[10] confirmed that the topographical factor varied with the variation in vitamin D levels in obese and the prevalence of VDD among African-American children and adolescents were 57% and 48.7% in Pennsylvania (latitude 40°N) and Wisconsin (latitude 43°N), respectively.

Going at par with VDD, obesity is also becoming a fast-growing health concern in Pakistan. Surplus body weight is a risk factor for many medical diseases, including cardiac disease, diabetes, arthritis and several cancers. According to recent studies, overweight and obesity have been shown to be related to low vitamin D status. For instance, according to a study in Oslo, Norway, the prevalence of VDD was highest in individuals with greater BMI regardless of their gender. Accumulating

Table 3 Multiple regression analysis of serum leptin determinants

Independent variables	Standardized β	T value	P value	95%CI for B	Collinearity statistics	
					Tolerance	VIF
BMI	0.017	1.057	0.292	-0.015-0.049	0.238	4.208
BMR	0.000	2.281	0.024	0.000-0.001	0.396	2.526
Muscle mass	-0.265	-2.995	0.003	-0.032-0.007	0.530	1.885
Body water (%)	0.308	1.875	0.063	-0.002-0.060	0.147	6.792
Body fat (%)	0.488	2.588	0.011	0.008-0.058	0.129	7.751
Bone mass	-0.191	-2.137	0.034	-0.032-0.007	0.523	1.912
Waist Circumference	-0.022	-0.201	0.841	-0.011-0.009	0.468	2.138
Log 25OHD	-0.253	-3.816	0.000	-0.720-0.232	0.944	1.060
Male gender	-0.186	-2.155	0.033	-0.419-0.018	0.522	1.916

Dependent variable: was log leptin. Univariate analysis was conducted and variables that had significant association with leptin in univariate analysis at $P < 0.2$ were entered in multivariable analysis shown above. Coefficients (r) and P values are calculated using Pearson's correlation analysis. BMI: Body mass index; BMR: Basal metabolic rate; 25OHD: 25-hydroxy vitamin D; HDL: High density lipoprotein.

epidemiological evidence suggests that hypovitaminosis D may be associated with obesity and related metabolic risks^[35]. The levels of 25OHD are associated with BMI, declining with increasing BMI^[36]. This is because adipocytes take up the Vitamin D from the blood, hence reducing the serum Vitamin D concentrations. This indicates that there is a link between Vitamin D levels and BMI which is a measure of relative weight based on an individual's mass and height. Hence, we hypothesized that lower Vitamin D levels would be noted with increasing BMI status among healthy individuals.

Similar to our study findings multiple studies have reported higher levels of leptin in females and this can be explained by differences in sex hormones^[37,38]. Our study findings showed that male gender inversely contributed towards leptin levels with standardized beta weight of -0.253.

The present study has few limitations. Firstly, it was a cross sectional study hence causal relationship between leptin and other variables could not be determined. Secondly, the sample size was limited to predict any valid conclusion for a large set of population. Some gender-specific interlink was also noted, but absence of data of follicle stimulating hormone, luteinizing hormone, estradiol and testosterone levels could not allow the confirmation of this hypothesis. However, the study overall adds to the scientific knowledge of the burden of vitamin D in relation to healthy individuals.

In conclusion, this cross sectional study confirmed the relation between basal metabolic rate, muscle mass, body fat percent, bone mass, serum 25OHD and serum leptin levels. Additional studies are obligatory to define the role of hypothalamic control of body in bone formation. Elucidation of such mechanism may lead to a novel therapeutic approach to osteoporosis. Another question that remains unanswered is whether overweight/obese requiring more intense vitamin D supplementation to achieve optimal levels of 25OHD. It will also be interesting how weight loss or vitamin D treatment could affect leptin levels in this population.

ARTICLE HIGHLIGHTS

Research background

Obesity is considered a leptin resistant state leading to elevated leptin levels. Obesity in turn has also been linked to Vitamin D deficiency (VDD).

Research motivation

Going at par with VDD, obesity is also becoming a rapidly growing health problem in Pakistan. Obesity has been associated with both leptin and VDD. Few studies have attempted to investigate the effect of body composition and leptin with vitamin D in this part of the world.

Research objectives

We investigated the relation of serum leptin with body composition, lipids and vitamin D in adults.

Research methods

In a cross sectional study design bioelectrical impedance analysis was performed on 167 apparently healthy adults along with recording their demographics and clinical history. Blood was drawn for biochemical analysis of serum leptin, total vitamin D, total cholesterol and HDL cholesterol.

Research results

Majority of the study group was vitamin D deficient. Females had higher median leptin levels compared to their counterparts. Overall 17 (10.1%) study subjects had raised serum leptin levels with 88.2% of these subjects being Vitamin D deficient. Basic metabolic rate, muscle mass, bone mass, body fat percent, lipids and 25-hydroxyvitamin D (25OHD) and gender were associated with serum leptin levels. Bone and muscle mass and serum 25OHD bore an inverse relation with serum leptin.

Research conclusions

The cross-sectional nature of this study could not elucidate causal relationships. However, it outlines important interplay between circulating leptin, vitamin D and body composition. The results indicate that basal metabolic rate, muscle mass, body fat percent, bone mass and serum vitamin D have an impact on serum leptin levels. 25OHD did not vary between obese and non-obese. This probably could be because > 80% of the study group was D deficient.

Research perspectives

Future studies addressing the causal relationships between these essential molecules, leptin, vitamin D and lipids is needed to better understand their use as biomarkers of risk for obesity and diseases associated with obesity.

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Evolving spectrum of diabetic nephropathy

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Abstract

Diabetes remains an important health issue as more patients with chronic and uncontrolled diabetes develop diabetic nephropathy (DN), which classically presents with proteinuria followed by a progressive decrease in renal function. However, an increasing proportion of DN patients have a decline in kidney function and vascular complications without proteinuria, known as non-proteinuric DN (NP-DN). Despite the increased incidence of NP-DN, few clinical or experimental studies have thoroughly investigated the pathophysiological mechanisms and targeted treatment for this form of DN. In this review, we will examine the differences between conventional DN and NP-DN and consider potential pathophysiological mechanisms, diagnostic markers, and treatment for both DN and NP-DN. The investigation of the pathophysiology of NP-DN should provide additional insight into the cardiovascular factors influencing renal function and disease and provide novel treatments for the vascular complications seen in diabetic patients.

Key words: Diabetic nephropathy; Non-proteinuric diabetic nephropathy; Diabetes; Kidney vascular complications

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Core tip: Diabetes remains an important health issue as more patients with chronic and uncontrolled diabetes develop diabetic nephropathy (DN). In recent years, an increasing proportion of DN patients have a decline in kidney function and vascular complications without proteinuria, known as non-proteinuric DN (NP-DN). This manuscript advances this discussion by examining the potential pathophysiological mechanisms, diagnostic markers, and treatments relevant to NP-DN. Furthermore, it illustrates the significance of

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INTRODUCTION: PATHOPHYSIOLOGY OF DIABETIC NEPHROPATHY

Diabetes remains an important health issue as an increasing number of patients with chronic and poorly controlled diabetes develop diabetic nephropathy (DN)^[1-4]. The main risk factors associated with the development of DN include hypertension, poor glycemic control, smoking, and dyslipidemia^[5]. Among several ethnicities, Native Americans have the highest incidence of DN followed by Asians, Hispanics, African-Americans, and Caucasians^[6]. Several genetic polymorphisms are also associated with development of DN, including angiotensin type 2 receptor and angiotensin converting enzyme (ACE)^[7-10]. In recent years, the number of patients seeking dialysis for kidney-related disorders has increased with the rise in DN^[11]. Specifically, DN remains the leading cause of all excess mortality among type I and II diabetic patients with microalbuminuria, macroalbuminuria, or end-stage kidney disease^[12,13]. Although kidney transplantation is an option, many DN patients have frequent post-operative complications associated with kidney transplant procedures, including cerebrovascular disease events and graft rejection^[14,15]. As a result, clinical studies examining the pathophysiology and therapeutic interventions for DN remain an important public health concern for reducing DN-associated end-stage renal disease and mortality.

DN begins with glomerular hyperperfusion and renal hyperfiltration and then progresses to microalbuminuria and a lowered glomerular filtration rate (GFR). Current guidelines define DN using four main criteria: a decline in renal function, diabetic retinopathy, proteinuria, and a reduction in GFR^[16]. Specifically, "Overt nephropathy is characterized by persistent proteinuria (> 500 mg/24 h) that usually precedes a fall in glomerular filtration rate (GFR) significant proteinuria has therefore long been regarded as the hallmark of DN"^[17]. DN is diagnosed by urinalysis and confirmed, if necessary, by a kidney biopsy, and its progression is monitored through regular measurements of microalbuminuria, serum creatinine, and calculated GFR^[1,18]. With advanced cases of DN, the kidney biopsy shows mesangial hypercellularity and expansion, thickening of the basement membranes, arteriolar hyalinosis, and interstitial fibrosis. In some cases, Kimmelstiel-Wilson lesion seen in DN kidney biopsies correlate with an increased risk of worsening renal function and retinopathy^[19]. However, several studies have reported substantial variability in patients with DN that deviates from accepted guidelines, which has encouraged clinicians to incorporate routine biopsy of DN patients^[20,21]. As a result, DN is now viewed as a spectrum of presentations with many authorities arguing for expanding the current pathological classification of DN to improve treatment strategies and outcomes^[16,22,23].

Among the parameters used to identify DN patients, the presence of proteinuria represents an important prognostic factor reflecting damage to the glomerular filtration barrier^[24]. However, several studies have described DN without significant proteinuria (> 500 mg/24 h) in over 50% of diabetic patients^[25-32]. Among the 15773 Type 2 diabetic patients with varying severity of renal insufficiency examined in the Renal Insufficiency and Cardiovascular Events Italian Multicenter Study, 56.6% were normoalbuminuric, 30.8% were microalbuminuric (30 to 300 mg/24 h), and 12.6% were macroalbuminuric (> 300 mg/24 h)^[33]. In some cases, the proteinuria vanishes with patients having normal albuminuria levels^[34-36]. For example, a six-year longitudinal study conducted by the Joslin Clinic showed that 58 percent of the 386 patients who had microalbuminuria eventually had normal albuminuria levels^[34].

Compared with patients with type II diabetes and DN, patients with type I diabetes and DN with normoalbuminuria had more of glomerular lesions, such as increased glomerular basement membrane thickness and more Kimmelstiel-Wilson nodules, and more frequent progression of DN^[28]. As shown in **Table 1**, a new classification was created to characterize DN patients with a decline in kidney

function and vascular complications without proteinuria, known as non-proteinuric DN (NP-DN)^[37,38]. Robles summarized these recent studies with this observation, “There have now been reports that in both type 1 and type 2 diabetes mellitus, a proportion of patients may have renal impairment without significant proteinuria or albuminuria, with a variable percentage of patients in these reports having advanced (stage 3–5) kidney disease. It could be interpreted as an accelerated kidney sclerosis due to the interaction of diabetes with other cardiovascular risk factors”^[17]. Furthermore, a recent clinical study reported NP-DN is an increasing cause of chronic kidney disease globally^[17]. At present, increasing age, repeated cardiovascular injury, such as hypertension, cardiovascular disease, and dyslipidemia, to the kidney, and an over-suppressed renal-angiotensin system have been proposed as potential mechanisms for NP-DN^[17].

Despite the increased incidence of NP-DN, few clinical or experimental studies have thoroughly investigated the pathophysiological mechanisms and targeted treatment of NP-DN. As the nephrologist Jean Halimi summarized, “it is not clear why some patients develop the ‘classical’ diabetic nephropathy with significant proteinuria, while others have impaired renal function associated with very low levels of proteinuria that sometimes persist as late as end-stage renal disease”^[38]. Furthermore, a clinical review published by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative showed that “there is insufficient evidence to assume that interventions that prevent or reverse microalbuminuria will necessarily lead to improvement in clinical outcomes and conversely that failure to reduce microalbuminuria precludes a beneficial effect of treatment on diabetic kidney disease”^[39]. In this review, we will discuss the differences between DN and NP-DN and consider potential pathophysiological mechanisms, diagnostic markers, and treatment for NP-DN.

POTENTIAL MECHANISMS OF NP-DN

Current research suggests that increased vascular resistance in renal interlobar arteries can damage glomerular and non-glomerular nephron structures and contribute to the onset and progression of NP-DN^[37,40]. A recent study reported an elevation in arterial stiffness, measured using aortic and brachial-ankle pulse wave velocity, in NP-DN patients, which was strongly associated with increased atherosclerosis and cardiovascular morbidity and mortality and decreased renal function^[40–47]. Thus, NP-DN patients likely have more atherosclerosis and increased vascular resistance which reduce glomerular function and damage glomerular-tubular structures.

Several studies examining NP-DN found elevated serum uric acid levels, which were strongly associated with the development of kidney disease^[48–50]. Although an antioxidant in the blood, uric acid is also a potent pro-oxidant and damages mitochondria through the stimulation of NADPH oxidases^[51]. Elevated uric acid levels could also damage vascular elements and induce endothelial dysfunction through various mechanisms, including activation of Toll-like receptor pathways^[51,52]. Furthermore, uric acid induces renal inflammation, vascular smooth muscle cell proliferation, and activation of the renin-angiotensin system^[53–56]. Prolonged elevation of uric acid levels in NP-DN patients can produce significant vascular changes that impair renal function leading to NP-DN^[57]. Therefore, elevated uric acid levels in NP-DN patients can produce more vascular damage than in DN patients. In recent years, sodium-glucose 2 (SGLT2) inhibitors were shown to increase uric acid excretion through the proximal tubule transporter, SLC2A9 (GLUT9), which improved glycemia control, weight loss, and blood pressure control among DN patients^[58–60]. Future clinical studies should include serial measurements of uric acid and uric excretion between DN and NP-DN patients prescribed SGLT2 inhibitors to investigate this mechanism.

Patients with NP-DN have elevated concentrations of serum tumor necrosis factor alpha (TNFα) and Fas-pathways^[61]. TNFα is a key mediator of inflammation through the induction of chemokines, IFN-γ inducible protein-10, intercellular adhesion molecule-1, and vascular adhesion molecule-1, which increase glomerular vasoconstriction and albumin permeability^[61]. Furthermore, TNFα is involved in the acute kidney injury, regulation of blood pressure, blood flow, and inflammation within the renal vasculature^[62–64]. TNFα and Fas also have important roles in apoptosis^[61]. The FasL-Fas system regulates renal cell apoptosis during immune and inflammatory responses through the activation of renal cell Fas receptors^[65]. In addition, murine models that block the FasL-Fas system prevent renal and tubular cell injury during ischemia-reperfusion experiments^[65]. Thus, increased levels of TNFα

Table 1 Pathophysiology of diabetic nephropathy and non-proteinuric diabetic nephropathy

Clinical parameter	Diabetic nephropathy	Non-proteinuric diabetic nephropathy
Proteinuria	Present	Absent
Regression of proteinuria	Present	Absent
Histology	Abnormal	Normal or abnormal
Glomerular filtration rate	Decreased	Decreased
Increased risk of chronic kidney disease	Present	Present

and Fas in NP-DN can alter renal vasculature and damage the kidney.

NP-DN patients also have elevated levels of osteoprotegerin and vascular endothelial growth factor (VEGF), which function in inflammation and angiogenesis, respectively^[66]. Interestingly, VEGF levels are inversely related to proteinuria levels in DN patients^[67]. In the presence of TGF- β , VEGF signaling leads to apoptosis and potentially cause glomerular vascular atrophy^[68]. Elevated serum VEGF levels in murine models initiate a feedback inhibition of VEGF production by podocytes leading to glomerular injury^[69]. In addition, osteoprotegerin is associated with chronic kidney disease in diabetic patients, leading to calcification of vascular tissue, glomerular damage, and proteinuria^[70,71].

In summary, the pathogenesis of NP-DN appears to involve vascular and soluble elements circulating in the blood, as shown in **Figure 1**. Comparisons between DN and NP-DN patients should provide insight into the functions of these receptors and other inflammatory responses occurring within the kidney. Furthermore, additional studies investigating non-enzymatic glycation of proteins, metabolic stress, hypertension, N-terminal fragment of pro brain natriuretic peptide and glomerular vascular injury can provide additional insight into the pathogenesis of NP-DN^[72]. This information may provide unique insights and possibilities for developing novel treatment for DN and NP-DN.

DIAGNOSTIC MARKERS FOR NP-DN

Given the recent identification of NP-DN, current guidelines should be expanded to include NP-DN and other forms of DN. Kidney biopsies are readily available and provide a detailed analysis of a patient's renal disease. However, complications, such as infection, bleeding, and other vascular injuries, limit its wider use by physicians^[73]. Furthermore, kidney biopsies may not fully detect the vascular changes occurring in NP-DN and DN patients. As a result, the development of safer and accessible diagnostic markers is critical for improving early diagnosis and treatment of conventional DN and NP-DN patients.

Ultrasound technology is one alternative which has provided opportunities for diagnosing and monitoring the progression of DN. Unlike renal biopsies, ultrasound represents an inexpensive and non-invasive method for examining and grading the progression of DN and other related renal pathologies, such as renal cysts or stones^[74]. Ultrasound technology can provide measurements on renal anatomy and function associated with DN, acute renal failure, and cirrhosis^[75]. Recent studies using ultrasound have provided an additional method for evaluating renal function in DN patients at various stages of the disease^[75-79]. Specifically, an increase in the Renal Resistive Index (RRI), which measures renal vascular resistance, has been shown to reliably detect and monitor the progression of DN and NP-DN^[75,80,81]. For example, a study in diabetic patients showed that RRI values were elevated in diabetic patients without overt proteinuria or renal atherosclerosis^[82]. Therefore, ultrasound sonography provides an effective method to screen, identify, and monitor hemodynamic and morphologic changes in DN patients^[82]. Furthermore, diabetic patients identified as high risk for DN could qualify for preventative pharmacologic treatment, which might prevent the onset of DN before the appearance of proteinuria^[83]. Recent reports with ultrasound technology and DN strongly suggest that this technology could be used to differentiate DN and NP-DN for diagnostic and screening purposes^[82]. In addition, only a few studies have systematically compared the renal function, prognosis, and various blood and urine components in conventional DN and NP-DN patients. More studies examining changes in the levels of TNF α , TGF- β , endothelin, and other interleukins in the blood and urine of DN and NP-DN might provide additional diagnostic criteria and potential insight into the pathophysiological mechanisms of NP-DN. More analysis comparing both groups

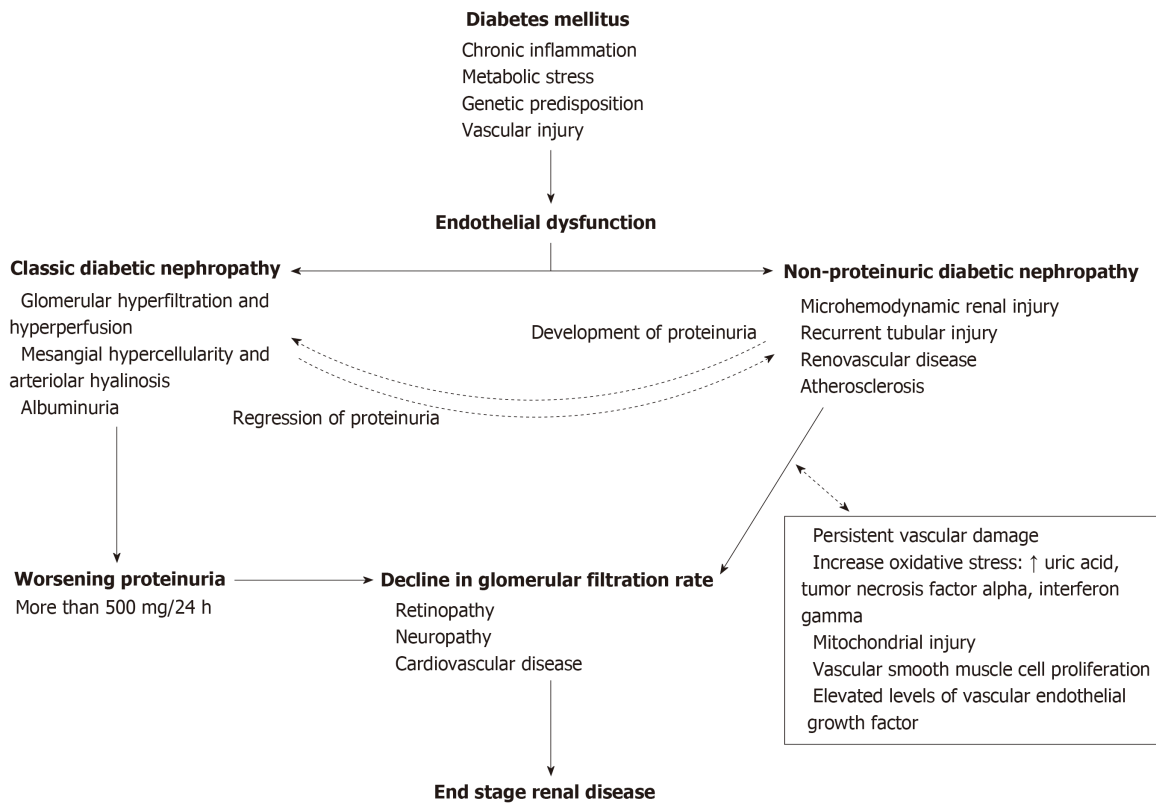


Figure 1 Pathophysiology of diabetic nephropathy and non-proteinuric diabetic nephropathy.

should help clarify distinct pathological and diagnostic criteria for DN and NP-DN.

POTENTIAL TARGETED THERAPIES FOR NP-DN

Uncontrolled hypertension produces hemodynamic stress that causes fibrinoid necrosis of small blood vessels leading to acute renal failure. The current pharmacological treatment for hypertensive disorders and glomerular vascular syndromes includes thiazide diuretics, beta blockers, ACE inhibitors, angiotensin II receptor blockers, calcium antagonists, and α_1 blockers. However, these anti-hypertensive drugs fail to prevent progressive declines in GFR and renal disease^[84]. As a result, the development of new pharmaceutical regimens for managing DN and NP-DN are needed^[85,86]. Several studies have found ACE-inhibitors lisinopril and enalapril and the angiotensin II receptor antagonist losartan were effective in treating patients with normoalbuminuric type II diabetes through reductions in albuminuria excretion, blood pressure, creatine clearance^[87-89]. In recent years, pharmacological alternatives for DN, such as heparin and antibody therapy, have been proposed for treating glomerular vascular syndromes.

Heparin is a potent glycosaminoglycan and anticoagulant used to treat and prevent deep vein thrombosis, pulmonary embolism, and arterial thromboembolism. Patients with diabetes have abnormal metabolism and catabolism of glycosaminoglycans^[90]. Diabetic mouse models treated with heparin sulfate and glycosaminoglycan had significant improvement in morphological and functional renal abnormalities^[90]. Unlike antihypertensive drugs, heparin reduces proteinuria and improves GFR without interacting with the renin-angiotensin-aldosterone system^[91]. Similarly, sulodexide, a heparin derivative, reduced proteinuria and improved renal function in murine models when given orally, intramuscularly, or intravenously^[92,93]. Clinical trials with long-term low-dose sulodexide have reported reduced proteinuria and renoprotective properties in DN, chronic kidney disease, hypertensive nephropathy, and primary glomerulonephritis^[93,94]. Thus, heparin could provide an effective additive for reducing proteinuria and GFR in conventional DN and NP-DN patients on conventional antihypertensive therapy.

Given the inflammatory activities associated with diabetes, some anti-inflammatory drugs, such as pentoxifylline, have been studied in the treatment of DN^[95,96]. Pentoxifylline is a methylxanthine derivative and a non-specific phosphodiesterase

inhibitor of TNF- α . Several studies with pentoxifylline have shown a decrease or stabilization in the progression of DN with additional reno-protective effects, such as decreased C-reactive protein, TNF- α , and risk for long-term dialysis^[97-100]. In addition, pentoxifylline attenuates the progression of glomerular crescents, sclerosis, mesangial expansion, and interstitial fibrosis seen in DN patients^[101]. Patients with NP-DN have elevated levels of TNF- α and other cytokines and could respond to pentoxifylline with improvement in renal vasculature and glomerular structures. Additional studies investigating other anti-inflammatory drugs in DN and NP-DN patients would provide an alternative first-line treatment in conjunction with current anti-hypertensive therapy. Table 2 shows the summary of NP-DN literature.

In summary, inflammation remains a central factor involved in the onset and pathogenesis of diabetes and diabetes-related complications. With the increase in NP-DN cases, new treatment and diagnostic markers are needed to understand the pathogenesis of both DN and NP-DN. New therapies beyond current anti-hypertensive therapy regimens hold promise in providing an effective measure for the prevention and treatment of DN and NP-DN. More clinical studies are needed to examine the differences between DN and NP-DN in pathogenesis, diagnosis, and treatment. Specifically, additional studies examining the use of allopurinol to reduce uric acid levels among NP-DN patients would provide a readily accessible treatment for both clinicians and patients. Furthermore, studies examining RRI can yield additional anatomical and pathophysiological data distinguishing NP-DN and DN. Despite these challenges, investigation of the pathophysiology of NP-DN requires further analysis into the cardiovascular factors influencing renal function and disease and identify novel treatment for the vascular complications seen in diabetic patients.

Table 2 Summary of non-proteinuric diabetic nephropathy literature

Field	Summary of non-proteinuric diabetic nephropathy literature
Prevalence	57% of diabetic nephropathy patients
Pathogenesis	Vascular and soluble elements, such as uric acid, TNF α , and VEGF, affecting renal microhemodynamics
Diagnosis	(1) Increased renal resistive index; (2) Alterations in TNF α , TGF- β , endothelin, and other interleukins
Treatment	(1) Enalapril; (2) Losartan; (3) Heparin; (4) Pentoxifylline

TNF α : Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor; TGF: Transforming growth factor.

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Management of diabetic dyslipidemia: An update

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Abstract

Diabetic dyslipidemia is a cluster of lipoprotein abnormalities characterized by increased triglyceride level, decreased high-density lipoprotein-cholesterol levels and increase in small dense low-density lipoprotein (LDL) particles. It is extremely common in type 2 diabetes (T2DM) affecting around 70 % of patients. Diabetic is a significant risk factor for atherosclerotic cardiovascular disease (ASCVD) which is the most common cause of death in the United States and LDL-cholesterol is the number 1 predictor of ASCVD events in T2DM. The purpose of this review is to discuss the pathophysiology and treatment of diabetic dyslipidemia. In this review, we have discussed both non-pharmacological and pharmacological treatment modalities including major treatment trials which have impacted the cardiovascular outcomes in patients with diabetes. Statin therapy is the mainstay of treatment to reduce ASCVD by decreasing LDL-C by 30%-49% or at least 50% depending on risk level. Attractive adjunctive therapies include Ezetimibe which is more cost effective and PCSK9 inhibitors which display potent LDL-cholesterol lowering and ASCVD event reduction. For severe hypertriglyceridemia, to avert the risk of pancreatitis, both fish oil and fenofibrate in concert with diet is the best strategy.

Key words: Diabetes; Dyslipidemia; Statins; Atherosclerosis; Ezetimibe; PCSK9

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Core tip: Atherosclerotic cardiovascular disease (ASCVD) is the major cause of mortality in diabetes. Low-density lipoprotein (LDL)-cholesterol lowering with statins reduce ASCVD and is the mainstay of therapy. Also, both ezetimibe and PCSK9 inhibitors are useful strategies when statins cannot be tolerated or the LDL-cholesterol goal is not achieved.

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INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is the commonest cause of death in the United States and western world^[1]. It claims around 2300 lives in the United States every day^[2]. Diabetes is a significant risk factor for ASCVD and it is the leading cause of mortality. Diabetic patients are 2-4 times more likely to die from ASCVD as compared to non-diabetic patients. The rapidly increasing burden of diabetes from 108 million in 1980 to 442 million in 2014 poses a significant threat globally^[3].

Diabetes can cause microvascular complications (retinopathy, neuropathy, nephropathy) and macrovascular complications (ASCVD) manifesting as coronary artery disease, stroke and peripheral arterial disease^[4]. Dyslipidemia in diabetes is common and is characterized by hypertriglyceridemia (HTG) with decreased levels of high-density lipoprotein (HDL)-cholesterol. Whilst low-density lipoprotein (LDL)-cholesterol levels are usually not elevated there is a preponderance of small dense LDL particles which appear to be more atherogenic^[5-6]. Furthermore, there is an increase in particle number as evidenced by increased apolipoprotein B levels and non-HDL-cholesterol levels^[5-6].

The 2 major sequelae of diabetic dyslipidemia are premature ASCVD from the elevated apolipoprotein B carrying particles and pancreatitis with severe HTG > 1000 mg/dL.

PATHOPHYSIOLOGY

Dyslipidemia is very common in type 2 diabetes (T2DM) mellitus affecting around 72%-85% patients^[7].

The exact mechanism of lipoprotein abnormalities in diabetes is not very well understood. Insulin resistance, rather than hyperglycemia, has been implicated in the pathogenesis of diabetic dyslipidemia because lipoprotein changes including an increase in triglycerides (TG), increase in VLDL particles, small dense LDL particles and a decrease in HDL level have been shown in patients with impaired fasting glucose and impaired glucose tolerance and T2DM^[6-8].

Lipoprotein abnormalities in diabetes can be divided into quantitative and qualitative. Quantitative changes include an increased triglyceride level and decreased HDL-C level. Qualitative changes include an increase in small dense LDL particles and large very-LDL sub fraction (VLDL1) that predisposes to the formation of small dense LDL particles^[7].

HTG occurs due to both increased production and decreased clearance, and it is the most common abnormality of diabetic dyslipidemia.

Insulin resistance causes increased production of VLDL. VLDL can be further divided into large VLDL1 (triglyceride-rich) and small, dense VLDL2.

Insulin resistance causes an increase in VLDL1 levels which worsens HTG^[7,9].

In addition to increased secretion of VLDL, there is decreased clearance of VLDL due to decreased hepatic uptake and impaired activity of lipoprotein lipase^[7,9,10].

HTG increases the activity of cholesterol ester transfer protein which leads to transfer of triglyceride to HDL and LDL from triglyceride-rich lipoprotein^[11]. This causes an increase in the TG content of HDL and LDL.

Small dense LDL particles are more prone to post-secretory modifications such as glycation and oxidation and permeate the intima more easily where they are trapped by proteoglycans^[6,7]. Thus whilst the LDL-cholesterol level is not overly increased there is an increase in the more atherogenic small dense LDL particles. In addition there is an increase in chylomicron and VLDL remnant particles in T2DM which are also atherogenic^[7,12].

DIABETIC DYSLIPIDEMIA AND CARDIOVASCULAR DISEASE

Epidemiological studies have shown a correlation between increased TG level and cardiovascular disease (CVD), and recent studies have established a cause and effect relationship between TG rich lipoproteins and CVD *via* mutations in apolipoprotein C3^[13,14].

The role of HDL in CVD is unclear. Studies have shown an inverse relationship between HDL and CVD^[15]. However as will be discussed under therapy there is no benefit to raising HDL-cholesterol in T2DM with niacin therapy^[16].

LDL-cholesterol has been the primary predictor of CVD. Multiple studies have shown a strong relationship between LDL and CVD. In diabetes, LDL concentration may or may not be increased, but there is an increase in the concentration of small dense LDL particles which are considered more atherogenic than large LDL particles^[6,7,17]. Also, in the UKPDS study, Turner *et al*^[18] showed that LDL-cholesterol was the number 1 predictor of ASCVD risk in T2DM following adjustment for both age and sex^[18].

TREATMENT TARGETS BASED ON GUIDELINES

Treatment strategy has significantly changed over the last two decades, but LDL-cholesterol has remained the cornerstone of treatment.

In 2013 the American College of Cardiology (ACC)/American Heart Association (AHA) published guidelines for the management of cholesterol to reduce ASCVD. These guidelines recommended using high, moderate or low-intensity statins depending upon the 10-year CV risk score and presence or absence of ASCVD. These guidelines did not recommend specific cholesterol targets. The ACC/AHA recommended that any patient with diabetes mellitus type 1 or 2 aged 40-75 should be treated with moderate intensity statins with a goal reduction in LDL-C of 30%-49%. High-intensity statins were recommended if the 10-year CV risk score is $\geq 7.5\%$ or if ASCVD was present with a target LDL-C reduction of $>$ or equal to 50%^[19].

In 2017 American Association of Clinical Endocrinologists guidelines categorized diabetic patients as high, very high and extreme risk patients for CVD. It recommended that patients with high risk [≥ 2 risk factors and 10 year risk 10%-20%, or chronic kidney disease (CKD) stage 3-4 with no other risk factors], very high risk [established acute coronary syndrome (ACS) or recent hospitalization for ACS, peripheral arterial disease, carotid, coronary artery disease, 10-year risk $\geq 20\%$, CKD stage 3-4 with 1 or more risk factors, heterozygous familial hypercholesterolemia], extremely high risk (progressive ASCVD, coronary artery disease with CKD stage 3-4, diabetes or heterozygous familial hypercholesterolemia, history of premature ASCVD in female with age < 65 or males with age < 55 years) should be treated for LDL targets of < 100 , < 70 and < 55 mg/dL respectively^[20].

The American Diabetes Association 2019 guidelines recommend that all diabetic patients with ASCVD or patients with a 10-year atherosclerotic cardiovascular risk $> 20\%$ should be treated with high-intensity statins (goal of 50% reduction in LDL-cholesterol) in addition to lifestyle modification^[21]. Diabetic patients aged < 40 with additional atherosclerotic cardiovascular risk factors (LDL-C ≥ 100 mg/dL, hypertension, CKD, smoking, albuminuria and FH of premature ASCVD), diabetic patients age 40-75 years without ASCVD or 10 year ASCVD risk $< 20\%$ and diabetic patients > 75 years old should be treated with moderate intensity statins with a goal of 30%-49% LDL-C reduction^[21].

Most recently, the new ACC/AHA guidelines were published^[22]. Diabetes was defined as a high risk condition for ASCVD. In addition they provided diabetes specific Risk Enhancers which included: Diabetes duration of >10 years in T2DM and >20 years duration for T1DM, Albuminuria > 30 mg/G creatinine, an estimated GFR < 60 mL/min /1.73m², retinopathy, neuropathy and an ankle-brachial index (ABI) < 0.9 . In adults 40-75 years with diabetes regardless of 10-year risk initiate moderate intensity statin. In adults with diabetes with ASCVD or multiple ASCVD risk factors it is reasonable to prescribe high intensity statin to lower LDL-C by 50% or more. In adults > 75 years on a statin it is reasonable to continue statin therapy. In adults 40-75 years old with LDL-C between 70-189 mg/dL without ASCVD the 10-year risk should be assessed using the age and race based robust pooled cohort equation (PCE) which uses age, smoking, hypertension, serum cholesterol, HDL-C, and presence or absence of diabetes to compute the 10-year risk^[22]. If the risk is 20% or higher, then therapy should aim for an LDL-C reduction of 50% or greater. In diabetics between 20-39 years of age it is reasonable to institute moderate intensity statin therapy if the following are present: T2DM with duration $>$ or equal to 10 years, T1DM with duration $>$ or equal to 20 years, albuminuria > 30 mg/G creatinine, e-GFR < 60 mL/min, retinopathy, neuropathy, ABI < 0.9 ^[22].

Since the occurrence of a first ASCVD event in diabetic patients 40-75 years old is associated with increased morbidity and mortality compared to non-diabetic patients high intensity statin therapy is reasonable as they age (men > 50 and women > 60 years) or develop the risk modifiers including T2DM with duration > or equal to 10 years, T1DM with duration > or equal to 20 years, albuminuria > 30mg/G creatinine , e-GFR < 60 mL/min, retinopathy, neuropathy, ABI < 0.9^[22]. Also, it is prudent to consider statin therapy in diabetic patients > 75 years taking into account side effects and co-morbidities and the life span of the patient.

THERAPEUTIC STRATEGIES

Diabetic dyslipidemia treatments can be divided into non-pharmacological and pharmacological. Non-pharmacological treatment includes medical nutrition therapy, weight loss, and physical activity.

Diabetic patients should increase the intake of plant stanols/sterols, viscous fiber (legumes, citrus, oats), n-3 fatty acids and decrease the intake of saturated and trans-fatty acids. American Diabetes Association recommends the Mediterranean diet or DASH (Dietary Approaches to Stop Hypertension) diet^[21-23].

Tree nuts, peanuts, grains are a good source of unsaturated fat, and decrease cholesterol, blood pressure and risk of CVD and diabetes.

Consumption of a walnut-rich diet in a randomized study showed improvement of non-HDL cholesterol and apolipoprotein B^[24]. An epidemiological association between nut consumption and decrease death due to CVD and overall mortality has been shown but randomized clinical trial data is still lacking^[25].

Around a 5% reduction in body weight is associated with improvement in lipid profile, insulin resistance and glycemic control^[26]. Weight loss decreases triglyceride level, raises HDL-C levels and can also improve blood pressure^[27]. Even though weight loss was shown to improve multiple risk factors, such as hemoglobin A1C and blood pressure, the Look AHEAD study did not show improvement in the cardiovascular events (CVE) after long term weight loss with intensive lifestyle change^[28], indicating the need for pharmacotherapy along with lifestyle modification to reduce ASCVD^[23].

Pharmacological therapy includes statins, cholesterol absorption inhibitors, niacin, fibrates, bile acid sequestrants (BAS), PCSK9 inhibitors and omega-3 fatty acids^[22]. The drugs that effectively and safely lower LDL-cholesterol are depicted in Table 1.

Statins

Statins inhibit 3-hydroxymethylglutaryl coenzyme A which is a rate-limiting step in the synthesis of cholesterol in the liver. Statins are used for primary and secondary prevention of CVD and stroke. Decreased cholesterol level in the liver leads to an upregulation of LDL receptors which leads to a decrease in plasma LDL cholesterol^[29]. In addition to the decrease in LDL cholesterol, statins lower the level of TG and increase the level of HDL-cholesterol^[30].

Statins also have pleiotropic effects and have been shown reduction of hsCRP and other markers of inflammation that help to stabilize plaque, improve endothelial function and decrease vascular inflammation and oxidative stress^[30,31]. Statins are divided into high-intensity (atorvastatin 40-80 mg, rosuvastatin 20-40 mg) which can decrease LDL- C by approximately 50% or more; moderate-intensity (Atorvastatin 10-20 mg, rosuvastatin 5-10 mg, simvastatin 20-40 mg, pravastatin 40 mg, lovastatin 40 mg, Fluvastatin 80 mg, pitavastatin 2-4 mg) which can decrease LDL-C by approximately 30%-50% ; and low-intensity (Simvastatin 10mg, Pravastatin 10-20 mg, Lovastatin 20 mg, Fluvastatin 20-40 mg, Pitavastatin 1 mg) which decrease LDL-C by < 30%^[19,22].

Trials have shown a reduction of CVE in diabetic patients with use of statins including the Heart Protection Study which reported a 22% reduction in CVE including ischemic stroke^[32] and The Collaborative Atorvastatin Diabetes Study^[33,34] which reported a 37% reduction in the primary end point of CVE also including ischemic stroke. Meta-analysis of 14 randomized clinical trials including over 18000 patients showed statin therapy reduced CVE by 21% and vascular mortality by 13% for every 39 mg/dL decrease in LDL-C during an average follow up of 4.3 years^[34,35].

Statins can cause side effects but are well tolerated in general. Myalgia is the most common side effect, affecting 5%-10% patients^[36]. Statin-induced necrotizing autoimmune myopathy and rhabdomyolysis are rare^[36]. Risk factors for myopathy include age, female sex, low BMI, high risk medications such as azole antifungals, macrolides, protease inhibitors, cyclosporine, fibrates, nicotinic acid, renal disease, Asian descent, excess alcohol intake, trauma^[19,22]. Statins can also cause new onset

Table 1 Summary of low-density lipoprotein-cholesterol lowering medications

Drug class	Mechanism of action	Clinical efficacy	Adverse reactions
Statins	Inhibition of HMG coenzyme A Reductase	Highly effective	Myalgia, myositis, rhabdomyolysis, elevation in liver enzymes, new onset diabetes
Ezetimibe	Decrease intestinal cholesterol absorption by binding to Niemann-Pick C1-like 1 protein	Moderately effective; Safe addition to statin therapy	Worsening of liver function, myopathy or rhabdomyolysis if added to statins; Nasopharyngitis, diarrhea, upper respiratory tract infection
PCSK9 inhibitors	Inhibition of Proprotein Convertase Subtilisin/Kexin Type 9	Very highly effective in combination with statin therapy	Injection site reaction including itching, swelling, erythema and pain
Bile acid sequestrants	Bind bile acids in the small intestine and prevent reabsorption	Moderately effective, safe addition to statin therapy, not desirable if triglycerides are > 300 mg/dL	Constipation, abdominal pain, bloating, drug malabsorption

HMG: Hydroxymethylglutaryl; PCSK9: Proprotein convertase subtilisin/kexin type 9.

diabetes; the exact underlying mechanism is not clear. The JUPITER (Justification for the use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) trial was the first trial to show an increased risk of diabetes. In this trial the risk of diabetes in the rosuvastatin group was increased by 0.6% compared to placebo group^[37]. The risk is higher with higher doses and in patients with Metabolic syndrome, BMI > 30 and A1c > 6%^[22]. The benefits of reducing CVE far outweigh the low risk for diabetes which can be prevented with diet and exercise.

Cholesterol absorption inhibitors (Ezetimibe)

Ezetimibe decreases cholesterol level by inhibiting intestinal absorption of cholesterol. It is used in combination with statins to achieve significant LDL-C reduction, or in patients who are not able to tolerate the required dose of statins.

In the IMPROVE-IT trial, 18144 patients with the ACS and LDL cholesterol between 50-125 mg/dL were randomized to simvastatin 40 mg with ezetimibe 10 mg or simvastatin 40 mg with placebo. During a median follow up of 6 years, patients who received simvastatin and ezetimibe had a significant reduction in LDL cholesterol compared to the simvastatin only group, 54 mg/dL *vs* 70 mg/dL respectively^[38]. There was 6.4% reduction in the primary composite endpoint (myocardial infarction, cardiovascular death, coronary revascularization in 30 d, hospitalization for unstable angina, and stroke) demonstrating the additional benefit of adding ezetimibe to a statin^[38]. More importantly in the patients with diabetes (27% of patients) there was a greater benefit on the primary end point with a 14% risk reduction. The combination of ezetimibe and simvastatin has been showed to decrease the risk of recurrent ischemic stroke when compared with simvastatin in patients with T2DM^[39] underscoring the importance of ezetimibe in diabetic patients with CVD.

Fibrates

Fibrates include bezafibrate, gemfibrozil, ciprofibrate, and fenofibrate. Fibrates activate nuclear peroxisome proliferator-activated receptor alpha which causes a reduction in triglyceride level by stimulating lipoprotein lipase activity. Fibrates can decrease fasting plasma triglyceride level by 30%-50% and can also decrease postprandial lipemia by decreasing the synthesis of fatty acids. Fibrates increase HDL level by upregulation of apoA-1 and A-II^[40]. Fibrates have also been shown to decrease small dense LDL level in some studies^[41].

In the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention trial, gemfibrozil compared to placebo resulted in a 31% reduction in TG, 4% reduction in cholesterol and a 6 % increase in HDL-cholesterol. Nonfatal myocardial infarction or death from coronary artery disease was decreased by 4.4%^[42] in these patients with ASCVD and low HDL-cholesterol. However, these patients did not have a high-risk LDL-C and did not appear to receive statin therapy.

The Fenofibrate Intervention and Even Lowering in Diabetes (FIELD) study evaluated the effect of treatment with fenofibrate in reducing macrovascular and microvascular complications in 9795 patients with T2DM. After 5-year follow-up period, treatment with fenofibrate was associated with no significant reduction in the primary end point^[43].

Also, in the ACCORD trial, a combination of simvastatin and fenofibrate in 5518 patients with T2DM, did not decrease the rate of nonfatal myocardial infarction, fatal

CVE or nonfatal stroke compared to simvastatin only group^[44].

Fibrates are metabolized in the kidney and should be avoided or used with caution in patients with CKD. The combination of gemfibrozil and statin predisposes to a greater risk for myopathy as is essentially contra-indicated.

The major indication of fibrates is to reduce TG in patients with very high TG at risk for pancreatitis. This diabetic HTG has been reviewed by the principal author^[45]. Briefly, in patients with severe HTG > 1000 mg/dL, secondary causes such as excess alcohol intake, drugs (steroids, oral estrogen, protease inhibitors *etc.*) and kidney disease should be ruled out. In these patients in addition to good glycemic control and reduction in fat and total calories in the diet, fibrates and or fish oils 4 g/d therapy needs to be initiated to lower TG levels < 500 mg/dL to avert the risk of pancreatitis.

Niacin

Niacin is a very potent drug for increasing HDL-cholesterol levels. Niacin also lower TG and LDL-cholesterol. However, the combination of statin and niacin did not show any additional cardiovascular benefit when compared with statin alone.

The AIM- HIGH trial did not show any cardiovascular benefit after adding niacin in high-risk patients who were already receiving simvastatin and ezetimibe^[46]. Heart Protection Study 2- Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) randomized 25673 patients with atherosclerotic vascular disease to receive niacin/laropiprant versus placebo. The treatment group did not show any cardiovascular benefit but there was a significant increase in new onset diabetes, bleeding and infections^[46]. No guidelines recommend niacin-statin combination therapy in patients with diabetes and patients with ASCVD since there is the potential for harm with no benefit.

Proprotein Convertase Subtilisin/Kexin Type 9 inhibitors

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK 9) inhibitors Alirocumab and Evolocumab are very potent drugs and can decrease LDL-C significantly when used as monotherapy or in combination with statins. PCSK9 inhibitors by binding PCSK9 prevents PCSK9 from binding LDL receptors and targeting them for intrahepatic lysosomal degradation. This leads to increased expression of LDL receptors causing a reduction in LDL-C level^[47]. These are given as subcutaneous injections every 2-4 wk.

PCSK9 inhibitors are indicated in patients with ASCVD who are on maximum tolerated statin therapy with or without ezetimibe but have LDL-C \geq 70 mg/dL or non-HDL-C \geq 100mg/dL. They are also indicated in patients with LDL \geq 190 mg/dL with underlying homozygous familial hypercholesterolemia or heterozygous familial hypercholesterolemia^[47].

In 2015, ODYSSEY long term trial enrolled 2341 adults who were at high risk for CVE due to history of established coronary artery disease or had presence of Heterozygous Familial Hypercholesterolemia, or coronary risk equivalent states (ischemic stroke, peripheral arterial disease, moderate CKD with GFR 30-59 or diabetes mellitus with two additional risk factors). These subjects had LDL-C level \geq 70 mg/dL despite being on maximum tolerated dose of statin and were randomized to receive alirocumab 150 mg or placebo. Alirocumab therapy decreased LDL-C from 122.8 mg/dL to 53 mg/dL at 48 mo^[48].

In ODYSSEY outcomes trial, use of alirocumab was studied in patients who have had ACS. This was a randomized, multicenter, double blind, placebo control trial of 18924 patients who had an episode of ACS within last 1-12 mo. These patients had an LDL-Cholesterol level of at least 70 mg/dL, an apolipoprotein B level of at least 80 mg/dL or a non-HDL cholesterol level of at least 100 mg/dL. These patients were already receiving maximum tolerated dose of statin or high intensity statin and were randomized to receive alirocumab 75 mg subcutaneously or placebo. After follow up 2.8 years there was a 15% reduction in the primary end point (composite of death from coronary heart disease, fatal or nonfatal ischemic stroke, nonfatal myocardial infarction or unstable angina requiring hospitalization), $P < 0.001$ ^[49]. Diabetic patients comprised 29% of the cohort and appear to have accrued a benefit but this was not detailed.

OSLER-1 and OSLER-2 evaluated the PCSK9 inhibitor Evolocumab. 4465 patients were randomly assigned in a 2:1 ratio to receive Evolocumab with standard therapy or standard therapy alone. Evolocumab decreased LDL-C from a median of 120 mg/dL to 48 mg/dL (61% reduction) as compared to standard therapy alone^[50].

PCSK9 inhibitors induce atheroma regression and decrease atheroma volume. In the Glagov randomized clinical trial, 968 patients were randomized to receive Evolocumab 420 mg subcutaneous injection monthly or placebo. Evolocumab decreased percent atheroma volume by 0.95% and total atheroma volume decreased by 5.8 mm³^[51].

In FOURIER trial 27564 patients with ASCVD and LDL level \geq 70 mg/dL while

being on maximally tolerated statin were randomized to evolocumab subcutaneous injection (140 mg every 2 wk or 420 mg every mo) or placebo. At 48 wk, the mean percent reduction in LDL-C was 59% in the treatment group compared to placebo with an achieved LDL-C of 30mg/dL. There was a 15 % relative risk reduction in the primary end -point (composite of cardiovascular death, stroke, myocardial infarction, coronary revascularization and hospitalization from unstable angina), $P < 0.001$ ^[52]. There was no increase in new onset diabetes. In a subsequent report in the 11031 diabetic patients they also showed a significant risk reduction in the above composite primary end point of 17%, $P = 0.0008$. There was no increase in new onset diabetes or any deleterious effect on glycaemia. However this was a study in diabetic patients with ASCVD so the role of PCSK9 inhibitors in primary prevention of ASCVD in diabetics remains unknown^[53].

PCSK9 inhibitors are very expensive with the annual cost of > \$14500^[54] which is more than 100 times higher than generic statin and can be a significant economic burden even in developed countries. These drugs are well tolerated, but the patient can develop an injection site reaction.

BAS

Bile acids are the end product of cholesterol catabolism. Cholestyramine, colestipol, and colesevelam are commonly used BAS. These bind to bile acid in the intestinal lumen and decrease their enterohepatic circulation which leads to increased production of bile acid in the liver causing a decrease in cholesterol level.

Use of cholestyramine in men over the long term has been shown to decrease total cholesterol and LDL cholesterol level by 13.4% and 20.3% respectively and also to decrease coronary heart disease by 19% when compared to placebo^[55]. Hence, they are a useful adjunct to statins in reducing LDL-C further. They are contra-indicated if TG levels are > 400 mg/dL since they can increase the risk of pancreatitis^[45].

Multiple studies have shown improved glycemic control with colesevelam in T2DM and hence they have the benefit of reducing both LDL-C and HbA1C levels, however there is no data to support further reduction in CVE^[56].

Omega-3 fatty acids

Omega-3 fatty acids are used as add on therapy to reduce triglyceride level. Omega-3 fatty acid formulations contain eicosapentaenoic acid (EPA) and docosahexaenoic acid.

Sub-analysis of the Japan EPA Lipid intervention trial showed that treatment with EPA of patients with impaired glucose metabolism and hypercholesterolemia resulted in a 22% reduction in coronary artery disease incidence compared to normoglycemic patients^[57]. However, in the ORIGIN trial, the use of omega-3 fatty acids (1.0 g/d) did not show cardiovascular benefit compared to placebo in patients with impaired glucose tolerance, diabetes or impaired fasting glucose^[58].

Recently, Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia (REDUCE-IT), double-blind, randomized multicenter, placebo control trial of 8179 patients with established CVD or diabetes and other risk factors was published. In this study, patients were already being treated with statins and had a fasting TG level of 135-499 mg/dL and LDL- cholesterol level between 41-100 mg/dL. They were randomized to receive either a total daily dose of 4 mg icosapent ethyl or placebo. The primary endpoint was a composite of cardiovascular death, nonfatal stroke, nonfatal myocardial infarction, coronary revascularization or unstable angina with a median follow-up of 4.9 years. There was a 25 % reduction in the primary end point with icosapent ethyl versus placebo, $P < 0.001$ ^[59]. Diabetics constituted around 58% of the patients and they appeared to accrue a similar benefit to non-diabetics. There was also a decrease in total mortality of 13% but an increase in hospitalizations for atrial fibrillation or flutter. However, before we can make any serious recommendations for diabetics, we need to see the publication in the diabetic sub-group but it could emerge as first line therapy for severe HTG and an adjunct to statins in patients with ASCVD and increased TG. Interestingly in the primary prevention cohort including diabetics there appears to be no significant benefit: Hazards Ratio of 0.88 (0.7-1.10).

CONCLUSION

Diabetic dyslipidemia is a prevalent condition and patients with diabetic dyslipidemia are at particularly high risk for ASCVD. For the majority of patients' statin therapy in concert with therapeutic life style changes remain first line. There are, however, many other lipid lowering medications available to treat individuals who do not attain LDL-C goals on statins such as ezetimibe and PCSK9 inhibitors.

EPA could also become another adjunctive therapy in diabetics with ASCVD.

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Novel pharmacological therapy in type 2 diabetes mellitus with established cardiovascular disease: Current evidence

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Abstract

Cardiovascular diseases (CVDs) remain the leading cause of death in the world and in most developed countries. Patients with type 2 diabetes mellitus (T2DM) suffer from both microvascular and macrovascular diseases and therefore have higher rates of morbidity and mortality compared to those without T2DM. If current trends continue, the Center for Disease Control and Prevention estimates that 1 in 3 Americans will have T2DM by year 2050. As a consequence of the controversy surrounding rosiglitazone and the increasing prevalence of diabetes and CVDs, in 2008 the Food and Drug Administration (FDA) established new expectations for the evaluation of new antidiabetic agents, advising for pre and, in some cases, post-marketing data on major cardiovascular events. As a direct consequence, there has been a paradigm shift in new antidiabetic agents that has given birth to the recently published American Diabetes Association/European Association for the Study of Diabetes consensus statement recommending sodium-glucose cotransporter-2 inhibitors (SGLT2i) and glucagon like peptide-1 receptor agonists (GLP-1RA) in patients with T2DM and established CVD. As a result of over a decade of randomized placebo controlled cardiovascular outcome trials, the aforementioned drugs have received FDA approval for risk reduction of cardiovascular (CV) events in patients with T2DM and established CV disease. SGLT2i have been shown to have a stronger benefit in patients with congestive

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heart failure and diabetic kidney disease when compared to their GLP-1RA counterparts. These benefits are not withstanding additional considerations such as cost and the multiple FDA Black Box warnings. This topic is currently an emerging research area and this mini-review paper examines the role of these two novel classes of drugs in patients with T2DM with both confirmed, and at risk for, CVD.

Key words: Type 2 diabetes mellitus; Glucagon-like-peptide 1 agonists; Sodium-glucose cotransporter-2 inhibitor; Cardiovascular disease; Major adverse cardiovascular event

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Core tip: Cardiovascular diseases are of significant concern in patients with type 2 diabetes mellitus. Novel therapies offer a new opportunity for cardiovascular risk reduction and add complexity in terms of selecting antihyperglycemic treatment. These pharmacological therapies, however, also have additional considerations.

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INTRODUCTION

Cardiovascular disease (CVD) is the most important cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM), with approximately 20% of the individuals with this condition suffering from established atherosclerotic disease^[1-4]. The cardiovascular (CV) risk seems to be driven largely by coexisting conditions in addition to the independent risk related to hyperglycemia^[5].

Modern medicine uses a polypharmacy approach due to the nature of the disease. Lipid lowering agents have been studied for years and data supports their cardiovascular benefit in selected patient with and without T2DM^[6]. Antithrombotic therapies for primary and secondary prevention are a topic of much debate in recent years, with data both supporting^[7], and refuting^[8] the idea of one-size-fits-all in patients with T2DM. Blood-pressure goals have also been a point of controversy, as demonstrated by Effects of Intensive Blood-pressure Control in T2DM trial [by the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study group]^[9] and by the variation of goal blood pressures in major guidelines.

Intensive versus standard glycemic control has been a research question dating back to the 1990s with the United Kingdom Prospective Diabetes Study^[10] with 3867 patients with T2DM and the Diabetes Control and Complications Trial^[11] with 1441 patients with type 1 diabetes mellitus (T1DM). These trials demonstrated reduced microvascular endpoints but no difference in macrovascular endpoints with intensive glycemic control. Metformin use was associated with a reduction in DM-related complications and all-cause mortality. Fast-forward to 2008-09 and we have the large Action in Diabetes and Vascular Disease ADVANCE^[12] trial with 11140 patients and the Veterans Affairs Diabetes Trial^[13] with 1,791 patients, both showing intensive glycemic control having no impact on macrovascular outcomes in patients with T2DM.

Given the heterogeneity of diabetes, caution must be had in extrapolating results of one trial to a population with different baseline characteristics, whether that be the type of diabetes or the CVD risk. For example, the Epidemiology of Diabetes Interventions and Complications trial in 2005^[14] showed that patients with T1DM had diminished rates of CVD with more stringent HbA1C targets. Then the same intervention of stringent HbA1C target resulted in the opposite outcome in those with T2DM in the large ACCORD trial in 2008^[15] with 10251 patients, demonstrating increased mortality and no CV benefit.

With hypoglycemia identified as a driving factor for the increased rate of CV events and related mortality^[16], our HbA1c targets became more liberal with many guidelines recommending HbA1c of 7%. As a result of the perceived need to avoid hypo-

glycemia, a new drug class, the dipeptidyl peptidase-4 inhibitors, became available in the United States in 2007. They have shown non-inferiority in atherosclerotic CVD, yet, saxagliptin in particular has shown a potential risk in congestive heart failure. For the purposes of this mini-review, this class will not be covered in detail as there are no studies showing superiority in preventing major cardiovascular events (MACE) (Table 1).

One provocative event was when Rosiglitazone had a post-marketing meta-analysis showing an increased risk of CV events in T2DM patients using this medication. With that debacle and the increasing prevalence of T2DM and CVDs, in 2008 the Food and Drug Administration (FDA) issued new mandates on MACE safety for new antidiabetic drugs. Studies had to be presented prior to approvals and these would be followed by post-marketing cardiovascular outcome trials (CVOTs). This decision has helped bring data that, otherwise, would not have been available.

The paradigm shift has been to have antihyperglycemic agents show, not only noninferiority, but superiority in reducing MACE. As a result of over a decade of randomized placebo controlled CVOT, drugs in two classes have received FDA approval for risk reduction of CV events in patients with T2DM and established CV disease; glucagon like peptide receptor agonists (GLP1RA) and sodium-glucose cotransporter-2 inhibitors (SGLT2i).

The decision for clinicians in selecting a second antihyperglycemic agent after metformin in T2DM has become significantly more complex with much more data to consider (Tables 2 and 3). We will review the pharmacology followed by the current evidence of cardiovascular, renal, blood pressure, weight and other effects of GLP1RAs and SGLT2is.

PHARMACOLOGY

Glucagon like peptide-1 receptor agonists (GLP-1RAs) have been available in the market since 2005, however it has taken over a decade to understand their effects. As an endogenous substance, its insulinotropic effect when associated with glucose-dependent insulinotropic polypeptide is very well established, giving rise to the incretin effect^[17] (Figure 1), which is significantly reduced in T2DM. Moreover, the discovery of receptors in the periphery^[18] sensitive to GLP1 have raised several questions regarding the reach in which our exogenous, man-made GLP-1RA can have a positive impact in the health of patients with T2DM^[19] given their increased potency and half-life compared to endogenous GLP1.

SGLT2 inhibitors have been available in our armamentarium since 2012 and were first used unrelated to β -cell function and insulin sensitivity^[20]. Originating from observations and studies made on patients with Familial Renal Glucosuria^[21], the effects of inhibiting SGLT2 are still under thorough investigation given the presence of such molecules not only in the proximal tubule of the nephron, but also on the glomerular basement membrane and in the heart^[22].

CURRENT EVIDENCE

Cardiovascular effects

Agents in both GLP-1RA and SGLT2i classes have obtained approval by the FDA for the indication of CV risk reduction in patients with T2DM and established CVD. Current data has proven that these agents can reduce the risk of MACE (CV death, nonfatal myocardial infarction and nonfatal stroke) with questions remaining on the ideal level of cardiovascular risk to benefit from GLP1RA. The CVOT design was intended to have both treatment groups maintain similar glycemic control, to minimize this confounder. In addition to this, SGLT2i have shown evidence of reduced hospitalization due to heart failure. New submissions to the FDA for both drug classes are in process.

The first GLP-1RA CVOT was the Evaluation of Lixisenatide in acute coronary syndrome trial^[23] in 2015, studying the effects of lixisenatide in a high risk population with subjects that had an acute coronary syndrome in the 6 mo prior to the study with an average starting HbA1c of 7.7%, demonstrating noninferiority when compared to placebo but no superiority. One of the limitations of the trial was its short duration and the severity of the illness in this very high-risk population.

In 2016, GLP-1RA gained much more attention after the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER)^[24] trial demonstrated the superiority of liraglutide in the primary end point (PEP) when compared against placebo in subjects with T2DM and high risk for CV events. These

Table 1 Summary of dipeptidyl peptidase 4 cardiovascular outcome trials

Trial	NumberFollow up	CVD (baseline)	Characteristics (baseline)	Drug vs Placebo (%) PEP	Superiority
SAVOR-TIMI53 (Saxagliptin) 2013	<i>n</i> = 16492, 2.1 yr (median)	Pre-existing CV or high CV risk/multiple CV risk factors	65 y/o, DM duration: 10 yr; A1c: 8%; BMI: 31	7.3 vs 7.2	No
EXAMINE (Alogliptin) 2013	<i>n</i> = 5380, 1.5 yr (median)	Acute MI or HUA in previous 15 to 90 d	61 y/o, DM duration: 7 yr; A1c: 8%; BMI: 29	11.3 vs 11.8	No
TECOS (Sitagliptin) 2015	<i>n</i> = 14671, 3.1 yr (median)	Pre-existing CV disease (CAD, ischemic stroke, PAD)	65.5 y.o, DM duration: 11.6 yr; A1c: 7.2%; BMI: 30.2	11.4 vs 11.6 (4-point MACE)	No

Note, as a class dipeptidyl peptidase 4 inhibitor has no data for significant reduction in cardiovascular endpoints. The TECOS trial had a 4-point MACE, consisting of death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke or hospitalization for unstable angina. DPP4: Dipeptidyl peptidase 4; CVOT: Cardiovascular outcome trial; CVD: Cardiovascular disease; PEP: Primary end point; BMI: Body mass index; HUA: Hospitalization due to unstable angina; MI: Myocardial infarctions; PAD: Peripheral artery disease; CAD: Coronary artery disease; MACE: Major cardiovascular events.

patients had a lower rate of CV death, nonfatal myocardial infarctions (MI) and nonfatal strokes (but no statistical difference with all strokes). Starting average HbA1c was 8.7%. The rate of hospitalization due to heart failure remained statistically nonsignificant.

Also, in 2016, the Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2 Diabetes (SUSTAIN-6)^[25] compared the once-weekly injection of semaglutide to placebo and had similar outcomes to liraglutide on a patient with a sizable prevalence of ischemic heart disease and hypertension (60% and 93% respectively). This was achieved with fewer patients and less years of follow-up (2 compared to 4 in the LEADER trial). The once-weekly injection of semaglutide was FDA approved in 2017 and in 2019 Novo Nordisk filed for FDA approval for a new CV indication based on the SUSTAIN-6 trial. Simultaneously they filed for FDA approval for oral semaglutide^[26] which would be the first GLP1RA in a pill form and the pertaining CVOT PIONEER6 is discussed below.

Subsequently in 2016-2017, two CVOTs were published on another GLP1RA exenatide. The Exenatide Study of Cardiovascular Event Lowering Trial^[27] confirmed noninferiority with once weekly subcutaneous injection of exenatide but lacking superiority when comparing to placebo. The study had the largest population in CVOT at the time with 14752 patients, from which 70% had a previous CV event, including coronary artery disease, ischemic cerebrovascular disease or peripheral artery disease. On average, the starting HbA1c was 8%. The main pitfall of the study was the inclusion of a sizable number of patients using SGLT2i in the placebo group. A phase 3 safety trial, FREEDOM-CVO^[28], had more than 4,000 patients supplied with exenatide through a continuous implanted pump and announced non-inferiority in CV safety. The subcutaneous pump would potentially address the high rate of discontinuation with weekly exenatide, which was 43%.

Finally, in 2018, three more CVOTs with GLP-1RAs were announced and full results are yet to be reported. REWIND^[29], investigating a weekly dulaglutide with an international scope, 46% women, and including T2DM with coexisting CVD or 2 or more CV risk factors. Only 36% of the 9901 patients had established CVD, yet at a median follow-up of 5 years, dulaglutide was still showing significantly reduced MACE. Next, Albiglutide was studied in the HARMONY^[30] trial which was also international across 28 countries and enrolling 9463 participants but all had established CVD and it was superior to placebo in reducing MACE. Lastly, the PIONEER6^[31] examined oral semaglutide in patients with T2DM with high risk of CV events and showed non-inferiority but not superiority in MACE. Secondary outcomes though showed statistically significant reduction in CV death and all-cause mortality in those 3183 patients.

SGLT2i also had its first CVOT published in 2015, Empagliflozin, Cardiovascular Outcomes and Mortality in Type 2 Diabetes trial (EMPA-REG OUTCOME)^[32]. They enrolled 7028 patients with recognized CVD or elevated CV risk with an average starting HbA1c of 8%, demonstrating superiority over placebo, similar to the PEP of the LEADER trial with an additional benefit for hospitalization for heart failure and diabetic nephropathy. Later, the Canagliflozin Cardiovascular Assessment Study (CANVAS)^[33] and the Study of the Effects of Canagliflozin on Renal Endpoints in Adult Participants with T2DM (CANVAS-R) trials had similar results by examining approximately 10000 patients with established CVD in a younger population.

Table 2 Summary of the results of the most important Randomized Controlled Trials prior to the new classes of antidiabetic medications

Study	Effects on microvascular complications	Effects on macrovascular complications	Effect on total mortality
DCCT ^[10] (1993), T1DM	Reduced retinopathy, nephropathy, neuropathy	No difference on major cardiovascular and peripheral vascular events	No difference
UKPDS ^[9] (1998)	Reduced microvascular endpoints	No difference on myocardial infarctions	No difference
ACCORD ^[14] (2008)	Reduced retinopathy, nephropathy, neuropathy	No difference on MACE	Increased mortality
ADVANCE ^[11] (2008)	Reduced nephropathy	No effect on MACE	No difference
VADT ^[12] (2009)	Reduced progression of albuminuria	No effects on major cardiovascular events	No difference

Note the lack of difference in macrovascular complications despite reduced microvascular complications, which is consistent among all studies. MACE: Major adverse cardiovascular events; DCCT: Diabetes Control and Complications Trial; T1DM: Type 1 diabetes mellitus; UKPDS: United Kingdom Prospective Diabetes Study; ACCORD: Action to Control Cardiovascular Risk in Diabetes; ADVANCE: Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled Evaluation; VADT: Veterans Affairs Diabetes Trial.

However, the magnitude of the benefit with canagliflozin was smaller compared to other trials (only a third of 1%, meaning we would have to treat several hundred more patients to prevent a MACE). It also raised safety concerns by showing increased risk for lower limb amputations and fractures while also being consistent with previous CVOTs in regards of the increased risk for mycotic infections but no change in rates of Diabetic Ketoacidosis.

In 2019, the Dapagliflozin Effect on Cardiovascular Events trial (DECLARE – TIMI 58)^[34] studied dapagliflozin for primary and secondary prevention in patients with T2DM and CVD or at high-risk for CVD and was the largest CVOT to date with 17160 patients. It showed noninferiority in MACE without superiority. A reduction in hospitalization for heart failure and all-cause mortality was established with robust reductions in the renal composite endpoints, suggesting a delay in the development and progression of renal disease.

Results have for the most part been consistent, as was demonstrated by Cheng *et al*^[35], who analyzed a total of 12 double-blind randomized controlled trials, concluding that liraglutide, empagliflozin and canagliflozin to be superior in CV outcome in comparison to placebo in patients with T2DM and established or high-risk for CVD.

Renal effects

From the abundance of evidence, clinicians have already established that the intensification of glycemic control is the best approach to reduce microvascular complications. But when microvascular disease has already taken place, our options have remained limited, with our first line of defense consisting of angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers (ARB) in the case of nephropathy and blood pressure control, and symptomatic treatment for the case of neuropathy and retinopathy.

Most of the large RCTs involving either SGLT2i or GLP-1RA have demonstrated, to varying degrees, a reduction in microvascular endpoints and associated morbidity. This is especially relevant for patients with chronic kidney disease and albuminuria, who represent a vulnerable subset of patients who, until recently, lacked treatment options for both preventing the development of the disease and delayed the its progression when these two factors are already present.

SGLT2i have demonstrated effects in hyperglycemic states by enhancement of glycosuria and natriuresis^[36]. These effects may have a renal protective role by indirectly lowering blood pressure by competitively blockading the SGLT2 receptors in the proximal convoluted tubules in the kidneys, thus preventing reabsorption of the filtered glucose and sodium, decreasing the overall effective intravascular volume in addition to the intended antihyperglycemic effect.

This is further exemplified by a new prospective analysis by Sugiyama *et al*^[37]. In this study, dapagliflozin was used in patients with T2DM with ineffective glycemic control. Those patients who were treated with dapagliflozin had a significant decrease in urine albumin-to-creatinine ratio (UACR) and urine N-acetyl-β-glycosaminidase, a marker of kidney injury. We can speculate based on these findings that dapagliflozin might prevent the renal tubulointerstitial atrophy that is correlated with the development of chronic kidney disease (CKD) in patients with T2DM.

Patients with early and uncontrolled T2DM have an increased glomerular filtration

Table 3 Summary of glucagon-like-peptide-1 receptor agonists and sodium glucose cotransporter 2 inhibitors Randomized Controlled Trials

Trial	Number Follow up	CV disease (baseline)	Characteristics (baseline)	Drug vs Placebo (%) PEP	Superiority
ELIXA ^[22] (Lixisenatide) (2015)	<i>n</i> = 6068, 2.1 yr	Acute Coronary Events (previous 180 d)	Median age: 60; DM duration: 9.3 yr (median); A1c: 7.7%; BMI: 30.1	13.4 <i>vs</i> 13.2 (4-point MACE)	No
LEADER ^[23] (Liraglutide) (2016)	<i>n</i> = 9340, 3.8 yr (median)	> 50 y/o + > 1 CV condition/CKD or Chronic HF or > 60 y/o > 1 risk factor for CVD	mean age: 64; DM duration: 12.8 yr (median); A1c: 8.7%; BMI: 32.5	13.0 <i>vs</i> 14.9	Yes
SUSTAIN-6 ^[24] (Semaglutide) (2016)	<i>n</i> = 3297, 2.1 yr (median)	> 50 y/o + > 1 CV condition/CKD or Chronic HF or > 60 y/o > 1 CV condition	mean age: 65; DM duration: 13.9 yr (median); A1c: 8.7%; BMI: 30.1	6.6 <i>vs</i> 8.9	Yes
EXSCEL ^[26] (Exenatide) (2017)	<i>n</i> = 14752, 3.2 yr (median)	70% with previous CV events (CAD, ischemic cerebrovascular disease, or PAD)	mean age: 63; DM duration: 12 yr (median); A1c: 8.0%; BMI: 32	11.4 <i>vs</i> 12.2	No
REWIND (Dulaglutide) (2019)	?	?	?	?	?
EMPA-REG ^[31] (Empagliflozin) (2015)	<i>n</i> = 7020, 3.1 yr (median)	Established CV disease; high CV risk	mean age: 63; DM duration: > 10 yr 57%; 5-10 yr 25%; A1c: 8.07%; BMI: 30.6	10.5 <i>vs</i> 12.1	Yes
CANVAS ^[32] (Canagliflozin); ANVAS - R (Canagliflozin) (2017)	Total = 10142; CANVAS: <i>n</i> = 4330; CANVAS-R <i>n</i> = 5812; 3.6 yr (mean)	> 30 y/o at high CV risk (ASCVD) Or > 50 y/o > 2 CV risk factors	mean age: 63.3; DM duration: 13.5 yr (median); A1c: 8.2; %BMI: 32	9.8 <i>vs</i> 10.1	Yes
DECLARE ^[33] (Dapagliflozin) (2019)	<i>n</i> = 17160; 4.2 yr (median)	> 40 y/o established CVD or multiple risk factors	MEAN age: 64; DM duration: 11 yr (median); A1c: 8.3%; BMI: 32	8.8 <i>vs</i> 9.4	No

Not all the molecules currently available in the market have shown benefit for MACE. However, this can be explained by study design and/or random chance. More trials are needed to verify such findings. Note the CANVAS and CANVAS - R trials had to standardize their results to number of participants/1000 patient-yr. The results depicted in this table were converted to percentage. The REWIND trial results will become available on the ADA scientific meeting, 2019. The ELIXA trial had 4-point PEP that consisted in death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke or hospitalization for unstable angina. GLP-1 RA: Glucagon-like-peptide-1 receptor agonists; SGLT2i: Sodium glucose cotransporter 2 inhibitors; PEP: Primary end point; CV: Cardiovascular; MACE: Major cardiovascular events; CKD: Chronic kidney disease; CVD: Cardiovascular disease; HF: Heart failure; PAD: Peripheral artery disease; CAD: Coronary artery disease; ELIXA: Evaluation of Lixisenatide in acute coronary syndrome; LEADER: Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results; SUSTAIN-6: Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes; EXSCEL: Exenatide Study of Cardiovascular Event Lowering Trial; REWIND: Researching cardiovascular Events with a Weekly Incretin in Diabetes; EMPA-REG: Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes trial; CANVAS: Canagliflozin Cardiovascular Assessment Study; CANVAS-R: A Study of the Effects of Canagliflozin on Renal Endpoints in Adult Participants with Type 2 Diabetes Mellitus; DECLARE: Dapagliflozin Effect on Cardiovascular Events trial.

rate (GFR). This exposes the proximal tubule to insulin and other growth factors, leading to hyperplasia and hypertrophy in the tubular cells with significant hyperfiltration^[38]. In turn, hyperfiltration could be the leading cause of renal damage in people with T2DM. SGLT2i can reverse this hyperfiltration in certain patients by blocking the glucose reabsorption in the proximal tubule. A model of renal hyperfiltration developed with pharmacokinetics (PBPK) and pharmacodynamics (PD) by the Quantitative Systems Pharmacology Diabetes Platform have confirmed this hypothesis^[39].

The evidence evaluating renal benefits of SGLT2i until recently, was limited by the fact that there has been no RCT trial where the primary outcome is renal with SGLT2i (recently, this has changed, see below). A meta-analysis of the CVOTs of SGLT2i in patients with T2DM including 34322 patients performed by Zelniker *et al*^[40] in *Lancet* 2019 concluded that SGLT2i decreased the risk of progression of renal failure by 45% with lesser reductions in progression of renal disease in patients with more severe kidney disease at baseline.

Another 2019 systematic review of 27 studies totaling 7363 participants with T2DM and CKD^[41] found SGLT2is demonstrated a nonsignificant decline in estimated glomerular filtration rate (eGFR) slope, though a significantly reduced risk of the composite renal outcome. A retrospective analysis made by Kobayashi *et al*^[42], defined the renal effects of SGLT2i in Japanese patients with T2DM with CKD. Results were

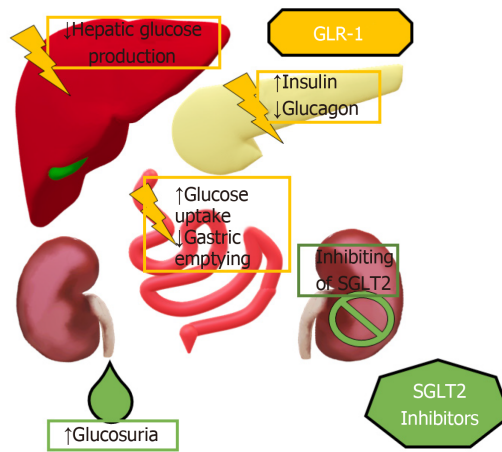


Figure 1 Mechanism of action of the sodium glucose cotransporter 2 inhibitors and the glucagon-like-peptide-1 receptor agonists. Glucagon-like-peptide-1 receptor agonists slows gastric emptying, suppresses glucagon secretion while also stimulating insulin secretion by inhibiting and stimulating, respectively, Alfa and Beta cells in the pancreas. This in turn inhibits hepatic gluconeogenesis with subsequent increase in glucose uptake in the skeletal muscles, diminishing hyperglycemia. Sodium glucose cotransporter 2 inhibitors reduce glucose reabsorption in the proximal convoluted tubule, inherently enhancing glucosuria. This created a global hypovolemic and hypocaloric state, which diminishes hyperglycemia. SGLT2: Sodium glucose cotransporter 2; GLP-1: Glucagon-like-peptide-1.

statistically significant for reduction in the UACR. GLP-1 RA have also demonstrated a certain degree of renal protection. Liraglutide and semaglutide have shown to decrease albuminuria while also halting the worsening of the eGFR^[43].

GLP-1 acts directly in the kidney by inhibiting the NH₃-dependent sodium reabsorption in the proximal tubule. The renal outcomes were a secondary outcome assessed in the LEADER trial^[23], showing a delay in new onset macroalbuminuria with a reduction of 26% in patients with liraglutide and a notable decrease in UACR^[44].

Due to lacking studies with primary renal outcomes, no GLP1RAs or SGLT2s have FDA approval for indication of renal benefits with T2DM. The recently published Canagliflozin and Renal Endpoints in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial^[45], whose data was published recently, might be a game changer. It is been almost 18 years since the advent of renin-angiotensin-aldosterone system blockers, the last advancement in the area. The study randomly assigned patients to receive canagliflozin or placebo on top of renin-angiotensin-aldosterone-system (RAAS) blocker therapy, observing an impressive 30% relative risk reduction in the primary endpoint consisting of end-stage kidney disease, doubling of serum creatinine, or renal or cardiovascular death that seems to be independent of the glucose lowering properties due to the minimal A1c difference at the end of the study (0.1%). This concept will be tested in the ongoing trials for dapagliflozin and empagliflozin (Dapa-CKD and EMPA-KIDNEY trials respectively) which have a sizable portion of participants without diabetes.

Additionally, it is also important to remember renal dosing requirements. SGLT2i require, in general, an eGFR greater than 45. For now, dulaglutide and liraglutide remain as the only novel medications that can be used in moderate to severe CKD given the evidence provided by the Dulaglutide versus insulin glargine in patients with type 2 diabetes and moderate-to-severe chronic kidney disease (AWARD-7) trial^[46] and the LEADER trial.

Blood pressure effects

Both SGLT2is and GLP1RA have shown reduction in blood pressure, independent from their hypoglycemic mechanisms^[47]. In Tikkanen's^[48] study of patients with T2DM and hypertension, at week 12 the mean difference versus placebo in mean 24-h systolic blood pressure was -3.44 mmHg and -4.16 mmHg with 10 mg and 25 mg of empagliflozin, respectively. Blood pressure can be reduced also in patients with nocturnal hypertension, as demonstrated in the SGLT-2i and ARB Combination Therapy in Patients with T2DM and Nocturnal Hypertension (SACRA) study, conducted in Japan. The reduction in nighttime systolic blood pressure (SBP) with the use of empagliflozin was associated with daytime reductions in SBP and 24-h SBP^[49].

The activation of the RAAS increases the SGLT2 mRNA expression in the proximal renal tubular epithelial cells with subsequent sodium intake. This causes an expansion

in the intravascular volume that leads to hypertension^[50]. Although the inhibition of SGLT2 will activate the RAAS, it is suggested to combine the SGLT2i with any RAAS blockers to suppress RAAS and thus prevent hypertension^[51].

Within GLP1RAs, exenatide and liraglutide have displayed a reduction in the systolic and diastolic blood pressure from 1 to 5 mmHg in comparison with other antidiabetic medications, like insulin, glimepiride, metformin, or placebo^[52]. Co-initiating the GLP1RA exenatide and the SGLT2i dapagliflozin compared to either agent alone, the DURATION-8^[53] trial showed the combination lowered the systolic blood pressure 4.1 mmHg, which was greater than either agent alone. Similar to renal outcomes, blood pressure has been a secondary outcome yet a beneficial one in alleviating some burden of hypertension with an antidiabetic agent^[54].

Weight effects

Increasing BMI can lead to the development of T2DM and poses a greater risk of CVD and all-cause mortality. Weight loss in patients with T2DM is critical in the improvement of hyperglycemia and cardiovascular comorbidities like hypertension and hyperlipidemia^[55].

Currently, the American Diabetes Association and the European Association for the Study of Diabetes have made an emphasis in the importance of lifestyle modifications, diet and exercise in patients with T2DM. Unfortunately, many of our antihyperglycemic agents are associated with weight gain including thiazolidinediones, sulfonylureas and insulin. Though modest at 1-3kg weight loss^[56], this has made the SGLT2i and the GLP-1RAs benefits in weight loss even more exciting.

In the DURATION-6^[57] trial, extended-release exenatide demonstrated an average weight loss of about 2.87 kg, and liraglutide^[58] has shown to reduce 4 to 6 kg of weight loss. The SCALE^[59] trial evidenced an 8.4 kg weight loss compared to a placebo group of 2.8 kg when treating patients without T2DM with a high-dose of 3.0 mg injected liraglutide as an adjunct to diet and exercise. One must keep in mind the CVOT trials were mostly the 1.8 mg dosing and the applicability of these results to the 3.0 mg dose is unknown.

Moreover, in 2017, semaglutide^[23] was associated with significant weight loss, which has shown to be superior to liraglutide in its dose for treatment for T2DM. Even though semaglutide not been approved for pharmacological weight loss therapy, it opens the possibility for one more GLP-1 RA being used as an anti-obesity drug that would prevent cardiovascular events in patients with T2DM. SGLT2i are associated with a more modest reduction of body weight, with dapagliflozin showing a mean 1.63 kg reduction compared with placebo. The DURATION-8^[52] clinical trial confirmed that a combination of dapagliflozin and exenatide, plus metformin as a background therapy, resulted in a secondary outcome of weight loss of 3.4 kg, which was greater than either drug alone. Nevertheless, the trend is that GLP1RAs offer more weight loss compared to SGLT2i, which is a relief compared to the classes that are associated with weight gain such as insulin and sulfonylureas.

While these medications are helpful in weight management, it is important to keep in mind this does not triumph over comprehensive lifestyles changes with aerobic exercise and dietary changes. Also, equally important, weight loss using GLP-1 RA should be monitored at least every 3 mo from the starting of the treatment due to side effects^[60].

Additional considerations

Several concerns exist with both GLP-1RAs and SGLT2is which must be weighed against the benefits detailed above. Like with any agent, discussion of risks and benefits when starting treatment is recommended. GLP-1RAs most common adverse effect is gastrointestinal with nausea, vomiting and diarrhea^[61]. Commonly, nausea tends to wane over time. Patients should be informed about this as well as the possibility of injection site reactions when beginning therapy. An increased rate of acute and/or chronic pancreatitis has been established with the available RCTs, but there is no firm evidence pointing towards causality^[62]. Preclinical data from studies done in rodents^[63] raised the possibility of a medication induced carcinogenesis, specifically medullary thyroid cancer (MTC), however, these effects may be irrelevant in humans^[64]. Nevertheless, a Black Box warning remains with GLP1RAs and risk of thyroid c-cell tumors, including a contraindication in patients with personal or family history of MTC or in patients with Multiple Endocrine Neoplasia syndrome type 2.

SGLT2is most common adverse effect is genitourinary. There is a fivefold increased risk of genital fungal infections with SGLT2i, including an FDA warning about rare occurrences of Fournier's gangrene^[65]. Multiple RCTs show increased risk of bacterial urinary tract infection versus placebo, which can prove to be a challenge when considering treatment^[66]. No clinical trial has examined special circumstances (indwelling bladder catheterization, benign prostatic hypertrophy, chronic

obstruction or ureteral reflux) but caution under these circumstances is advised. Given the inherent diuretic effect, patients who are prone to volume depletion (use of loop diuretics, the elderly) are at increased risk of complications, including hypotension^[67].

The FDA^[68] has issued a warning regarding SGLT2i users being more prone to DKA, secondary to the intrinsic shift in the metabolism of glucose to fat oxidation with the promotion of hyperglucagonemia and ketosis^[69]. Canagliflozin has been associated with an increased risk for fractures^[70] and lower-limb amputations^[32], including a Black Box warning for the amputation risk. There is need for further research on whether these side effects are a class effect or unique to canagliflozin.

One of the most controversial topics is the cost-effectiveness and the prohibitive out-of-pocket costs of both drug classes. Studies with reliable results on the long-term economic burden are scarce. The number needed to treat on both classes is in the hundreds based on the CVOTs from which the indication for cardiovascular prevention was approved by the FDA. GLP-1RAs demonstrated CV benefit after several years of median follow up, compared to SGLT2is, specifically empagliflozin, which demonstrated a divergence in survival curve for MACE at 3 mo in the EMPA-REG study. Some of the drugs that had a statistically significant benefit in the PEP for cardiovascular outcome had a very small percentage of benefit over placebo (*i.e.*, canagliflozin with 0.3% benefit over placebo). Side effects like lower-limb amputations, DKA and pancreatitis can be economically damaging and add several thousand dollars to the already high economic burden.

CONCLUSION

In summary, given the current data, both GLP-1 RA and SGLT2i have, to varying degrees, a benefit in renal and cardiovascular protection independent of their glucose-lowering potential in patients with T2DM and high risk of CVD. Additionally, they have more modest benefits in blood pressure and weight control. The low risk for hypoglycemia is appealing.

When starting therapy, the cost-effectiveness is a concern shared by clinicians and patients. The number needed to treat to prevent MACE in both drug classes are in the hundreds and the economic burden is in the thousands to millions per patient per year. Considering the benefits in each study were observed after several years of follow up, the out-of-pocket expense could be prohibitively high.

Both common and rare adverse effects are also a consideration. SGLT2i carry an increased risk for mycotic urinary tract infections, dehydration and DKA. Canagliflozin additionally has the concerns of bone fractures and lower-limb amputations. GLP-1RAs have been associated with both acute and chronic pancreatitis, as well as a common side effect of nausea. The evidence for pancreatitis is debatable and weak since it is not supported by trials or a meta-analysis. Reports of increased MTC risk in rodents has the resultant black box warning of thyroid c-cell tumors. Specific trials designed to take a closer look at these effects will be necessary in the future to prepare a better risk-benefit assessment. The economic burden needs to be added to the equation.

As more studies concerning different agents on the same class of drugs are added to the already existing data, the question on whether each new finding is a class affect or a molecule-based outcome will be determined. With the current evidence at our disposal, we cannot guarantee that GLP-1 RAs all have the same benefits and what the ideal patient population is to maximize those benefits. SGLT2is, on the other hand, seem to offer a more homogenous effect with certain differences that can be attributed to each individual study to a certain extent, but more research is necessary.

As clinicians, we are moving a step forward in T2DM management. Now, patients can be offered antihyperglycemic agents that will treat micro and macrovascular complications while also treating independent risk factors, maintaining an acceptable level of antihyperglycemic effect with a low risk for hypoglycemic events.

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Observational Study

Association of hypoglycaemia in screening oral glucose tolerance test in pregnancy with low birth weight fetus

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Abstract

BACKGROUND

Gestational diabetes mellitus (GDM) is a common metabolic derangement in pregnant women. In the women identified to be at high risk of GDM, a 75 g oral glucose tolerance test (OGTT) at 24-28 wk gestation is the recommended screening test in the United Kingdom as per National Institute for Health and Care Excellence (NICE). Hypoglycaemia following the glucose load is often encountered and the implication of this finding for the pregnancy, fetus and clinical care is unclear.

AIM

To determine the prevalence of hypoglycaemia at any time during the screening OGTT and explore its association with birth weight.

METHODS

All deliveries between 2009 and 2013 at the local maternity unit of the University hospital were reviewed. Of the total number of 24,154 women without pre-existing diabetes, those who had an OGTT for GDM screening based on NICE recommended risk stratification, who had a singleton delivery and had complete clinical and demographic data for analysis, were included for this study ($n = 3537$). Blood samples for fasting plasma glucose (FPG), 2-hour plasma glucose (2-h PG) and HbA_{1c} had been obtained. Birth weight was categorised as low (≤ 2500 g), normal or Macrosomia (≥ 4500 g) and blood glucose ≤ 3.5 mmol/L was used to define hypoglycaemia. Binary logistic regression was used to determine the association of various independent factors with dichotomized variables; the differences between frequencies/proportions by χ^2 test and comparison between group means was by one-way ANOVA.

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RESULTS

Amongst the study cohort (3537 deliveries), 96 (2.7%) women had babies with LBW (< 2500 g). Women who delivered a LBW baby had significantly lower FPG (4.3 ± 0.6 mmol/L, $P = 0.001$). The proportion of women who had a 2-h PG ≤ 3.5 mmol/L in the LBW cohort was significantly higher compared to the cohorts with normal and macrosomic babies (8.3% *vs* 2.8% *vs* 4.2%; $P = 0.007$). The factors which predicted LBW were FPG, Asian ethnicity and 2-h PG ≤ 3.5 mmol/L, whereas maternal age, 2-h PG ≥ 7.8 mmol/L and HbA_{1c} were not significant predictors.

CONCLUSION

A low FPG and 2-h PG ≤ 3.5 mmol/L on 75-gram OGTT are significantly associated with low birth weight in women identified as high risk for GDM. Women of ethnic backgrounds (Asians) appear to be more susceptible to this increased risk and may serve as a separate cohort in whom we should offer more intensive follow up and screening for complications. Cost implications and resources for follow up would need to be looked at in further detail to support these findings.

Key words: Hypoglycemia; Glucose tolerance test; Low birth weight; Pregnancy

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Core tip: Hypoglycaemia following a glucose load in a oral glucose tolerance test is often encountered whilst screening for Gestational diabetes mellitus in pregnant women categorized as high risk and our study with a large cohort, confirms an association between hypoglycaemia and low birth weight (LBW) delivery. In addition to this, our study also finds that Asian ethnicity confers a risk for LBW babies.

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INTRODUCTION

It is estimated that 700000 women give birth in England and Wales each year and 5% of these are complicated by diabetes mellitus. Gestational diabetes mellitus (GDM) accounts for the vast majority of this cohort (87.5%)^[1]. A 2-h 75 g oral glucose tolerance test (OGTT) is undertaken at 24-28 wk gestation in women at high risk as a screening test for GDM, in line with National Institute for Health and Care Excellence (NICE) recommendations^[1]. Women diagnosed with GDM based on this test have specialist antenatal intervention during pregnancy with improved maternal and neonatal outcomes^[2].

A small proportion of women experience hypoglycaemia during the screening OGTT in pregnancy, which on a routine basis is not considered abnormal, and does not usually have an impact on antenatal care. This is despite such women being deemed "high risk" based on initial NICE risk stratification to necessitate an OGTT in the first place. Maternal hypoglycaemia during pregnancy in women with pre-existing diabetes mellitus is associated with intrauterine growth retardation and pre-eclampsia^[3,4]. Low maternal glucose might hinder growth-promoting aspects of the fetus' environment, a mechanism that is not clearly understood, that could potentially explain the lower birth weight fetus in women with hypoglycemia during pregnancy. Low levels of human placental lactogen has been linked to intra uterine growth retardation and other suggested mechanism include a reduced insulin level in fetus of a mother with low blood sugar levels^[4]. It is unclear if hypoglycemia during a screening OGTT in high risk women is associated with adverse perinatal outcomes with some studies potentially suggesting such an association^[3-8]. Maternal hypoglycaemia during a glucose challenge test has been linked to intra uterine growth retardation and low birth weight (LBW) as early as 1970's^[5,6] and a number of

subsequent studies have shown similar link^[7-9], however, a study by Weissman *et al*^[10] showed no increase in small for gestational age infants in this group.

We aimed to determine the prevalence of hypoglycaemia on OGTT (both fasting and 2-h PG) in women screened for GDM at 24-28 wk gestation in our hospital and explore the association between maternal hypoglycaemia during OGTT screening and birth weight.

MATERIALS AND METHODS

Patient selection

We reviewed all deliveries in the maternity unit of our University hospital over a consecutive 4-year period between years 2009 and 2013, identifying 24154 women without pre-existing diabetes mellitus. Utilising the risk stratification recommended by National Institute of Clinical Excellence (2008), 7207 women were categorized as at "high risk" for GDM, who were then offered an OGTT at 24-28 wk as part of GDM screening. HbA_{1c} estimation is undertaken simultaneously with all OGTTs as per the local trust guidelines^[11]. Laboratory data that was obtained from the clinical biochemistry department was thereafter linked to the clinical information that was taken from the electronic patient records in the obstetric registry on the dataset.

Those women with singleton pregnancy delivered on or after 37-wk gestation were identified ($n = 6716$) for the purpose of this study to avoid the impact of the preterm deliveries on birth weight during analysis. No other selection criteria were used however complete demographic and clinical data was available in 3537 women and these women formed the cohort used for analysis.

Categorisation by birth weight and glycaemic parameters

Birth weight definitions: LBW: ≤ 2500 g^[12]; Normal birth weight: 2501- 4499 g; Macrosomia: ≥ 4500 g^[13].

Glycaemic parameters: A fasting plasma glucose (FPG) ≥ 5.6 mmol/L and/or a 2 h plasma glucose (2-h PG) post 75 g glucose load ≥ 7.8 mmol/L in the OGTT were the cut offs used to diagnose GDM. Blood glucose value ≤ 3.5 mmol/L was classed as hypoglycaemia. Based on the 2-h PG, the cohort was categorised into "low" 2-h PG (≤ 3.5 mmol/L), "normal" 2-h PG (3.6-7.7 mmol/L) and "high" 2-h PG (≥ 7.8 mmol/L).

Analytical methods

OGTT was performed after a minimum of 8-h overnight fast as per standard protocol. A blood sample for FPG was obtained each participant was given a glucose drink (75 g of D-dextrose powder dissolved in 200 mL of water). Samples for FPG and 2-h PG were obtained by taking 2 mL of venous blood in tubes containing sodium fluoride. A sample for HbA_{1c} estimation was obtained along with the sample for FPG. HbA_{1c} was measured using high performance liquid chromatography on a Tosoh G7 analyser (Tosoh Bioscience Ltd., Worcestershire, United Kingdom). The performance scores in the United Kingdom National External Quality Assurance Scheme were: A scores < 100 and B scores $< 2\%$. The between-batch coefficient of variation was 1.8% and 1.4% for an HbA_{1c} of 5.7% and 9.5% respectively.

The International Federation of Clinical Chemistry (IFCC) units for HbA_{1c} levels were introduced in the United Kingdom since 1st June 2009. Locally, the IFCC reference system was adopted and the dual reporting of HbA_{1c} with IFCC units and the corresponding calculated Diabetes Control and Complications Trial value was available during the period and utilised for the analysis of data among the participants.

Statistical analysis

Data were analysed using SPSS version 21 (SPSS Inc., Chicago, IL). Data are presented as mean \pm SD unless otherwise stated. All statistical tests were considered significant at $P < 0.05$. Comparison between multiple group means was by one-way ANOVA and the differences between frequency/proportions by Chi-square test. Binary logistic regression analysis was undertaken to determine the association of independent factors with dichotomised variable (birth weight).

RESULTS

The demographic details and the glycaemic parameters of the cohort ($n = 3537$) of women are shown in Table 1. The proportions of women with LBW and macrosomic

babies were each 2.7%, and remaining 94.6% had babies with normal birth weight. In total 130 women (3.7%) had hypoglycaemia (blood glucose ≤ 3.5 mmol/L) on the OGTT, majority on the 2-h PG value ($n = 107$ (3.0%)).

Women who delivered LBW fetus had a significantly lower FPG compared to women delivering babies with normal birth weight or macrosomic babies (Table 1). The mean 2-h PG was similar in the three cohorts by birth weight, however the proportion with 2-h PG ≤ 3.5 mmol/L in the LBW cohort was significantly higher compared to the other two cohorts (8.3% *vs* 2.8% *vs* 4.2%; $P = 0.007$).

On binary logistic regression independent predictors of LBW were FPG (OR = 0.52, 95% CI: 0.32-0.86; $P = 0.010$, B = minus 0.654), Asian ethnic origin (OR = 2.36, 95% CI: 1.45-3.84; $P = 0.001$) and 2-h PG ≤ 3.5 mmol/L (OR = 2.52, 95% CI: 1.11-5.72; $P = 0.028$). Maternal age, 2-h PG ≥ 7.8 mmol/L and HbA_{1c} were not significant predictors of LBW.

Comparing the "low" *vs* "normal" *vs* "high" 2-h PG cohorts (Table 2), women in "low" 2-h PG cohort, compared to "normal" and "high", were younger (27.2 ± 5.8 *vs* 28.4 ± 5.7 and 30.6 ± 5.5 years, $P < 0.001$), with more Caucasians (86% *vs* 82% and 73%, $P < 0.001$). Birth weight (mean \pm SD) for "low", "normal" and "high" 2-h PG cohorts were 3357 ± 591 *vs* 3480 ± 515 *vs* 3349 ± 459 g, being significantly lower in "low" cohort compared to "normal" (mean difference in weight = -122.9 g, Std. error 50.33 g; $P = 0.015$), but comparable to the "high" 2-h PG cohort. "Low" 2-h PG cohort had a significantly higher proportion of LBW compared to those with "normal" and "high" 2-h PG (7.5% *vs* 2.6% *vs* 2.5%; $\chi^2 = 13.9$, $P = 0.008$). The still-birth rates were similar in the three cohorts of 2-h PG.

DISCUSSION

Our study on a large cohort of pregnant women at high risk of GDM, delivered at 37 wk gestation or later, demonstrates that low FPG and/or 2-h PG ≤ 3.5 mmol/L on OGTT at 24-28 wk gestation, both independently predict LBW baby. This supports the previous smaller studies that found a relation between maternal hypoglycaemia during OGTT and LBW^[7-9]. Melamed *et al*^[12] have calculated that a threshold of 88.5 mg/dl (4.9 mmol/L) following 100 g glucose challenge will predict a birth-weight $< 10^{\text{th}}$ percentile. In a recent study^[13] on women who had postprandial hypoglycaemia on OGTT comparing with GDM and normoglycaemic groups, when subsequently monitored with self-monitoring of blood glucose, nearly half of them had elevated FPG readings above 5.1 mmol/L on at least 2 occasions in the 1-wk period were in the GDM range when using the Australian Diabetes in Pregnancy Society criteria. However, the study did not find any differences in the pregnancy outcomes amongst the groups studied or enough evidence to recommend use of self-blood glucose monitoring in this cohort^[13].

Women who had babies with a LBW were more likely to have blood glucose of ≤ 3.5 mmol/L compared to those who had babies with normal birth weight or macrosomia. This highlights the importance of not dismissing this important finding in a pregnant woman with a low blood glucose value detected on OGTT.

This study also highlights the importance of ethnicity when assessing risk, as we have found that the women of Asian ethnicity were at a greater risk of delivering a baby of LBW babies (29%). A study of pregnant women in India showed a higher incidence of LBW in those with fasting hypoglycaemia and this increased risk was found across different nutritional and pre-eclamptic statuses^[14]. Therefore, women of Asian ethnicity may be a sub-group who require more closer follow-up.

In our analysis maternal age did not appear to be a factor associated with LBW, contrary to the previous study^[15] which found that the women with hypoglycaemia were younger and had lower pre-pregnancy body mass index (BMI). Maternal BMI is associated with increase in insulin resistance predominantly in the skeletal muscle and adipose tissue potentially increasing risk of impaired glycaemia on OGTT and risk GDM.

The findings of our study may have implications in terms of obstetric follow up and further investigations for growth and assessment of those mothers identified with low blood glucose values on their OGTT. This would hold particularly true for those women of Asian descent and this group should have lower threshold to investigate fetal growth and optimize neonatal outcomes. The findings of our study and the fact that these women are considered "high risk" as per NICE criteria for needing the OGTT screening, this cohort of "high risk" women with hypoglycaemia may need appropriate intensive antenatal care with fetal growth monitoring, rather than being discharged due to the fact that OGTT does not suggest GDM.

One of the limitation of this study is that body mass index was not available and

Table 1 Demographics and glycaemic parameters for the cohort categorised by birth weight

	Birth Weight			
	< 2500 g (LBW) (n = 96)	2500-4500 g (normal BW) (n = 3346)	> 4500 g (macrosomia) (n = 95)	
Maternal age (yr)	28.6 ± 5.6	28.7 ± 5.6	29.0 ± 5.4	P = 0.85
Proportion Asians	29%	15%	1%	P = 0.001
FPG (mmol/L)	4.3 ± 0.6	4.5 ± 0.6	4.7 ± 0.5	P = 0.001
2-h PG (mmol/L)	5.5 ± 1.9	5.8 ± 1.6	5.8 ± 1.3	P = 0.26
Proportion with 2-h PG ≤ 3.5 mmol/L	8.3%	2.8%	4.2%	P = 0.007
HbA _{1c} IFCC (mmol/mol)	34.5 ± 3.4	34.3 ± 4.3	34.3 ± 0.4	P = 0.92

LBW: Low birth weight; FPG: Fasting plasma glucose; 2-h PG: 2 h plasma glucose on the oral glucose tolerance test.

could potentially impact on the association we report. Shinohara *et al*^[16] studied the pre-pregnancy BMI in the context of hypoglycaemia in OGTT and found that the hypoglycaemia was significantly associated with small for gestational age babies among underweight women (BMI < 18.5 kg/m²).

In conclusion, low FPG and/or 2-h blood glucose ≤ 3.5 mmol/L on 75-g OGTT is significantly associated with LBW in women identified as high-risk for GDM. Women of ethnic backgrounds (Asian) appear to be more susceptible to this increased risk and may serve as a separate cohort in whom we should offer more intensive follow up and screening for complications may need to be offered. Cost implications and resources for follow up would need to be looked at in further detail to support these findings.

Table 2 Demographics and clinical parameters for the cohorts categorised by 2-h plasma glucose

	2-h PG category (mmol/L)			
	Low (≤ 3.5) <i>n</i> = 107	Normal (3.6-7.7) <i>n</i> = 3066	High (≥ 7.8) <i>n</i> = 364	
Maternal age (yr)	27.2 \pm 5.8	28.4 \pm 5.7	30.6 \pm 5.5	<i>P</i> < 0.001
Proportion caucasians (%)	86	82	73	<i>P</i> < 0.001
Birth weight in grams	3357 \pm 591	3480 \pm 515	3349 \pm 459	¹ <i>P</i> < 0.001
Proportion with LBW (%)	7.5%	2.6%	2.5%	<i>P</i> = 0.008

¹Overall *P* < 0.001; on *post hoc* tests there was a significant difference only between the "low" 2-h PG cohort compared to "normal" 2-h PG (mean \pm SE = 122.9 \pm 50.3 g, *P* = 0.015). There was no difference in the birth weight between "low" 2-h PG and "high" 2-h PG cohorts. LBW: Low birth weight; 2-h PG: 2 h plasma glucose on the oral glucose tolerance test.

ARTICLE HIGHLIGHTS

Research background

Screening for gestational diabetes in high risk women during pregnancy is undertaken with oral glucose tolerance test (OGTT). This paper is an observational study auditing the prevalence of significant hypoglycaemia on the screening OGTT during pregnancy and exploring its impact on the birth weight, if any association with low birth weight (LBW). Currently those women identified as with hypoglycaemia on OGTT do not have any additional antenatal monitoring. Any association of such hypoglycaemia noted on the screening OGTT with LBW might help in targeting antenatal care in such women towards improving pregnancy outcomes.

Research motivation

The results of our study support allocation of resources for antenatal monitoring of women noted to have hypoglycaemia, especially the Asian ethnic cohort who appeared to be at higher risk of having babies with low birth-weight.

Research objectives

This study was undertaken to determine the prevalence of hypoglycaemia on the OGTT during screening for gestation diabetes in high risk women and explore any association with fetal birth weight.

Research methods

We audited data on all woman deemed high risk and had the screening OGTT during pregnancy identifying 3537 women who met the criteria and had the required complete data for analysis. Having defined hypoglycaemia (blood glucose ≤ 3.5 mmol/L) and categorizing birth weight as low (≤ 2500 g), normal (2500 to 4499 g) or Macrosomia (≥ 4500 g) we analysed the prevalence of hypoglycaemia on the OGTT screening and its association with birth weight using ANOVA to compare group means and logistic regression analysis to assess the factors independently predicting the low birth-weight.

Research results

In this audit on 3537 women deemed high risk as per NICE criteria and who had the OGTT screening, the proportion who has hypoglycaemia was 3.7%, majority of the hypoglycaemia being on the 2-h plasma glucose (2-h PG) value. 2.7% women had babies with LBW and this cohort had significantly lower fasting glucose (4.3 \pm 0.6 mmol/L, *P* = 0.001) and a higher proportion of this cohort had 2-h PG ≤ 3.5 mmol/L compared to the cohorts with normal and macrosomic babies (8.3% *vs* 2.8% *vs* 4.2%; *P* = 0.007). The factors which predicted LBW were fasting plasma glucose, Asian ethnicity and 2-h PG ≤ 3.5 mmol/L. Maternal age, 2-h PG ≥ 7.8 mmol/L and HbA_{1c} were not significant predictors of LBW.

Research conclusions

We observed the prevalence of hypoglycaemia in the screening OGTT during pregnancy to be about 3.7%. Such hypoglycaemia appears to be independently associated with risk of fetal LBW, and Asian ethnic origin being another risk factor for fetal low birth.

Research perspectives

This study on a large cohort of high risk women may improve awareness amongst clinicians about the potential impact of hypoglycaemia on birth weight and potentially help in considering assessment of fetal weight with serial growth scans as a part of antenatal care towards improving pregnancy outcomes. Future studies incorporating other risk factors associated with the fetal birth weight and studies looking at resource implications to implement the required fetal growth monitoring for such at-risk women with hypoglycaemia would be recommended.

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Association between sarcopenic obesity and higher risk of type 2 diabetes in adults: A systematic review and meta-analysis

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Abstract

BACKGROUND

The coexistence of sarcopenia and obesity is referred to as sarcopenic obesity (SO) and it has been hypothesized that the two components of SO may synergistically increase their negative effects. However, many uncertainties still surround this condition especially with regard to its potential negative effects on health outcomes.

AIM

To conduct a systematic review to determine the prevalence of sarcopenia among adults with overweight and obesity and to investigate whether SO was associated with a higher risk of type 2 diabetes (T2D).

METHODS

This study was conducted in adherence with the Preferred Reporting Items for Systematic Review and Meta-Analyses guidelines. Literature searches, study selection, methodology development and quality appraisal were performed independently by two authors and the data were collated by means of meta-analysis and narrative synthesis.

RESULTS

Of the 606 articles retrieved, 11 studies that comprised a total of 60118 adults with overweight and obesity of both genders met the inclusion criteria and were reviewed, revealing two main findings. First, the overall prevalence of sarcopenia is 43% in females and 42% in males who are with overweight and obesity. Secondly, the presence of SO increases the risk of T2D by 38% with respect to those without SO (OR = 1.38, 95% CI: 1.27-1.50).

CONCLUSION

A high prevalence of sarcopenia has been found among adults with overweight and obesity regardless of their gender and this condition seems to be associated

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with a higher risk of T2D. Clinician should be aware of this scenario in their clinical practice for the better management of both obesity and T2D.

Key words: Obesity; Overweight; Sarcopenia; Type 2 diabetes; Reduced lean body mass

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Core tip: The coexistence of sarcopenia and obesity is referred to a phenotype termed sarcopenic obesity, defined as the increase in body fat deposition, and the reduction in lean mass and muscle strength. Since many uncertainties still surround this condition, especially with regard to its potential negative effects on health outcomes, we conducted this systematic review and found a high prevalence of sarcopenia among adults with obesity. Moreover, this condition seems to be associated with a higher risk of type 2 diabetes (T2D). Clinicians should be aware of this scenario in their clinical practice for better management of obesity and T2D.

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INTRODUCTION

A condition that occurs because of the coexistence of sarcopenia and obesity has been termed sarcopenic obesity (SO)^[1-7]. Many uncertainties still surround this phenomenon with regard to its definition and its potential negative effects on health outcomes, especially those related to obesity, namely the so-called cardio-metabolic diseases^[8,9] such as type 2 diabetes (T2D), cardiovascular diseases, dyslipidaemia and metabolic syndrome^[5,6,10-13]. In fact, it has been hypothesized that the two components of SO may synergistically increase their negative effects on health, however this is still a matter of debate^[14-16].

Several studies have been conducted with a specific focus on determining the association between SO and T2D, however data regarding the contention that individuals with SO are likely to have poorer glycaemic profiles (*i.e.*, hyperglycaemia, high HbA1c, insulin resistance, *etc.*,) are still contradictory and require further clarification^[11,17-23]. Moreover, to the best of our knowledge no systematic review posing this issue as a primary outcome has yet been conducted in order to provide an unbiased interpretation of the evidence published to date. In light of these considerations, we set out to systematically review the published literature with the aim of determining the prevalence of sarcopenia among adults with overweight and obesity and to investigate whether SO was associated with higher risk of T2D, in accordance with the PICO process^[24-26] as detailed below: P – population: Individuals in the overweight or obese categories, however they were defined [*i.e.*, body mass index (BMI), body fat percentage, waist circumference, *etc.*,]^[27]; I – seeking treatment (*i.e.*, weight-loss or any other treatment if recruited from a clinical setting), otherwise non-treated if subjects were recruited from the general population; C – comparison: Comparison between individuals with sarcopenia and those without SO and with the healthy control group (when available); O – outcome: (i) Prevalence of SO however it was defined in the studies' Methods section (*i.e.*, low muscle mass, low muscle strength, low physical performance, increased visceral adiposity, increased waist circumference *etc.*,) and assessed [*i.e.*, bioelectric impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA), handgrip, *etc.*,] among the entire obesity groups in the two genders; (ii) The prevalence of T2D however it was defined in the studies' Methods section (*i.e.*, fasting plasma glucose and glycated hemoglobin A1c, oral glucose tolerance test *etc.*,) in the SO and non-SO groups.

MATERIALS AND METHODS

The review conformed to the Preferred Reporting Items for Systematic Review and

Meta-Analyses guidelines^[28-30] and was registered in the PROSPERO Registry, No. CRD42018111931^[31].

Inclusion and exclusion criteria

All studies that evaluated SO and T2D in adults were included, provided that they met the following criteria: (i) Studies written in English; (ii) Original research with a cross sectional or longitudinal design; and (iii) Prospective or retrospective observational (analytical or descriptive), experimental or quasi-experimental controlled or non-controlled studies, documenting clearly the prevalence of SO, as well as the association or relationship between SO and T2D. No reviews or non-original articles (*i.e.*, case reports, editorials, "Letters to the Editor" and book chapters) were included.

Information source and search strategy

The literature search was designed and performed independently in duplicate by two authors, namely the principal (DK) and the senior investigator (ME). The PubMed and Science direct databases^[32] were systematically screened using the MeSH terms and a manual search was carried out to retrieve other articles that had not been identified via the initial search strategy. Publication date was not considered an exclusion criterion for the purposes of this review.

Study selection

Two independent authors (DK and ME) screened the articles for their methodology and suitability for inclusion. The quality appraisal was conducted according to the Newcastle-Ottawa Scale (NOS), which relies on a 9-star system whereby scores of 0-3, 4-6 and 7-9 are considered poor, moderate and good quality, respectively^[33]. Consensus discussion was used to resolve disagreements between reviewers.

Data collection process and data items

The title and abstract of each paper were initially assessed by two independent authors (DK and ME) for language suitability and subject matter relevance, the selected studies were then assessed in terms of their suitability for inclusion and the quality of the methodology. The studies that passed both rounds of screening are presented in [Table 1](#).

Data synthesis

The 11 studies that met the inclusion criteria have been presented as a narrative synthesis. In addition, a meta-analysis was conducted on the included studies using Med Calc. software^[34]. The Mantel Haenszel fixed and random effect models were used to estimate the overall effect size and 95%CI. The pooled estimate and 95%CI of the prevalence of SO among males and females in the included studies was estimated similarly.

RESULTS

The initial search retrieved 606 papers. After the first round of screening, 366 papers were excluded for: (i) Languages other than English; (ii) Non-human studies; and (iii) Dealing with obesity without sarcopenia, or the latter without the former. The second round of screening excluded 229 articles due to: (i) Inappropriate paper type, not original research articles (*i.e.*, clinical reviews, Letters to the Editor, chapters in a book and case reports); (ii) Descriptions of SO, but not health-related outcomes; and (iii) An unclear definition of SO or identification of individuals with this condition. Accordingly, following the screening process, 11 articles were included in the systematic review and underwent narrative synthesis and meta-analysis ([Figure 1](#)). The NOS checklist proved that the studies were of a high quality ($n = 11$) (mean score = 7.36 points) ([Table 2](#)).

Narrative synthesis

In 2012, Sénéchal *et al*^[35] conducted a cross-sectional evaluation in which the authors assessed dynapenic obesity, defined as low leg muscle strength combined with abdominal obesity, in 1963 individuals with abdominal obesity. Of these patients, 566 had dynapenic obesity (data per gender is not available). Regardless of gender the mean age and mean BMI in the dynapenic obesity and non-dynapenic obesity groups were 65.4 ± 9.9 years and 29.9 ± 4.6 kg/m² and 65.5 ± 9.6 years and 30.8 ± 4.5 kg/m² respectively. Furthermore, 130 of the 566 individuals with dynapenic obesity had T2D compared to 196 of the 1397 individuals in the non-dynapenic obesity group.

One year later, Lu *et al*^[18] completed a cross sectional study in which they assessed

Table 1 Studies included in the systematic review

Study	Design	Definition of SO	Body composition	Gender	Sample	Mean age	Mean BMI	Prevalence Sarcopenic Obesity	Prevalence of Diabetes
Sénéchal <i>et al</i> ^[35] , 2012	Cross sectional	Dynapenic obesity, defined as low leg muscle strength, combined with abdominal obesity	Kin- Com dynamometer	M-F	T = 1963	Non DO: 65.5 ± 9.6; DO: 65.4 ± 9.9	Non DO: 30.8 ± 4.5; DO: 29.9 ± 4.6	DO: <i>n</i> = 566/1963 (Did not distinguish in gender)	T2D: Non DO: <i>n</i> = 196; DO: <i>n</i> = 130
Lu <i>et al</i> ^[18] , 2013	Cross sectional	Defined by combination of total skeletal muscle mass/wt. (100) and BMI ≥ 25 kg/m ²	BIA	M-F	T = 180; M = 60; F = 120	Non SO: 69.9 ± 7.3; SO: 61.1 ± 9.9	Non SO: 26.8 ± 1.6; SO: 27.8 ± 2.6	<i>n</i> = 35/60 in males; <i>n</i> = 80/120 in females	T2D: Non SO: <i>n</i> = 12/65; SO: <i>n</i> = 17/115
Poggiogalle <i>et al</i> ^[36] , 2015	Cross sectional	Defined by ASMM/h ² or ASMM/wt. < 2SD of sex specific mean combined with assessment of FM and FFM	DXA	M-F	T = 727; M = 141; F = 586	46.49 ± 13.73; 46.99 ± 13.76	38.85 ± 5.88; 38.84 ± 5.79	SO: <i>n</i> = 68/141 in males; <i>n</i> = 350/586 in females	Pre-diabetes or T2D: Non-SO: <i>n</i> = 69; SO: <i>n</i> = 155
Ma <i>et al</i> ^[37] , 2016	Retrospective; Cross sectional	SO: BMI > 30 kg/m ² and 24 h- UC < median	Sex-specific 24-h urinary creatinine excretion	M-F	T = 310; M = 144; F = 166	71.8 ± 7.6	34.1 ± 4.0	SO: <i>n</i> = 44/144 in males; <i>n</i> = 52/166 in females	T2D: Non SO: <i>n</i> = 51; SO: <i>n</i> = 40
Xiao <i>et al</i> ^[38] , 2017	Retrospective	FMI/FFMI ratio > 95 percentile of sex, BMI and ethnicity specific population-representative references	BIA	M-F	T = 144; M = 45; F = 99	Non SO: 56.6 ± 12.7; SO: 54.6 ± 10.1	Non SO: 44.0 ± 7.6; SO: 49.1 ± 8.3	SO: 73/144 in total; (Did not distinguish in gender)	T2D: Non SO: <i>n</i> = 36/71; SO: <i>n</i> = 34/71
Kang <i>et al</i> ^[39] , 2017	Cross sectional	ASM/Wt < 1 SD the mean of the reference group, and BMI ≥ 25 kg/m ²	DXA	F	T = 1555	Non SO: 61.05 ± 0.44; SO: 62.91 ± 0.44	Non SO: 26.80 ± 0.07; SO: 27.93 ± 0.11	SO: <i>n</i> = 855/1555 (All females)	T2D: Non SO: <i>n</i> = 105/700; SO: <i>n</i> = 165/855
Aubertin-Leheudre <i>et al</i> ^[40] , 2017	Cross sectional	Dynapenic obesity, defined as low handgrip strength (≤ 19.9 in females; ≤ 31.9 in males), combined with BMI ≥ 30 kg/m ²	Jamar Handheld Dynamometer	M-F	T = 670; M = 213; F = 457	Non SO: 76.3 ± 4.7; SO: 78.0 ± 4.6	Non SO: 35.6 ± 4.8; SO: 34.9 ± 4.8	SO: <i>n</i> = 77/213 in males; <i>n</i> = 179/457 in females	T2D: Non SO: <i>n</i> = 133/414; SO: <i>n</i> = 81/256
Park <i>et al</i> ^[41] , 2018	Cross sectional	SO defined by combination of SMI < 2 SD and WC ≥ 90 cm for men and ≥ 85 cm women	BIA	M-F	T = 53818; M = 38820; F = 14998	Non SO: 40.5 ± 9.2; SO: 40.0 ± 11.3	Non SO: 26.9 ± 2.2; SO: 30.7 ± 3.4	<i>n</i> = 6513; M = 3341; F = 3172	T2D: Non-SO: <i>n</i> = 2176; SO: <i>n</i> = 391
Kreidieh <i>et al</i> ^[42] , 2018	Cross sectional	ALM/BMI < 0.512	BIA	F	T = 154	33.26 ± 14.65	31.42 ± 4.94	<i>n</i> = 31	T2D: Non SO: <i>n</i> = 3/123; SO: <i>n</i> = 4/31

Khazem <i>et al</i> ^[43] , 2018	Cross sectional	ALM/BMI < 0.789, (ALM/Wt.) × 100% < 25.72, and (ALM/Wt.) × 100% < 29.60	BIA	M	T = 72	32.79 ± 13.65	33.69 ± 5.85	23.9%-69.4%	T2D: Non SO: n = 1/22; SO: n = 3/50
Scott <i>et al</i> ^[46] , 2018	Cross sectional (includes a longitudinal part)	ALM/height < 7.26 kg/m ² combined with handgrip strength < 30 kg and/or low gait speed ≤ 0.8 m/s. Obesity was defined as body fat percentage ≥ 30%	DXA Handgrip strength Gait speed	M	T = 525	Non SO: 75.9 ± 4.7; SO: 80.3 ± 6.5	Non SO: 30.7 ± 3.4; SO: 27.2 ± 2.3	n = 80	High fasting glucose or diabetes medications: Non SO: n = 177/445; SO: n = 29/80

SO: Sarcopenic obesity; DO: Dynapenic obesity; BMI: Body mass index; M: Male; F: Female; BIA: Bioelectric impedance analysis; T2D: Type 2 diabetes; DXA: Dual-energy X-ray absorptiometry.

SO defined as the coexistence of obesity (BMI ≥ 25 kg/m²) and sarcopenia based on the skeletal muscle index estimated by BIA. A sample of 180 individuals with obesity (60 males and 120 females) was recruited. Of the 60 males included in the sample 35 had SO compared to 80 of the 120 females. Regardless of gender the mean age and BMI in the SO group were 61.1 ± 9.9 years and 27.8 ± 2.6 kg/m², and 69.9 ± 7.3 years and 26.8 ± 1.6 kg/m² in the non-SO group. Moreover, 12 of the 65 patients in the SO group had T2D compared to 17 of the 115 patients in the non-SO group.

In early 2016, Poggiogalle *et al*^[36] conducted a cross sectional study in which the authors assessed SO using DXA, with SO defined as the coexistence of obesity (BMI ≥ 30 kg/m²) and sarcopenia (ASMM: height² < 6.54 and < 4.82 kg/m² for males and females respectively) or (ASMM: weight < 0.2827 and < 0.2347 for males and females respectively). This study enrolled a sample of 727 individuals with obesity (141 males and 586 females), with mean ages of 45.63 ± 13.53 and 45.76 ± 13.58 years, and mean BMIs of 37.56 ± 5.99 and 37.80 ± 5.77 kg/m² respectively for each gender. Of the 141 male patients 68 had SO, while 350 of the 586 females had the condition. In addition, 155 of the 418 patients had pre-diabetes or T2D in the SO group compared to 70 of the 309 patients in the non-SO group.

In the same year, Ma *et al*^[37] performed a cross-sectional evaluation on SO defined by BMI and sex-specific 24-h urinary creatinine excretion, in 310 patients (166 females and 144 males) with obesity (BMI ≥ 30 kg/m²). Fifty-four of the 144 males and 52 of the 166 females had SO. The mean BMI and age of the SO group were 34.1 ± 4.0 kg/m² and 71.8 ± 7.6 years, while they were 34.9 ± 4.4 kg/m² and 67.8 ± 6.8 years in the non-SO group, respectively. Furthermore, 40 of the 106 patients had T2D in the SO group in comparison to 51 of the 204 patients in the non-SO group.

In 2017, Xiao *et al*^[38] performed a retrospective study on the prevalence of SO and its association with health outcomes in patients seeking weight loss treatment in a bariatric surgery setting. Body composition analysis was conducted by means of BIA and SO was defined by a fat mass:fat-free mass index (FMI: FFMI) ratio greater than the 95th percentile of sex, BMI and ethnicity-specific population-representative references. A sample of 144 adults with obesity (99 females and 45 males) were enrolled, with a mean age of 55.6 ± 11.5 years and a mean BMI of 46.6 ± 8.4 kg/m². Of the 144 patients included in the sample 73 had SO (data per gender is not available). The mean age and BMI of the individuals with obesity only were 56.6 ± 12.7 years and 44.0 ± 7.6 kg/m², compared to 54.6 ± 10.1 years and 49.1 ± 8.3 kg/m² in those with SO. Furthermore, 34 of the 73 patients had T2D in the SO group in comparison to 36 of the 71 patients in the non-SO group.

In 2017, Kang *et al*^[39] conducted a large cross-sectional study to assess the association between SO and metabolic syndrome in postmenopausal women. SO was defined by the co-existence of sarcopenia (ASM/weight < 1 standard deviation below the mean of the reference group) and a BMI cut-off point for obesity which referred to a score of 25 kg/m² on the basis of the Asia-Pacific obesity criterion. The study included 1555 females with obesity, of whom 855 had SO, with a mean age of 62.91 ± 0.44 years and a mean BMI of 27.93 ± 0.11 kg/m². On the other hand, 700 did not have SO and had a mean age of 61.05 ± 0.44 years and a mean BMI of 26.80 ± 0.07 kg/m². In

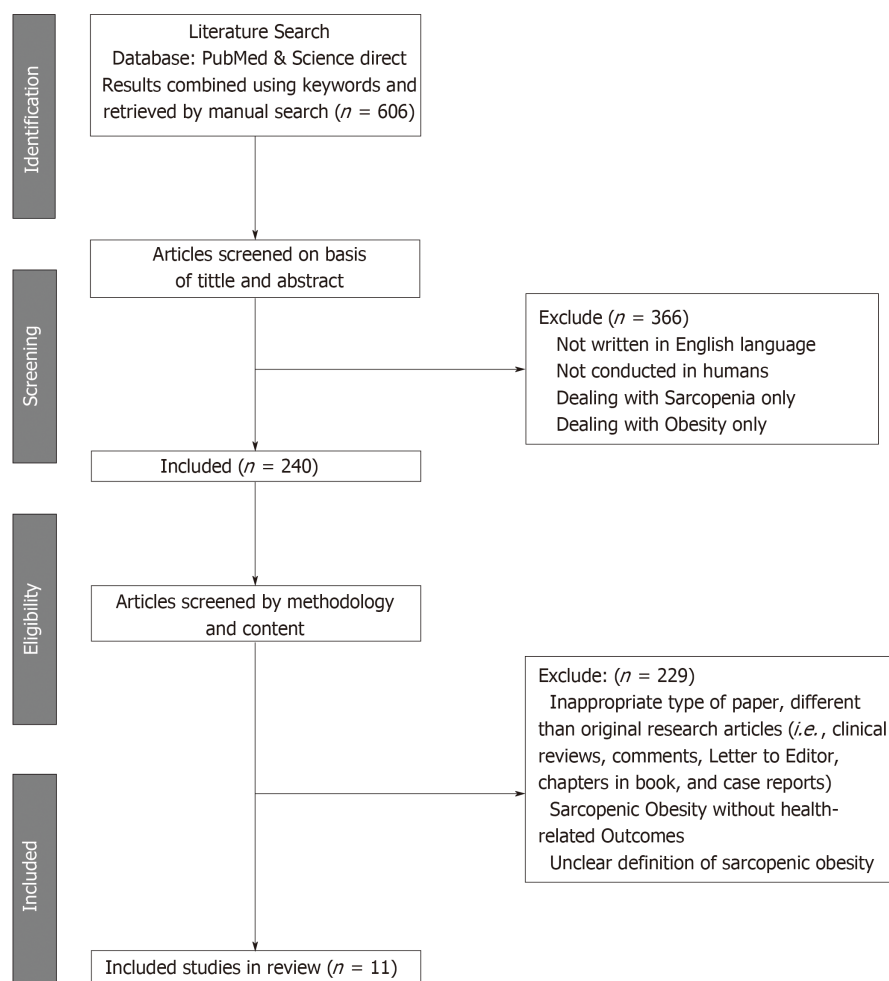


Figure 1 The flowchart summarizing the study selection procedure.

addition, 165 of the 855 patients had T2D in the SO group while 105 of the 700 patients in the non-SO group had T2D.

In the same year, a cross-sectional study by Aubertin-Leheudre *et al*^[40] aimed to examine the association between dynapenic obesity and metabolic risk factors in older adults (age ≥ 70 years). Dynapenic obesity was defined as low handgrip strength (u 19.9 in females; ≤ 31.9 in males) combined with a BMI of ≥ 30 kg/m². The study included 670 participants with obesity (213 males and 457 females), of whom 256 had dynapenic obesity, with a mean age of 78.0 ± 4.6 years and a mean BMI of 34.9 ± 4.8 kg/m², and 414 did not have dynapenic obesity, with a mean age of 76.3 ± 4.7 years and a mean BMI of 35.6 ± 4.8 kg/m². Furthermore, 81 of the 256 individuals in the dynapenic obesity group had T2D while 133 of 414 individuals in the non-dynapenic obesity group had T2D.

In 2018, Park *et al*^[41] conducted a large cross sectional study in two sites, which included a total of 53818 adults with overweight and obesity of both genders (38820 males and 14998 females), of whom 6513 had SO defined as below two standard deviations of the mean of the skeletal muscle mass index for young adults assessed by BIA and a waist circumference of ≥ 90 cm for men and ≥ 85 cm for women. The mean age and BMI of the individuals with obesity only were 40.5 ± 9.2 years and 26.9 ± 2.2 kg/m² compared to those with SO who had a mean age of 40.0 ± 11.3 years and a mean BMI of 30.7 ± 3.4 kg/m². Moreover, 391 of the 6513 patients had T2D in the SO group compared to 2176 of the 47305 patients in the non-SO group.

In 2018, Kreidieh *et al*^[42] conducted a cross sectional controlled study in which body composition measurements were conducted by BIA using a definition that in addition to appendicular lean mass (ALM) also involved BMI, and patients were considered affected by SO if $ALM:BMI < 0.512$. The study included 154 females with overweight and obesity with a mean age of 33.26 ± 14.65 years and a mean BMI of 31.42 ± 4.94 kg/m². Of the 154 female patients 31 had SO. Moreover, four of the 31 patients had T2D in the SO group compared to three of the 123 patients in the non-SO group.

In 2018, Khazem *et al*^[43] performed a cross-sectional controlled study on 72 adult

Table 2 Quality assessment of the included studies

Author	Sénéchal <i>et al</i> ^[35] , 2012	Lu <i>et al</i> ^[18] , 2013	Poggiogale <i>et al</i> ^[36] , 2016	Ma <i>et al</i> ^[37] , 2016	Xiao <i>et al</i> ^[38] , 2017	Kang <i>et al</i> ^[39] , 2017	Aubertin- Leheudre <i>et al</i> ^[40] , 2017	Park <i>et al</i> ^[41] , 2018	Scott <i>et al</i> ^[46] , 2018	Kreidieh <i>et al</i> ^[42] , 2018	Khazem <i>et al</i> ^[43] , 2018
Selection											
Represents cases with independent validation	1	1	1	1	1	1	1	1	1	1	1
Cases are consecutive or obviously representative	1	1	1	1	1	1	1	1	1	1	1
Controls from the community	1	1	1	1	1	1	1	1	1	1	1
Controls have no history of sarcopenic obesity	1	1	1	1	1	1	1	1	1	1	1
Comparability											
Controls are comparable for the most important factors	1	1	1	1	1	1	1	1	1	1	1
Control for any additional factor	0	0	1	0	0	1	0	1	0	0	0
Ascertainment of exposure											
Secured record or structured interview where blind to /control status	1	1	1	1	0	1	1	1	1	1	1
Same method of ascertainment for cases and controls	1	1	1	1	1	1	1	1	1	1	1
Cases and controls have completed follow up	0	0	0	1	0	0	0	0	1	0	0
Total score	7	7	8	8	6	8	7	8	8	7	7

Newcastle-Ottawa Scale (NOS) for longitudinal and cross-sectional studies. Yes = 1, No (not reported, not available) = 0; Studies with scores of 0-3, 4-6, 7-9 were considered as low, moderate and high quality, respectively.

males with overweight and obesity with a mean age of 32.79 ± 13.65 years and a mean BMI of 33.69 ± 5.84 kg/m². In this study the authors used three different definitions proposed by Batsis *et al*^[44], Levine and Crimmins^[21], and Oh *et al*^[45] based on ALM: BMI

and $(\text{ALM: weight}) \times 100\%$ to define SO. Body composition was assessed by BIA. Based on each formula the prevalence of SO varied between 23.9% and 69.4%. However, based on the definition that was revealed to be more useful from the clinical perspective, 50 of the 72 patients had a reduced lean body mass with a prevalence of 69.4%. Moreover, three of the 50 patients had T2D in the SO group in comparison to one of the 22 patients in the non-SO group.

Finally, in 2018 Scott *et al*^[46] conducted a large sampled study that aimed to investigate the cross-sectional association between SO and components of metabolic syndrome in community-dwelling older men. SO was defined by the co-existence of sarcopenia as $\text{ALM/height} < 7.26 \text{ kg/m}^2$ combined with handgrip strength $< 30 \text{ kg}$ and/or low gait speed $\leq 0.8 \text{ m/s}$, while obesity was defined as a body fat percentage of $\geq 30\%$. The study included 525 males with obesity, of whom 80 had SO, with a mean age of 80.3 ± 6.5 years and mean BMI of $27.2 \pm 2.3 \text{ kg/m}^2$ and 445 did not have SO, with a mean age of 75.9 ± 4.7 years and mean BMI of $30.7 \pm 3.4 \text{ kg/m}^2$. Furthermore, 29 of the 80 individuals in the SO group had T2D in comparison to 177 of the 445 individuals in the non-SO group.

Meta-analysis

The meta-analysis estimated the overall prevalence of SO among males and females. With high heterogeneity among the included studies, a random effect model was considered for the estimation of the overall prevalence of SO. The forest plots in Figures 2 and 3 show that SO affected 43% (95%CI: 28-59) of females and 42% (95%CI: 31-53) of males. In addition, the overall odds ratios of T2D in patients with SO as compared to those without SO are presented in Figure 4. The fixed effect weighted pooled odds for T2D in patients with SO indicated an increased risk of T2D of approximately 38% compared to those without SO (OR: 1.38, 95%CI: 1.27-1.50). The heterogeneity analysis revealed moderate variability ($I^2 = 60\%$).

DISCUSSION

This systematic review aimed to provide benchmark data on the prevalence of sarcopenia in individuals with overweight and obesity and to assess any potential association between SO and T2D in this population. The major finding is that sarcopenia seems to affect approximately 40%-45% of individuals with overweight and obesity of both genders, and the co-existence of both conditions, namely sarcopenia and excess weight/obesity increases the risk of T2D by nearly 38% when compared with those who had excess weight or obesity alone. The underlying mechanism behind this association is still unclear, however it seems that there is a bi-directional interaction between obesity, chronic inflammation, insulin resistance and sarcopenia^[19]. In fact, the chronic inflammation plays an important role in the pathogenesis of T2D. For this reason we speculate that coexistence of both obesity and sarcopenia under the so-called phenotype "SO", may have a synergistic effect with chronic inflammation being a common "denominator" seen in both conditions, which seems to exacerbate further glucose metabolism impairment (*i.e.*, insulin resistance, pre-diabetes and T2D)^[19].

The clinical implication of this review's findings is the awareness of the high prevalence of sarcopenia in the overweight/obese population that should be raised among clinicians and patients. Secondly, these results reveal the importance of screening for SO in individuals affected by excess weight and obesity, since this condition also seems to be strongly associated with T2D.

This systematic review has certain strengths. To the best of our knowledge this is the first systematic review to assess the overall prevalence of SO in males and females with overweight and obesity. In fact, the studies that have been conducted on this topic reported varying levels of prevalence that ranged between 0 and 100%, depending on the applied definition of SO^[47,48]. Higher prevalence tends to be reported in studies that accounted for body mass (*i.e.*, BMI), whereas a lower prevalence is reported in those that did not^[43,49]. A low prevalence may also be explained by the use of definitions that have primarily been developed from studies in older cohorts and these may not be applicable to younger adults^[47].

However, this systematic review also has certain limitations. Foremost, our results need to be interpreted with caution with regard to the association between SO and the prevalence of T2D, since the cross-sectional design of the studies (*i.e.*, non cohort), included in our systematic review indicates only simple associations between SO and T2D at best and does not provide solid information regarding any causal relationships between the two conditions^[50,51]. In other words, these studies lack evidence to determine if SO may lead to the onset or deterioration of T2D, since very few studies

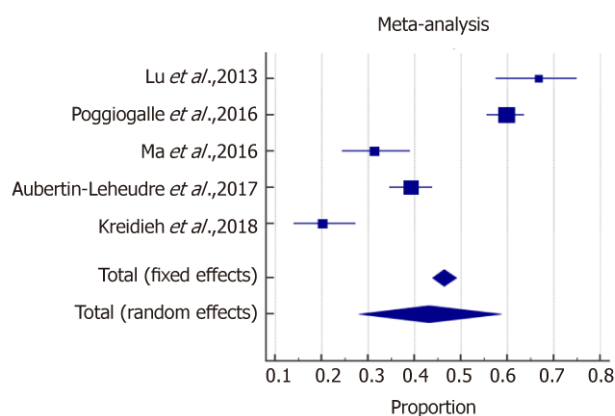


Figure 2 Forest plot for the pooled estimate of proportion of females with sarcopenic obesity.

have longitudinally investigated the “real” effects of SO on health^[52]. These shortcomings in the current research indicate the need to design longitudinal studies to clarify the real effect of SO on the onset and progression of T2D.

In conclusion, a high prevalence of sarcopenia has been found among adults with overweight and obesity regardless of their gender, and this condition seems to be associated with a higher risk of T2D. Clinicians should be aware of this scenario in their clinical practice for better management of both obesity and T2D.

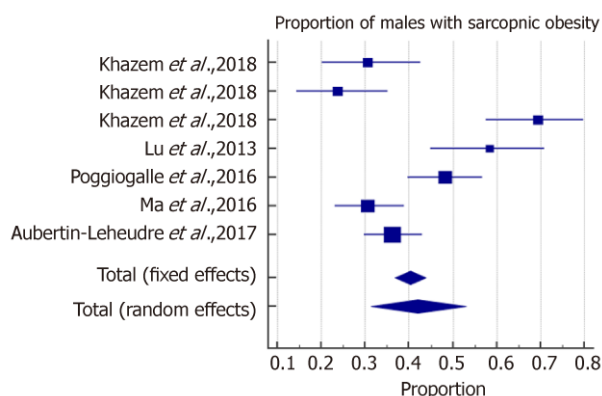


Figure 3 Forest plot for the pooled estimate of proportion of males with sarcopenic obesity.

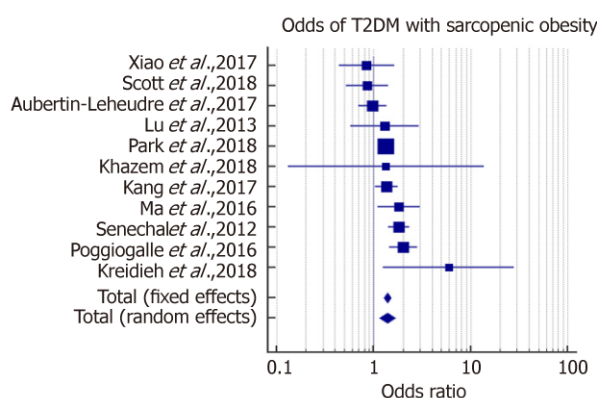


Figure 4 Forest plot for the pooled estimate of the odds of type 2 diabetes with sarcopenic obesity.

ARTICLE HIGHLIGHTS

Research background

The coexistence of sarcopenia and obesity has been termed as sarcopenic obesity (SO). Several studies have been conducted in order to determine any potential association between SO phenotype and type 2 diabetes (T2D). However, the available data are still contradictory and require further clarification.

Research motivation

To our knowledge no systematic review on the primary outcome related to the association between SO and T2D has been conducted yet to provide an unbiased interpretation of the evidence published to date.

Research objectives

We set out to systematically review the published literature with the aim of determining the prevalence of sarcopenia among adults with overweight and obesity and to investigate whether SO was associated with higher risk of T2D.

Research methods

The review conformed to the Preferred Reporting Items for Systematic Review and Meta-Analyses guidelines, and data were collated by means of narrative synthesis and meta-analysis.

Research results

The prevalence of SO in adult with overweight and obesity is 43% in females and 42% in males, and the presence of this condition increases the risk of T2D by 38% with respect to those without SO.

Research conclusions

A high prevalence of sarcopenia has been found among adults with overweight and obesity regardless of their gender, and this condition seems to be associated with a higher risk of T2D. The clinical implication of our findings is to raise awareness of the high prevalence of this phenotype in the overweight/obese population, and the importance of screening for SO in

individuals affected by excess weight, since this condition seems to be strongly associated with T2D. However, our results need to be interpreted with caution with regard to the association between SO and the prevalence of T2D, since the cross-sectional design of the studies included in our systematic review indicates only associations between the two conditions and that does not provide information regard the causal relationships.

Research perspectives

The current research indicates the need to design longitudinal studies to clarify the real effect of SO on the onset and progression of T2D. In other words, the available studies lack in evidence to determine if SO may lead to the onset or deterioration of T2D, since very few studies have longitudinally investigated the “real” effects of SO on health.

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Prevention of macrovascular complications in patients with type 2 diabetes mellitus: Review of cardiovascular safety and efficacy of newer diabetes medications

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Abstract

Lack of conclusive beneficial effects of strict glycemic control on macrovascular complications has been very frustrating for clinicians involved in care of patients with diabetes mellitus (DM). Highly publicized controversy surrounding cardiovascular (CV) safety of rosiglitazone resulted in major changes in United States Food and Drug Administration policy in 2008 regarding approval process of new antidiabetic medications, which has resulted in revolutionary data from several large CV outcome trials over the last few years. All drugs in glucagon-like peptide-1 receptor agonist (GLP-1 RA) and sodium-glucose cotransporter-2 (SGLT-2) inhibitor classes have shown to be CV safe with heterogeneous results on CV efficacy. Given twofold higher CV disease mortality in patients with DM than without DM, GLP-1 RAs and SGLT-2-inhibitors are important additions to clinician's armamentarium and should be second line-therapy particularly in patients with T2DM and established atherosclerotic CV disease or high risks for CV disease. Abundance of data and heterogeneity in CV outcome trials results can make it difficult for clinicians, particularly primary care physicians, to stay updated with all the recent evidence. The scope of this comprehensive review will focus on all major CV outcome studies evaluating CV safety and efficacy of GLP-1 RAs and SGLT-2 inhibitors.

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Core tip: Multiple cardiovascular (CV) outcome trials performed mainly to meet regulatory requirements by United States Food and Drug Administration have provided very important findings on CV safety and efficacy of newer anti-diabetic drugs. All drugs in glucagon-like peptide-1 receptor agonist (GLP-1 RA) and sodium-glucose cotransporter-2 (SGLT-2)-inhibitor classes have shown to be CV safe with heterogeneous results on CV efficacy. Abundance of data and heterogeneity in CV outcome trials results can make it difficult for clinicians to stay updated with all the recent evidence. The scope of this comprehensive review will focus on all major CV outcome studies evaluating CV safety and efficacy of GLP-1 RAs and SGLT-2 inhibitors.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is associated with long-term complications, which can be broadly classified as macrovascular and microvascular complications. The UK Prospective Diabetes Study (UKPDS) provided much needed information on glycemic goals for T2DM management and demonstrated that strict glycemic control significantly reduces microvascular complications, but failed to show beneficial effects on macrovascular complications^[1]. A 10-year post-trial follow up of UKPDS subjects showed a 15% reduction in risk for myocardial infarction (MI) in the intensive therapy group, despite loss of glycemic differences after the first year of conclusion of the UKPDS trial^[2]. This ongoing benefit is now widely known as a “legacy effect” of early strict glycemic control. Interestingly, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study around the same time showed an unexplained increase in mortality with intensive glycemic control in older patients with long standing T2DM with no significant reduction of major cardiovascular (CV) events^[3].

Lack of conclusive beneficial effects of strict glycemic control on macrovascular outcomes has been very frustrating for clinicians involved in care of patients with diabetes mellitus (DM). Due to conflicting results and uncertainty on beneficial effects of glucose lowering therapies on major CV events, there has been growing interest in determining how glucose-lowering pharmacotherapies impact risk for major CV events. Sulfonylureas and rosiglitazone have shown association with an increased risk of adverse CV events and mortality^[4,5]. Nissen *et al*^[6] showed 43% increased risk of MI with rosiglitazone treatment, which led to highly publicized controversy surrounding CV safety of rosiglitazone. This resulted in major changes in United States Food and Drug Administration (US FDA) policy in 2008 regarding approval process of new antidiabetic medications. Improved glycemic control alone is no longer sufficient and US FDA has requested CV outcome data from randomized, controlled trials for approval of new drugs for treatment of DM^[6].

Changes in FDA approval policy for new antidiabetes drugs has resulted in revolutionary data from several large CV outcome trials over the last few years^[7]. The primary composite endpoint for majority of CV outcome trials has been major adverse cardiovascular events (MACE), a composite of death from CV causes, nonfatal MI, or nonfatal stroke. Abundance of data and heterogeneity in CV outcome trials results can make it difficult for clinicians, particularly primary care physicians, to stay updated with all the recent evidence. The scope of this review will focus on all major CV outcome studies evaluating CV safety and efficacy of glucagon-like peptide-1 receptor agonists (GLP-1 RA) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors.

GLP-1 RA

Intestinal L-cells secrete GLP-1, a potent incretin hormone, in response to nutrient ingestion^[8]. Synthetic GLP-1 RA drugs are beneficial for patients with T2DM through their multiple mechanisms of action such as increasing glucose stimulated pancreatic insulin secretion, inducing expansion of insulin secreting pancreatic beta-cell mass, decreasing gastric emptying, inhibiting glucagon and gastric acid secretion and promoting satiety through GLP-1 effects on the central nervous system^[8]. GLP-1 RA's have gained popularity over the last decade due to their beneficial effects on metabolic endpoints aside from the reduction of blood glucose such as promoting weight loss, helping patients with portion control, favorable effects on blood pressure and cholesterol, and accumulated data over the last few years showing their CV safety and efficacy. There are currently five FDA approved GLP-1 agonists available for clinicians to help manage diabetes of their patients. These medications include exenatide (Daily injection approved in 2005 and once weekly injection approved in 2012), liraglutide (approved in 2010), dulaglutide (approved in 2014), lixisenatide (approved in 2016), and semaglutide (approved in 2017). Albiglutide was approved in 2014 for management of T2DM but was taken off market in May, 2018 due to limited prescribing of the drug. Therefore, we will not review details of albiglutide CV outcome trial (Harmony Outcomes) in this review article.

GLP-1 RA CV OUTCOME STUDIES

Lixisenatide in patients with type 2 diabetes and acute coronary syndrome (ELIXA) was the first CV outcome trial of GLP-1 RA's^[9]. Addition of lixisenatide to usual care did not significantly decrease the rate of major adverse cardiovascular events (MACE). ELIXA enrolled 6068 patients with T2DM who had a MI or who had been hospitalized for unstable angina within the previous 180 d. The median follow-up was only 25 mo. Patients were randomized to receive lixisenatide or placebo in addition to locally determined standards of care. Lixisenatide showed noninferiority to placebo in terms of MACE primary composite end point of CV death, MI, stroke, or hospitalization for unstable angina [13.4% *vs* 13.2% events; hazard ratio (HR), 1.02; 95% confidence interval (CI): 0.89 to 1.17; $P < 0.001$] but did not show superiority ($P = 0.81$). There was no significant decrease in the rate of hospitalization for heart failure or the rate of death. Failure to detect a benefit from lixisenatide for the primary MACE end point could have been due to enrollment of high risk patients with recent coronary artery disease and short duration of follow up.

Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes (LEADER) studied the CV effects of liraglutide and not only showed noninferiority, but superiority to placebo for MACE (composite of CV death, nonfatal MI, or nonfatal stroke), CV death and all-cause mortality^[10]. A total of 9340 patients with T2DM and high CV risks were followed for 3.8 years. Patients who received liraglutide had 13% relative risk reduction in the primary endpoint of MACE compared with placebo (13.0% *vs* 14.9% events; HR, 0.87; 95%CI: 0.78 to 0.97; $P < 0.001$ for noninferiority; $P = 0.01$). Beneficial effects of liraglutide on reducing MACE was primarily due to significant reduction in CV death (4.7% in liraglutide group *vs* 6.0% in placebo; HR, 0.78; 95%CI: 0.66 to 0.93; $P = 0.007$). Liraglutide also showed significant reduction in all-cause mortality (hazard ratio, 0.85; 95%CI: 0.74 to 0.97; $P = 0.02$). It's important to note that CV death and all cause death benefits were apparent after 12-15 mo and 18 mo of liraglutide treatment, respectively. More patients in the placebo arm required insulin and other oral anti-diabetes drugs such as sulfonylureas, to intensify their glycemic control. Unfavorable CV effects of other anti-diabetic drugs may have altered statistics in liraglutide's favor.

Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes (SUSTAIN-6) confirmed the noninferiority of semaglutide to placebo for the primary MACE endpoint, a composite of CV death, nonfatal MI, or nonfatal stroke (6.6% *vs* 8.9% events; HR, 0.74; 95%CI: 0.58 to 0.95; $P < 0.001$ for noninferiority) and nonfatal stroke (1.6% *vs* 2.7% events, HR, 0.61; 95%CI: 0.38 to 0.99; $P = 0.04$)^[11]. Unlike liraglutide, semaglutide treated patients lower risk of primary composite outcome (MACE) was predominantly driven by a significant decrease in the rate of nonfatal stroke and a nonsignificant decrease in nonfatal MI (HR ratio, 0.74; 95%CI: 0.51 to 1.08; $P = 0.12$). Rates of CV death were similar in semaglutide and control group. Notably, diabetic retinopathy complications occurred at significantly higher rate in semaglutide treated patients (HR, 1.76; 95%CI: 1.11 to 2.78; $P = 0.02$).

The fourth trial, Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes (EXSCEL) was different than previous CV outcome trials of GLP-1

agonists as it was performed in a usual-care setting among patients with T2DM at a wide range of CV risk^[12]. Unlike previous CV outcome trials studying GLP-1 RAs, where patients with high risks for CV disease were enrolled, 26.9% of subjects in EXSCEL trial did not have previous CV disease at randomization. After a median follow up of 3.2 years, once weekly exenatide was non-inferior to placebo for MACE (composite of CV death, nonfatal MI, or nonfatal stroke) but failed to show superiority (11.4% *vs* 12.2% events, HR, 0.91; 95%CI: 0.83 to 1.00; $P < 0.001$ for noninferiority and $P = 0.06$ for superiority). Even though there was 14% lower rate of death from any cause in the exenatide group compared to placebo (HR, 0.86; 95%CI: 0.77 to 0.97); this difference was not considered to be statistically significant on the basis of the hierarchical testing plan. A large proportion, 43%, of exenatide treated subjects prematurely discontinued the trial regimen, which authors speculated to be due to complexity of first generation exenatide injection device used in the trial and lack of run in period. Even with these limitations and a quarter of the study population without history of CV disease, treatment with exenatide almost reached statistical significance for primary endpoint MACE, and it's encouraging that direction of CV outcomes was consistent with beneficial effects seen in previous trials.

CV outcome trial (REWIND) for dulaglutide, has been completed but results are not published yet. However, the manufacturer of dulaglutide announced in a press release that patients who were treated with dulaglutide in REWIND trial had significantly reduced CV outcomes compared with placebo, meeting the primary trial endpoint^[13].

Meta-analysis of 4 major CV outcome trials of GLP-1 RAs, ELIXA (lixisenatide), LEADER (liraglutide), SUSTAIN 6 (semaglutide), and EXSCEL (extended-release exenatide) provided further valuable information regarding CV safety and efficacy of the GLP-1 RA drug class^[14]. A total of 33457 participants were included from four CV outcome trials in the meta-analysis. GLP-1 RAs as a class showed robust CV safety and efficacy. Patients treated with GLP-1 RAs demonstrated 10% reduced risk of MACE, a composite of CV death, nonfatal MI, or nonfatal stroke, (HR, 0.90, 95%CI: 0.82–0.99; $P = 0.033$), a 13% risk reduction in CV mortality (HR, 0.87; 95%CI: 0.79–0.96; $P = 0.007$), and a 12% relative risk reduction in all-cause mortality (HR, 0.88, 95%CI: 0.81–0.95; $P = 0.002$), compared to those treated with placebo.

GLP1 RA CV OUTCOME STUDIES DISCUSSION

Even though the statistical results differ in all four major CV outcome trials for GLP-1 RAs, the overall trend and magnitude of results were similar towards CV efficacy except in the ELIXA trial (Table 1). Liraglutide and semaglutide significantly reduced risk for primary endpoint of MACE (a composite of CV death, nonfatal MI, or nonfatal stroke). CV deaths and all-cause mortality risks were significantly lower with liraglutide use and semaglutide decreased risk for nonfatal stroke by 39% after 2 years of treatment. Lixisenatide and once weekly exenatide failed to show CV efficacy. Once weekly exenatide, however, decreased MACE by 9% and all-cause mortality risk by 14% after 3.2 years of treatment but just failed to reach statistical significance. Notably, CV benefits of GLP-1 RAs were shown even with patients receiving standard of care management for CV risk management including anti-platelet medications and treatment for hypertension and hyperlipidemia.

Differences in CV outcomes could be explained by differences in study population mainly in terms of CV disease risk, duration of follow up and adherence to trial drug. ELIXA had neutral results for CV efficacy, but this was the only trial that only enrolled subjects with recent MI or hospitalization for unstable angina^[9]. It can be argued that patients already had too far advanced atherosclerotic disease to benefit from drug. On the other hand, EXSCEL is the only trial that included patients with diverse CV risks (approximately 27% of patients without known CV disease), which makes its results more applicable to a broad range of patients with T2DM seen in usual clinical practice^[12]. However, including lower risk subjects also makes it more likely to not accrue sufficient adverse CV events in a timely manner to reach statistical significance. The other two trials recruited patients with T2DM who were at high risk for CV events and it can be argued that it may have helped to show superior CV safety with relatively short duration of follow up. It cannot be disputed that drug specific differences in GLP-1 RA class (structural similarities to human GLP-1, and short acting *vs* longer acting GLP-1 RAs) may also have contributed to variable CV efficacy outcome. However, there is robust evidence for CV safety of all GLP-1 RAs and it stands to reason that GLP-1 RA drug class has favorable effects on MACE (CV death, nonfatal MI and nonfatal stroke).

Table 1 Summary of cardiovascular outcome trials of glucagon-like peptide-1 receptor agonists

Drug	ELIXA	LEADER	SUSTAIN-6	EXSCEL
	Lixisenatide	Liraglutide	Semaglutide	Exenatide
Study design and salient features	Enrolled 6068 patients with T2DM and recent coronary event within 180 d; Median DM duration 9.2 yr; Median follow up 25 mo	Enrolled 9340 patients with T2DM and with high CV risks; Median DM duration 12.8 yr; Median follow up 3.8 yr	Enrolled 3297 patients with T2DM and established CV disease or with high CV risks; Median DM duration 13.2 yr and 14.1 yr in low dose and high dose treatment group, respectively; Median follow up 104 wk	Enrolled 14752 patients with T2DM at a wide range of CV risk; Approximately 27% of patients without known CV disease; Median DM duration 12 yr; Median follow up 3.2 yr; 43% subjects prematurely discontinued exenatide
Primary endpoint/MACE	No significant difference in MACE-4	13% reduction in MACE	26% reduction in MACE	9% reduction in MACE ¹
Secondary Outcomes	No significant difference in death from CV causes; No significant differences in rate of hospitalization for heart failure	22% reduction in death from CV causes ² ; 15% reduction in all-cause mortality ²	39% reduction in nonfatal stroke; 26% reduction in nonfatal myocardial infarction ³ ; No significant difference in CV death or all-cause mortality	14% reduction in all-cause mortality ⁴ ; No significant differences in death from CV causes

¹Nonsignificant reduction (hazard ratio, 0.91; 95% confidence interval, 0.83 to 1.00; $P < 0.001$ for noninferiority and $P = 0.06$ for superiority);

²Cardiovascular death and all-cause mortality benefits were apparent after 12-15 mo and 18 mo of liraglutide treatment, respectively;

³Nonsignificant reduction (hazard ratio, 0.74; 95% confidence interval, 0.51-1.08; $P = 0.12$);

⁴This difference was not considered to be statistically significant on the basis of the hierarchical testing plan. T2DM: Type 2 diabetes mellitus; DM: Diabetes mellitus; CV: Cardiovascular; MACE: Major adverse cardiovascular events, a composite of death from cardiovascular causes, non-fatal myocardial infarction, or nonfatal stroke; MACE-4: MACE endpoint as above and hospitalization for unstable angina.

SGLT-2-INHIBITORS

SGLT-2 proteins are expressed in the proximal convoluted tubule of the kidneys and are responsible for approximately 90% of renal glucose reabsorption^[15,16]. SGLT-2 inhibitors are FDA approved drugs for treatment of patients with T2DM that work through a unique mechanism of reducing renal threshold for glucose reabsorption, resulting in increased glycosuria and decreased blood glucose. There are currently four drugs in SGLT-2-inhibitor class approved by US FDA; Canagliflozin (approved in 2013), Dapagliflozin (approved in 2014), Empagliflozin (approved in 2014) and Ertugliflozin (approved in 2017). SGLT-2-inhibitor drugs are only approved for estimated glomerular filtration rate (GFR) > 45 . Several recent large-scale clinical trials have provided exciting data on CV safety and efficacy of empagliflozin, canagliflozin and dapagliflozin. A clinical trial looking at the CV safety of Ertugliflozin is currently ongoing and is expected to be completed in September, 2019.

SGLT-2-INHIBITORS CV OUTCOME STUDIES

Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPA-REG OUTCOME) studied CV safety and efficacy of empagliflozin in 7028 patients with T2DM and established CV disease^[17]. Patients were randomized in a 1:1:1 fashion to receive either empagliflozin 10 mg, empagliflozin 25 mg, or placebo. Pooled empagliflozin was superior to placebo for primary composite outcome of MACE (a composite of CV death, nonfatal MI, or nonfatal stroke). Results were similar for two doses of empagliflozin vs placebo for the primary endpoint. Pooled empagliflozin group showed 14% reduced relative risk for MACE (10.5% vs 12.1% events; HR, 0.86; 95%CI: 0.74 to 0.99; $P < 0.001$ for noninferiority; $P = 0.04$ for superiority), 38% relative risk reduction for CV deaths (3.7% vs 5.9%, $P < 0.001$), 35% relative risk reduction for CHF hospitalization (2.7% vs 4.1%, $P = 0.002$) and 32% relative risk reduction for all-cause mortality (5.7% vs 8.3%, $P < 0.001$). Heart failure hospitalization risk reduction results were similar in patients with and without CHF at baseline. Patients with established chronic kidney disease had numerically higher event rates for all outcomes than in patients with estimated GFR > 60 mL/min in both treatment and placebo groups.

The Canagliflozin Cardiovascular Assessment Study (CANVAS) comprised of two identical trials studying non-inferiority and superiority of canagliflozin compared with placebo on MACE (a composite of CV death, nonfatal MI, or nonfatal stroke); CV death and death from any cause^[18]. A total of 9,734 patients with T2DM and either established CV disease (age 30 years or above) or high risk of CV disease (age 50 years

or above with 2 or more risk factors) completed the trial with a mean follow up of 188.2 wk. Results showed a significant decrease in primary endpoint of MACE in canagliflozin treated individuals compared with placebo (26.9 *vs* 31.5 participants with an event per 1000 patient-years; HR, 0.86; 95%CI: 0.75 to 0.97; $P < 0.001$ for noninferiority; $P = 0.02$ for superiority). Patients treated with canagliflozin had significantly lower rates of hospitalization for heart failure (HR 0.67; 95%CI: 0.52–0.87). No significant difference was found in the two groups for outcomes of death from any cause and death from CV causes. There was a higher risk of amputation of toes, feet or legs (primarily at level of toe and metatarsal) with canagliflozin *vs* placebo (6.3 *vs* 3.4 participants with amputation per 1000 patient-years; HR, 1.97; 95%CI: 1.41 to 2.75). Of note, canagliflozin treated group showed 27% reduction in progression of albuminuria and 40% reduction in adverse renal outcome (a composite of sustained 40% reduction in the estimated GFR, the need for renal-replacement therapy, or death from renal causes). However, based on pre-specified hypothesis testing sequence, the renal outcomes were not considered statistically significant.

The Dapagliflozin Effect on Cardio-vascular Events–Thrombolysis in Myocardial Infarction 58 (DECLARE-TIMI 58) was a randomized, double-blind, placebo controlled, phase 3 trial that evaluated the non-inferiority of dapagliflozin on MACE (a composite of CV death, nonfatal MI, or nonfatal stroke) and a composite of CV death or hospitalization for heart failure, in patients with T2DM (40 years or older) and established atherosclerotic CV disease or multiple risk factors for atherosclerotic CV disease^[19]. A total of 13198 out of 17160 randomized participants completed the trial with a median follow up of 4.2 years. This trial included the highest proportion of patients (more than 10000 patients), without established atherosclerotic CV disease compared to previous CV outcome trials. Individuals with a creatinine clearance < 60 mL per minute were excluded from trial. Results showed that dapagliflozin was noninferior to placebo for MACE but failed to show superiority (8.8% *vs* 9.4% events; HR, 0.93; 95%CI: 0.84 to 1.03; $P = 0.17$). A significantly lower rate of hospitalization for heart failure was noted in the dapagliflozin group (HR, 0.73; 95%CI: 0.61 to 0.88); there was no difference in the rate of CV death in the two groups (HR, 0.98; 95%CI: 0.82 to 1.17). Diabetic ketoacidosis was more common in the dapagliflozin group than in the placebo group (0.3% *vs* 0.1%; hazard ratio, 2.18; 95%CI: 1.10 to 4.30; $P = 0.02$). There was no difference in the rates of amputations in the two groups.

Zelniker *et al*^[20] performed a meta-analysis of all major CV outcome trials of SGLT-2-Inhibitors in patients with T2DM. A total of 34322 patients were included from 3 major trials; 60.2% of patients had established CV disease and rest had multiple risks factors for CV disease. As a group, SGLT-2-inhibitors demonstrated 11% reduction in MACE, a composite of CV death, nonfatal MI, or nonfatal stroke (HR, 0.89; 95%CI: 0.83–0.96; $P = 0.0014$) but benefit was not seen in patients without established atherosclerotic CV disease. There was 23% relative risk reduction of CV death or hospitalization for heart failure in SGLT-2-inhibitors treated patients compared to placebo (HR, 0.77; 95%CI: 0.71–0.84; $P < 0.0001$), with favorable effects seen regardless of presence or absence of atherosclerotic CV disease or heart failure. Though beyond the scope this review article, it is important to mention that SGLT-2-inhibitors significantly reduced (45%) progression of renal disease irrespective of baseline atherosclerotic CV disease. Patients with worse renal function had greater benefit in terms of hospitalization for heart failure.

SGLT-2-INHIBITORS CV OUTCOME STUDIES DISCUSSION

Empagliflozin and canagliflozin both showed a 14% risk reduction in MACE (a composite of CV death, nonfatal MI, or nonfatal stroke) but dapagliflozin neither decreased nor increased the risk for MACE in patients with T2DM, compared to placebo (Table 2). Empagliflozin also showed robust risk reduction for CV death (38%), hospitalization for HF (35%) and all-cause mortality (32%) in patients with T2DM and established atherosclerotic CV disease after a median follow up of 3.1 year. Canagliflozin and dapagliflozin treatment resulted in a significantly lower rate of hospitalization for heart failure (33% relative risk reduction for canagliflozin and 27% for dapagliflozin) but failed to significantly decrease death from CV causes or death from any cause^[18,19]. As a group, SGLT-2-inhibitors have shown more robust and consistent effect on prevention of hospitalization for heart failure in patients with T2DM with or without history of heart failure or atherosclerotic CV disease^[20]. Beneficial effects on major adverse CV events was not only moderate, but also limited to patients with established CV disease.

Heterogeneity of CV efficacy outcomes for various SGLT-2-inhibitors may be

Table 2 Summary of cardiovascular outcome trials of sodium-glucose cotransporter-2-inhibitors

Drug	EMPA-REG outcome	CANVAS	DECLARE-TIMI 58
	Empagliflozin	Canagliflozin	Dapagliflozin
Study Design and salient features	Enrolled 7028 patients with T2DM and established CV disease; 100% subjects with established CV disease; DM duration: 57% > 10 yr and 25.1% 5-10 yr; Median follow up 3.1 yr	Enrolled 9734 patients with T2DM and either established CV disease or high risk of CV disease; 65.6% subjects with established CV disease; Mean DM duration 13.5 yr; Mean follow up 188.2 wk	Enrolled 17160 patients with T2DM and with variable CV risks; 40.5% subjects with established CV disease; Median DM duration 11 yr; Median follow up of 4.2 yr
Primary endpoint/MACE	14% reduction in MACE in pooled empagliflozin group	14% reduction in MACE	No significant difference in MACE
Secondary Outcomes	35% reduction in hospitalization for heart failure ¹ ; 38% reduction in death from CV causes; 32% reduction in all-cause mortality	33% reduction in hospitalization for heart failure; No significant difference in death from CV causes; No significant difference in all-cause mortality	27% reduction in hospitalization for heart failure; No significant difference in death from CV causes; No significant difference in all-cause mortality

¹Heart failure hospitalization risk reduction results were similar in patients with and without CHF at baseline. T2DM: Type 2 diabetes mellitus; DM: Diabetes mellitus; CV: Cardiovascular; MACE: Major adverse cardiovascular events, a composite of death from cardiovascular causes, non-fatal myocardial infarction, or nonfatal stroke.

explained by differences in individual drugs, but cannot be definitively stated due to lack of head to head randomized controlled trials. However, differences in CV outcomes of SGLT-2-inhibitor drugs can be explained, at least in part, due to differences in study design and patient populations studied. EMPA-REG OUTCOME only included patients with established CV disease but 65.6% patients enrolled in CANVAS program, and only 40.5% patients in DECLARE-TIMI 58 had established CV disease^[17-19]. Since reduction of MACE with SGLT-2-inhibitors was only seen in patients with established atherosclerotic CV disease, a lower proportion of this patient population in DECLARE-TIMI 58 may have resulted in failure to show superiority for the primary composite MACE endpoint. Also, DECLARE-TIMI 58 trial excluded patients with creatinine clearance < 60 mL per minute, but other two trials had a higher proportion of patients with renal insufficiency (eGFR < 60 mL/min per 1.73 m²); 25.9% in EMPA-REG OUTCOME and 20.1% in CANVAS. A lower proportion of individuals with renal insufficiency and established atherosclerotic CV disease may have been responsible for lower mortality rates in the placebo group in DECLARE-TIMI 58 compared to EMPA-REG OUTCOME. It cannot be assessed how much of these differences in patient population affected the final CV outcome, but these observations underscore the fact that clinicians should be cautious in generalizing results of these CV outcome trials to patients with diverse CV risk factors. However, the above reviewed CV outcome trials have confirmed CV safety of SGLT-2-inhibitors. Overall, the evidence is strong for beneficial effects of SGLT-2-inhibitors on reducing hospitalization for heart failure, and moderate reduction of major adverse CV events has only been clearly demonstrated in individuals with T2DM and established atherosclerotic CV disease.

CONCLUSION

Multiple CV outcome trials performed mainly to meet regulatory requirements by US FDA have provided very important findings on CV safety and efficacy of newer anti-diabetic drugs. All drugs in GLP-1 RA and SGLT-2-inhibitor classes have shown to be CV safe with heterogeneous results on CV efficacy. However, the overall trend and magnitude of CV outcomes is similar within the drug classes. GLP-1 RAs have beneficial effects on MACE (a composite of CV death, nonfatal MI and nonfatal stroke). SGLT-2-inhibitors have stronger and consistent evidence for prevention of hospitalization for heart failure than on atherosclerotic MACE, where beneficial outcome was only seen in patients with T2DM and established atherosclerotic CV disease. Given twofold higher CV disease mortality in patients with DM than without DM^[21], GLP-1 RAs and SGLT-2-inhibitors are important additions to clinician's armamentarium and should be second line-therapy particularly in patients with T2DM and established atherosclerotic CV disease or high risks for CV disease. In fact, the recent consensus statement from the ADA and EASD confirms this point and suggests GLP-1 RA's and SGLT-2 inhibitors after metformin in high CV-risk individuals^[22].

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Exploration of the shared pathophysiological mechanisms of gestational diabetes and large for gestational age offspring

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Abstract

Gestational diabetes mellitus (GDM) and large for gestational age (LGA) offspring are two common pregnancy complications. Connections also exist between the two conditions, including mutual maternal risk factors for the conditions and an increased prevalence of LGA offspring amongst pregnancies affected by GDM. Thus, it is important to elucidate potential shared underlying mechanisms of both LGA and GDM. One potential mechanistic link relates to macronutrient metabolism. Indeed, derangement of carbohydrate and lipid metabolism is present in GDM, and maternal biomarkers of glucose and lipid control are associated with LGA neonates in such pregnancies. The aim of this paper is therefore to reflect on the existing nutritional guidelines for GDM in light of our understanding of the pathophysiological mechanisms of GDM and LGA offspring. Lifestyle modification is first line treatment for GDM, and while there is some promise that nutritional interventions may favourably impact outcomes, there is a lack of definitive evidence that changing the macronutrient composition of the diet reduces the incidence of either GDM or LGA offspring. The quality of the available evidence is a major issue, and rigorous trials are needed to inform evidence-based treatment guidelines.

Key words: Gestational diabetes mellitus; Large for gestational age; Metabolism; Biomarkers; Glucose; Lipids

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Core tip: The prevalence of gestational diabetes is on the rise, warranting attention on the consequences for mother and offspring, as well as management options. One consequence is an increased risk of large for gestational age (LGA) offspring. While deranged macronutrient metabolism of carbohydrate and lipids in gestational diabetes may play a role in fetal overgrowth, there is a lack of conclusive evidence that dietary interventions employed as first-line gestational diabetes management reduces this risk of LGA offspring.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that develops after the first trimester of pregnancy and resolves post-delivery. Its prevalence has increased over recent years due to a variety of factors including a change in diagnostic criteria, increasing maternal obesity, and increasing maternal age. Although estimates vary according to the population and diagnostic tests used, in Australia the current prevalence is around 13% (reported in 2016)^[1] compared to 3.6% 20 years earlier^[2].

GDM increases the risk of a number of adverse outcomes for both mother and baby. A common concern is the increased risk of a large baby, referred to as “large for gestational age” or “LGA” - usually defined as birthweight above the 90th percentile for gestational age^[3]. A related term is macrosomia - usually defined as absolute birthweight greater than 4000 g or 4500 g^[4]. LGA and macrosomia in turn pose risks for delivery complications as well as potential longer-term metabolic complications.

While fetal growth is determined by the interaction of a number of factors, both genetic and environmental, the link between GDM and LGA suggests there may be shared pathogenic elements (Figure 1). Indeed, risk factors in common for both conditions include older maternal age, maternal obesity, gestational weight gain, and ethnicity^[4]. The aim of this paper is to review the key elements of maternal metabolism that are both altered in the GDM state and linked to higher birthweight in such pregnancies, and to discuss how they relate to nutritional guidelines for women with GDM.

GLYCAEMIA

Normal pregnancy involves a progressive increase in maternal insulin resistance from mid-pregnancy, promoting diversion of glucose to the fetus^[4,5]. Maternal and placental hormones such as progesterone, oestrogen, prolactin, human placental lactogen, human placental growth hormone, and cortisol, are the main drivers for this insulin resistance^[6,7]. Alterations in adipokines (proteins secreted by adipose tissue) such as tumour necrosis factor alpha, adiponectin, and leptin may also contribute^[7].

In GDM, the insulin resistance of pregnancy is exaggerated, and/or maternal pancreatic beta cells can not sufficiently compensate^[8]. GDM may therefore be the unmasking of underlying beta cell dysfunction. Other key elements of carbohydrate metabolism are also altered in GDM. For instance, compared to matched controls, women with GDM exhibit smaller increases in first-phase insulin response (the initial rapid insulin secretion in response to glucose ingestion that lasts only minutes) as pregnancy progresses^[8]. Insulin suppression of gluconeogenesis is also impaired in women with GDM^[9]. Overall, this leads to hyperglycaemia.

Maternal hyperglycaemia is believed to play a significant role in the development of LGA^[9]. According to the hyperglycaemia-hyperinsulinaemia hypothesis (also called the Pedersen hypothesis), maternal hyperglycaemia leads to fetal hyperglycaemia as glucose is transferred down the placental concentration gradient^[10]. This consequently stimulates the fetal pancreas to increase insulin production; the resulting hyperinsulinaemia leads to fetal overgrowth as insulin is a prominent growth hormone for the fetus^[11].

There is much data from human and animal studies indicating the importance of

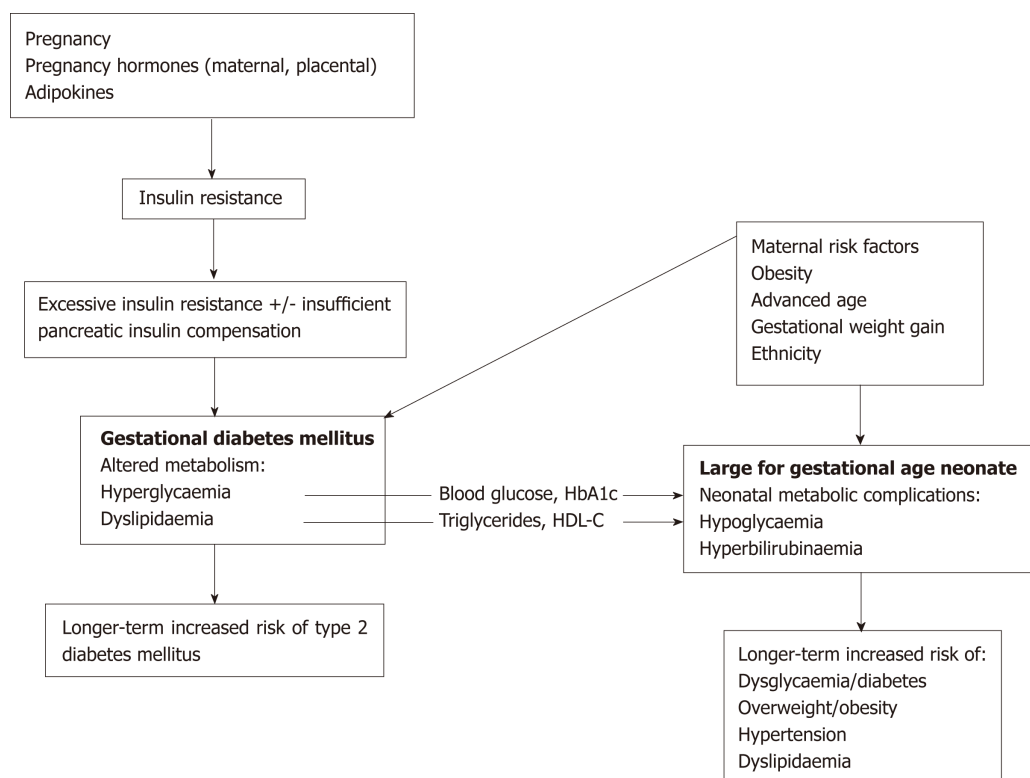


Figure 1 Gestational diabetes mellitus increases the risk of large for gestational age offspring, and both have potential long-term metabolic consequences. The two conditions also have shared maternal risk factors. Altered carbohydrate and lipid metabolism is present in pregnancies affected by gestational diabetes mellitus (GDM) and markers of this metabolism *e.g.*, maternal blood glucose, triglycerides and high-density lipoprotein cholesterol (HDL-C), have been associated with large for gestational age offspring in GDM pregnancies. Therefore, these biomarkers may provide a link between the two conditions. HbA1c: Glycosylated haemoglobin; HDL-C: High-density lipoprotein cholesterol; GDM: Gestational diabetes mellitus.

glycaemia management during pregnancy^[10]. The Hyperglycemia and Adverse Pregnancy Outcomes study was the seminal paper demonstrating the continuous relationship between glucose levels at 24–28 wk' gestation and pregnancy outcomes, including LGA offspring^[12]. A similar linear relationship between maternal glucose and C-peptide levels in infant cord blood was also evident in this population, corroborating the role of fetal hyperinsulinaemia. This study subsequently informed the development of GDM diagnostic criteria by demonstrating that hyperglycaemia below that of overt diabetes is associated with greater risk of adverse outcomes.

Moreover, other glycaemic markers have also been assessed for an association with LGA in GDM pregnancies. This includes glycosylated haemoglobin, 1,5-anhydroglucitol and fructosamine^[13]; however, there is limited support for these markers. Overall, the association between glucose control and LGA offspring is incomplete, suggesting that other factors may be involved.

LIPIDS

Adaptive changes occur in lipid metabolism during normal pregnancy. The first two trimesters of gestation comprise an “anabolic phase”, involving enhanced maternal fat storage^[14]. This occurs due to a combination of hormonal changes (*e.g.*, progesterone, prolactin, cortisol) promoting lipid synthesis and storage, inhibition of lipid breakdown, as well as hyperphagia^[15]. The final trimester is a “catabolic phase” where there is a net breakdown of fat stores. Insulin resistance plays an important role in this shift, by leading to enhanced lipolysis of adipose tissue and reduced lipoprotein lipase activity, thereby reducing overall lipid uptake^[15]. These changes are associated with an initial decrease in maternal circulating lipid levels in the first trimester, with subsequent gradual increase across gestation, reaching peak levels prior to delivery^[15]. The greatest increase is seen in triglyceride levels, which reach up to triple prepregnancy values^[16].

Lipids are vitally important in fetal development, as they are involved in key processes such as synthesis of cell membranes and steroid hormones^[17]. Fetal lipids are sourced through a combination of placental transfer and *de novo* synthesis. While

maternal cholesterol is transferred across the placenta, particularly early on in pregnancy, endogenous fetal cholesterol synthesis becomes more prominent in late gestation^[9,15]. On the other hand, triglycerides are the major lipid storage form in fetal adipocytes but maternal triglycerides do not cross the placenta intact. However, free fatty acids (FFAs) may be transferred across the placenta, with specific enzymes, receptors and binding proteins within the placenta thought to enable this process^[15]. Fetal triglycerides are synthesised using FFAs^[12,15].

In women with GDM, significantly higher triglyceride levels are present throughout pregnancy, and lower high-density lipoprotein cholesterol (HDL-C) levels are present across the final two trimesters, compared to those without GDM^[12]. The exaggerated insulin resistance in GDM again plays an important role, with one effect being reduced suppression of lipolysis^[18].

The effect of these alterations in lipid metabolism on fetal adiposity in pregnancies with GDM is increasingly being recognised^[12]. A meta-analysis involving GDM and non-GDM pregnancies indicated high maternal triglycerides and low HDL-C during pregnancy were associated with increased birthweight^[19,20]. Furthermore, alterations in placental structure and function which are seen in GDM pregnancies, such as the expression of lipid-related genes and enzymes, as well as differences in placental phospholipid composition, raise the possibility of altered transplacental lipid pathways which may promote increased lipid transfer in GDM^[21]. Indeed, the metabolic environment of GDM, involving hyperinsulinaemia, hyperlipidaemia, and hyperglycaemia, is proposed to contribute to enhanced transfer, synthesis and storage of lipids in fetal adipose tissue^[15]. This has even led to revision of the Pedersen hypothesis, with lipids being proposed as a driver of LGA in pregnancies with diabetes despite good glycaemic control^[15,20].

Several studies have investigated associations between maternal lipids and birthweight in pregnancies affected by GDM. A study by Schaefer-Graf *et al*^[22] showed third trimester maternal FFAs and triglycerides were associated with LGA newborns in GDM pregnancies, which remained significant after adjusting for confounding variables including glucose levels^[23]. Of note, cord blood lipids were also sampled, with positive correlations evident between maternal and cord blood levels of triglycerides, FFAs, and glycerol. There was also indication of greater insulin resistance in LGA newborns, as cord blood insulin-to-glucose ratio was significantly positively correlated to birthweight.

Greater insight has come from a subsequent study from Schaefer-Graf *et al*^[22], which found cord blood FFA levels were significantly lower in pregnancies without diabetes compared with those with GDM. This may be indicative of increased FFA transplacental transport in GDM pregnancies. In addition, in a study of 104 Korean women with GDM, maternal hypertriglyceridaemia (defined as triglycerides > 75th percentile value, 3.33 mmol/L) at 24-32 wk' gestation was significantly associated with LGA offspring, independent of maternal pre-pregnancy body mass index (BMI), gestational weight gain, maternal age or parity; although the model showed only a sensitivity of 48% and specificity of 83.5% for LGA prediction. This study found no significant association between total cholesterol or HDL-C levels and LGA newborns. In contrast, a European research group did find HDL-C was negatively associated with LGA newborns in GDM pregnancies^[24]. Triglycerides, however, were not significantly associated in this study, which may be related to the earlier timing of the sample collection (booking visit compared to 24-32 wk' gestation for the former study). Finally, a pilot study examining home monitoring of fasting and post-prandial triglyceride levels during late pregnancy has also been presented^[25]. While the triglyceride levels were not correlated with birthweight, the study was limited by small sample size (twelve participants) and heterogeneous population (eight participants had GDM). It does however provide a new avenue to gather more comprehensive data on triglyceride profiles during pregnancy. In sum, there is increasing support for a role of lipids contributing to fetal overgrowth in GDM pregnancies.

Lipids are also being investigated as predictive biomarkers for GDM. Lipidomics, using techniques such as liquid-chromatography-mass spectrometry and nuclear magnetic resonance imaging, have been employed to identify potential lipid biomarkers of GDM in maternal blood^[26]. However, limited data is available regarding optimal cut-off values^[27,28]. One study of low risk pregnancies found triglycerides and LDL-C were independent predictors of GDM, although the sensitivity was low^[5]. The ratio of triglycerides to HDL-C, proposed to be a clinical indicator of insulin resistance, may be superior than a single lipid biomarker^[29], and has been shown to have a high negative predictive value, identifying women with low risk of GDM development in early gestation^[30]. Ultimately, lipids may be most useful in a combined prediction model, including other biomarkers factors and clinical risk factors^[31].

GDM NUTRITIONAL GUIDELINES

Given the aforementioned links between maternal glycaemia and lipids with GDM, it is unsurprising that dietary modification is central to GDM management. Indeed, medical nutrition therapy is considered first-line treatment, along with physical activity^[32], aiming to achieve an appropriate balance between promoting normoglycaemia and adequate gestational weight gain, while allowing for optimal nutrition and fetal growth^[33].

Despite being a foundation of management, a prominent issue is the paucity of specific dietary recommendations, reflecting the state of evidence. Indeed, there is substantial heterogeneity across studies including differences in experimental designs, macronutrient compositions, outcome measures, prescribed versus self-reported intakes (and lack of adherence monitoring), and lack of control conditions. Other issues include the small sample sizes, late timing of initiation of the dietary intervention and the short duration. Consequently, there is a lack of consensus, with differing guidelines available^[34].

Carbohydrate intake has been the main focus of guidelines and research. Fasting and/or 2-h post-prandial glucose titres are key targets in the management of GDM^[35,36]. The recommended dietary reference intake of carbohydrate during pregnancy is a minimum of 175 g per day^[34], but the available evidence does not indicate the ideal carbohydrate intake for women with GDM. In part, this may be because the effect of carbohydrate intake may not only relate to the amount consumed (grams or percentage), but also to the type of carbohydrate [*e.g.*, fibre, low/high glycaemic index (GI)], timing of consumption and the protein/fats consumed at the same time, thus elucidating the impact across studies can be difficult^[35]. Indeed, recommendations on calorie intake vary, with studies examining a broad range from 1500 to 2800 kcal/d showing positive pregnancy outcomes^[36]. Some guidelines do recommend moderate calorie restriction (1600-1800 kcal/d) for overweight or obese women with GDM to improve glycaemic control and limit gestational weight gain^[29]. Currently, it is generally recommended that carbohydrate intake should be individualised with respect to amount and type of carbohydrate, according to ongoing assessment of nutrition and glycaemic management^[37].

Different dietary interventions with varying carbohydrate compositions have been proposed for GDM management. GI is a relative ranking of food items (on a scale of 0-100), based on how quickly blood glucose levels increase after ingestion of a standard quantity of the particular food^[38]. The GI is unchanged by pregnancy^[39]. Low GI diets for women with GDM have been associated with reductions in the proportion of women requiring insulin and in neonatal birthweight in randomised control trials (RCTs)^[37]. Meanwhile, a systematic review and meta-analysis of RCTs assessing different types of dietary interventions on maternal glycaemic control and birthweight in pregnancies with GDM concluded that on pooled analysis, dietary interventions demonstrated favourable effects compared with control diets^[40]. This included improved maternal glycaemic control from baseline (fasting and post-prandial glucose levels), lower birthweight and less macrosomia (although similar LGA risk). While the quality of the evidence was graded as low, it does indicate nutritional guidance can play an important role in GDM management and there is potential for improvement in outcomes with firmer evidence to guide recommendations.

A concern about the attention on reducing carbohydrate intake is the possibility that it may lead to a proportional increase in dietary fat intake. The impact of this on pregnancy outcomes is unclear, although it may be associated with worsening of insulin resistance^[34]. There has been limited specific investigation of maternal dietary lipid management during pregnancy^[35]. A study involving women without GDM found a high fat maternal diet was associated with increased neonatal adiposity^[41]. Meanwhile, a pilot study ($n = 12$) of women with diet-controlled GDM found a low fat/higher-complex carbohydrate diet was associated with lower insulin resistance of adipose tissue, compared with a higher fat/low-carbohydrate diet, although the birthweights of the two groups were not significantly different^[42]. Furthermore, a high monounsaturated fatty acid diet compared with a high carbohydrate diet in GDM pregnancies showed there was better insulin sensitivity in the latter group, although the overall glucose control was similar between the groups^[43]. There was no difference in birthweights, but the high monounsaturated fatty acid diet was associated with lower diastolic blood pressure. Moreover, a small study ($n = 10$) assessing the effect of the fat composition in a test meal on glycaemic parameters in women with GDM found that saturated fats were associated with significantly lower post-prandial glucose and insulin levels compared to monounsaturated fatty acids^[44]. Hence, while there is a suggestion that dietary fats may influence metabolism in GDM, much more research is needed to properly assess this. Further evaluation of potential treatment

options targeting maternal dyslipidaemia is also needed^[45].

Furthermore, gestational weight gain is a related important consideration, with higher gestational weight gain associated with both GDM and LGA^[41]. There are no specific guidelines for gestational weight gain in women with GDM, but since maternal overweight and obesity are also risk factors for adverse outcomes including GDM and LGA, the Institute of Medicine recommendations for appropriate gestational weight gain are different according to pre-pregnancy BMI^[46,47]. For women with obesity (BMI ≥ 30 kg/m²), 5-9 kg weight gain is recommended, compared to 11.5-16 kg for normal weight women (BMI 18.5-24.9 kg/m²). The importance of obesity in pregnancy is also highlighted by research demonstrating a synergistic relationship between obesity and GDM in increasing the risk of adverse pregnancy outcomes^[48]. Meanwhile, although numerous diet and/or lifestyle interventions aiming to promote appropriate gestational weight gain have been reported, the results have overall been mixed, with only modest effectiveness demonstrated^[49].

Finally, while many gaps remain in the current state of knowledge, technological advancements are likely to be a key driver of developments in this space. Indeed, contributions from fields such as metabolomics are already shedding light on the mechanisms underlying GDM^[50]. Analytical techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy have been employed to investigate metabolic profiles associated with GDM and hence determine the pathways leading to insulin resistance^[50]. Lipid and amino acid molecules have been most consistently identified by these processes thus far^[51]. Importantly, such research is enabling identification of potential therapeutic targets, which in turn may involve dietary intervention. However, given the metabolic heterogeneity within pregnancy, personalisation of interventions will be important^[52].

CONCLUSION

The relationships between GDM and LGA raise the possibility of shared mechanisms. Central components include abnormal carbohydrate and lipid metabolism, with insulin resistance recognised as a key instigating factor. While both glycaemic and lipid biomarkers have been associated with LGA, their utility for prediction in clinical practice is yet to be determined. Dietary modification is the cornerstone of GDM treatment, but there is insufficient data, particularly in the area of dietary lipids, to form definitive evidenced-based dietary recommendations. Hence there remains great need for rigorous studies investigating the impact of specific dietary interventions on pregnancy outcomes in women with GDM.

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Diabetes self-care in primary health facilities in India - challenges and the way forward

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Abstract

India has approximately 73 million people living with diabetes and another 37 million with prediabetes while nearly 47% of the diabetes cases are undiagnosed. The high burden of poor glycemic control and early onset of complications with associated economic costs indicates a high prevalence of poor self-management practices. It is well-established that achieving patient-centered primary care consistent with a chronic care model ensures optimum diabetes self-management support and improves long-term clinical and health outcomes in diabetes patients. The public sector primary care system in India provides services free of cost to beneficiaries but lacks patient-centered care that undermines diabetes self-management education and support. Furthermore, factors like poor patient knowledge of diabetes, suboptimal medication adherence, persistent clinical inertia, lack of data for monitoring and evaluation through clinical audit worsens the standards of diabetes care in primary care settings of India. There is a need for government initiatives to be directed towards the provision of comprehensive outpatient care that is inclusive of uninterrupted supply of drugs, provision of essential laboratory investigations, training and availability of qualified diabetes educators and availability of specialist support when required. Furthermore, the integration of depression screening and smoking cessation services at the primary care level is warranted.

Key words: Primary care; Diabetes; Self-care; Adherence; India

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Core tip: Public primary care facilities in India, especially in rural and suburban areas, are frequently unable to deliver patient-centered care for diabetes self-management through education and support due to the lack of trained diabetes educators and team-based support, and the absence of community linkages. Studies from Indian primary care facilities indicate the high prevalence of suboptimal medication adherence, poor glycemic status, clinical inertia, poor patient knowledge of diabetes, lack of depression

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screening and inadequate assistance for tobacco cessation. Developing prospective registries with predefined data standards in Indian primary care facilities is essential for enabling clinical audits and monitor the quality of patient care.

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INTRODUCTION

India has approximately 73 million people living with diabetes and another 37 million with prediabetes while nearly 47% of the diabetes cases are undiagnosed^[1,2]. The steady increase in diabetes burden in India since 1990 is attributed to the ongoing epidemiological, nutritional, social and economic transitions along with an increase in the prevalence of overweight and obesity^[3,4]. Diabetes and related complications impose a high-burden of catastrophic economic costs on the poorest populations in India by increasing out of pocket spending^[5]. However, India is committed to achieving universal health coverage for effective diabetes management by expanding services for diabetes prevention and control with targets to provide essential medicines and diagnostics to at-least 80% of the cases by 2025^[6].

Diabetes is a chronic disease requiring daily self-management by establishing and maintaining a continuum of care for the attainment of optimal health outcomes. Failure to do so increases the risk of early onset and development of microvascular and macrovascular complications of diabetes^[7]. Persons with diabetes themselves need to become caregivers and sustain a multitude of daily self-management decisions that include: (1) Adherence to medications in terms of the correct dose, frequency, route and protection against adverse effects; (2) Lifestyle modifications: Adequate physical activity and daily exercise with a healthy diet; (3) Cessation from smoking and harmful use of alcohol; (4) Self-monitoring of blood glucose; and (5) Foot-care^[8]. Diabetes self-management education and support (DSME/S) for educating the patient on diabetes self-care is an integral component of the chronic care model for primary-care clinics which is effective in improving diabetes-related health outcomes^[9,10]. The American Diabetes Association (ADA) has recommended measurement and monitoring of the key outcomes of DMSE/S including self-management, clinical outcomes, health status, and quality of life^[10].

There is also growing recognition of the need for high quality and patient-centered primary medical care focusing on both the primary and secondary levels of diabetes prevention^[11]. Primary care refers to the first level of contact with the health system that is equitable and accessible to individuals and provides integrated medical care services with scope for timely specialist referral thereby meeting the health needs of a majority of the population^[12,13]. According to the World Health Organization, people-centered primary-care should be comprehensive and continuous focusing on individual health-needs and preferences and enabling care from a trusted provider^[14].

The role of primary health care is particularly relevant in catering to the diabetes-related health needs of the Indian population due to its majority rural and large urban slum cluster population having a high diabetes burden^[15]. Moreover, there exists a large proportion of the functionally illiterate population with consequently poor health literacy undermining their ability for correct self-management of chronic illnesses like diabetes^[16]. However, the provision of high-quality diabetes care through primary care clinics and its physicians is a major public health challenge even in the developed world. A study among primary-care physician treated diabetes patients in the United States observed most had suboptimal health outcomes due to lack of medication intensification^[17]. Similarly, in the United Kingdom, more than one-third of type 2 diabetes patients on treatment have suboptimal glycemic control highlighting inadequate empowerment of patients towards self-care^[18,19]. The functioning of primary care systems in India, a developing, lower-middle income country, therefore, needs evaluation for identification of gaps, deficiencies and recommendations in promoting patient efficacy for diabetes self-care.

SEARCH STRATEGY

We summarized evidence regarding the standards of diabetes self-care in primary health facilities of India and challenges in achieving optimum patient-centered care (PCC) consistent with a chronic care model. We browsed PubMed and Scopus databases using the keywords with country (India) restricted searches applied: (Primary Health Care OR Primary Care) AND (Diabetes); (Diabetes) AND (Adherence OR Compliance); (Diabetes) AND (Self-care OR Self-management); (Diabetes) AND (Knowledge OR Awareness); (Diabetes) AND (Depression) We also screened the reference list of the selected articles to find other relevant articles.

STRUCTURE OF PRIMARY CARE HEALTH-DELIVERY MECHANISMS IN INDIA AND DIABETES-CARE

Public sector delivery of primary care services in India includes a distinctive primary health care approach in the rural areas and underserved regions of urban areas that provide free of cost services to all beneficiaries. The importance of primary care for meeting the healthcare needs of the rural population was recognized as early in 1946 in the Health Survey and Development committee report^[20]. The goal of PHC is emphasized as the provision of comprehensive health-care inclusive of adequate preventive, curative and promotive health services that are readily available, affordable and accessible for the target predominantly rural population. The expansion of PHC in India has continued over the years, but limited funding and persistent underutilization by target beneficiaries is a cause for concern^[21].

The public sector PHC delivery system includes a network of female voluntary community health workers termed Accredited Social Health Activists (ASHAs) with 1 ASHA catering to approximately every one thousand population. The community workers link the vulnerable population with the public health sector, government health initiatives and the national health programs. The PHC facilities in rural areas include sub-centers that are peripheral outposts (1 for every 3000-5000 population) and primary-health centers (1 for every 20000-30000 population). Community health centers and district hospitals have the capacity for providing secondary-care including referral services^[22]. Nevertheless, India also has a complex and diverse private healthcare sector that accounts for a majority of both outpatient and in-patient visits and also contributes substantially higher to the total health expenditure^[23]. However, the standards and quality of care in the private sector are highly variable depending upon location, cost of service and local factors. Moreover, the private sector contributes to escalating out of pocket expenses and is fundamentally inequitable leading to its inadequacy in attaining universal health coverage, especially amongst the economically disadvantaged populations.

A salient feature of the Indian public health-care system is its alignment with various vertical health programs. India also has a national program for prevention and control of cancer, diabetes, cardiovascular diseases and stroke (NPCDCS), operationalized across all the districts of the country in 2016. The NPCDCS prescribes a package of services for NCD control at each level of the public health-care delivery system in India inclusive of behavior change counseling, screening, diagnosis, clinical treatment and referral services. The program also recommends regular patient education for effective diabetes management during the initial and follow-up patient visits^[24].

CURRENT STATUS OF DIABETES SELF-CARE PRACTICES IN PRIMARY CARE FACILITIES IN INDIA

The heterogeneous health system in India means that primary medical care can be extended by various health facilities ranging from the primary health centers to secondary/district hospitals or even tertiary care hospitals depending upon accessibility, affordability, and patient preference.

A study from Northern India ($n = 385$) in three government health facilities had found 25.5% patients non-adherent to anti-diabetic medications, 29% were non-adherent to dietary recommendations and 52% non-adherent to exercise recommendations^[25]. Another study conducted at a community health center in Northern India ($n = 256$) reported non-compliance to medications in 46.5% patients and non-compliance to dietary recommendations in 23.5% diabetes patients^[26]. A study from a rural health facility in Western India ($n = 307$) reported poor pharmacological

compliance in 23.7% and poor non-pharmacological compliance in 49.1% diabetes patients^[27]. A study from Southern India ($n = 162$) reported 95.6% of diabetes patients were adherent to medications and 82.1% were adherent to exercise^[28]. There exist very few studies that have assessed self-care and adherence characteristics in diabetes patients from rural India.

CHALLENGES IN THE PROVISION OF DIABETES SELF-MANAGEMENT SUPPORT THROUGH PUBLIC SECTOR PRIMARY CARE IN INDIA

Lack of readiness for PCC for diabetes self-management

PCC promotes a non-authoritarian patient guided shared decision-making approach in patient-provider relationships^[29]. PCC includes respect for individual patient preferences, integration of care, information and education, access to care, the involvement of family and outlining care continuity and transition^[30]. Achieving high-quality diabetes PCC requires effective DMSE/S that is respectful and responsive to individual patient preferences for the realization of desired health goals. DMSE delivery is indicated at four key time-points: (1) At the time of diagnosis; (2) During an annual assessment; (3) At the time when complications arise; and (4) When there is a transition in care^[31]. Studies have shown that PCC empowers patients and increases their efficacy of self-care for medication adherence and healthy lifestyle choices^[32]. In contrast, ineffective communication between health-care providers and diabetes patients results in suboptimal diabetes care and lowers patient adherence to their prescribed self-care practices^[33].

Adopting a patient-centered approach also enables health-system planning for reducing the risk of diabetes complications by appropriate monitoring and adequately treating comorbidities like hypertension, hyperlipidemia and risk factors like tobacco smoking and obesity^[31].

The prerequisites for implementing PCC includes a team-based approach inclusive of trained diabetes educators, community involvement and maintenance of updated treatment registers (documenting demographic details, blood pressure and glycemic status readings, patient follow-up, referral, and complication data) consistent with a chronic care model^[9].

The Indian NPCDCS also recommends training of nurses as diabetes educators^[24]. Nevertheless, the implementation mechanisms for attaining optimal glycemic control in diabetes patients through effective DMSE remains a low priority across the healthcare delivery spectrum in India including specialist tertiary care hospitals^[25,34]. An important reason for ineffectual DMSE in primary care settings in India is the inadequate availability of trained diabetes educators rendering patient education as an additional function for physicians who can be ill-equipped for the task in the absence of any curricular or formal training and certification^[35]. Moreover, in the Indian context, the counseling of young diabetes patients is particularly challenging due to disease-related stigma^[16]. There also can exist diminished motivation for physicians to engage in patient education due to preexisting heavy patient load and congestion at clinic sites that limit avenues for patient-provider communication^[36]. Specialist referral for diabetes management is also a fundamental challenge in remote and rural primary care facilities, often lacking trained diabetes specialists^[37,38].

Poor patient knowledge of diabetes

It is well-established that patient knowledge of diabetes improves health outcomes in diabetes patients which include improved glycemic control and reduced complications^[39]. Studies from India have mostly reported poor patient knowledge of diabetes. A study in government health facilities of Northern India ($n = 385$) found only 38.5% diabetes patients were aware of symptoms of hypoglycemia while 74% were aware of plasma glucose levels indicating good glycemic control^[40]. A study from Western India ($n = 400$) reported only 29 (9.4%) diabetes patients' had good diabetes-related knowledge, whereas 219 (71.3%) had moderate and 59 (18.2%) patients had poor knowledge^[27]. A study in a rural community in Southern India found nearly half (48.8%) diabetes patients were unaware that diabetes is an incurable disease and almost none of the patients were aware of the importance of foot-care for diabetes patients^[41]. However, another study from a rural hospital in Southern India found nearly 75% of diabetes patients having good foot-care knowledge scores^[42]. Low educational status is consistently reported as a predictor of poor knowledge of diabetes^[27,40].

Suboptimal medication adherence and clinical inertia

Medication Adherence is the “extent to which a patient acts by the prescribed interval, and a dose of a prescribed regimen”^[43]. Suboptimal medication adherence in diabetes patients worsens glycemic control and other therapeutic outcomes by precluding the full benefit of treatment and increases the need for hospital admissions^[44,45]. Both patient-related and healthcare system related factors influence medication adherence^[46]. There is a need to improve medication adherence assessment measures in India by developing psychometrically valid scales particularly when measuring insulin adherence. The newer scales need to be culturally valid and focus upon constructs like belief in medications and traditional concepts relating to “hot” and “cold” medicines^[34].

In comparison to community-based studies in rural and underserved areas, public health facility-based studies conducted in India usually show higher rates of medication adherence in diabetes patients probably due to a regular supply of free of cost medications which are particularly beneficial for patients belonging to the lower socioeconomic classes^[25,34,41,47,48]. However, the high proportion of adherent patients is often found not correlating with their glycemic status^[25,34]. A multicenter study reported a mean HbA1c of 9.2% in a large cohort of 20554 Indian diabetes patients^[49]. Clinical inertia, the failure to intensify the treatment of a diabetic patient despite not meeting recommended glycemic targets also contributes to poor glycemic control. This phenomenon has been linked to limited drug armamentarium and failure of a timely switch to insulin therapy by Indian physicians^[35,49,50]. Reasons for delayed insulin initiation by Indian physicians include concerns over side-effects like hypoglycemia and ethical concerns due to doubtful patient self-efficacy^[35,51]. Moreover, lower SES patients may be unable to afford glucometers and strips that are not provided by the public health system^[34].

Lack of diabetes clinical audits and prospective registries

Diabetes is a “whole-life” disease requiring a sustained continuum of care. The ADA recommends the standards of diabetes care should include a 3-6 monthly clinical review of the diabetes patient that includes plasma glucose and blood pressure examination, HbA1c investigation at least twice a year, annual foot examination and chronic kidney disease diagnosis. Furthermore, patients are expected to assess their therapeutic response to anti-diabetic therapy by regular self-monitoring of blood glucose^[31]. Facilities for these laboratory investigations are not regularly available in Indian primary health facilities thereby needing a further referral and associated with risk of patient non-compliance. Clinical audits of diabetes registries is a valuable tool for increasing health-system accountability, monitoring health outcomes, efficiency and improving the quality of care^[52]. A record based audit of a primary health facility in Southern India reported an increase in patients with ideal monitoring from 3.2% in the first year to 48.2% patients in the second audit year^[38].

The Indian NPCDCS recommends the maintenance of NCD treatment registers from primary-care level onwards although no data standards are specified^[24]. Moreover, in the absence of electronic or digital health records, lack of clinical audits and a scarcity of published literature, there is limited scope for ascertaining treatment outcomes of diabetes patients treated in primary care settings.

Lack of integration of mental health services with diabetes care

Patients with type 2 diabetes mellitus (T2DM) are two times more likely to have depression compared to the general population^[53]. Depression tends to lower adherence to self-care practices and is associated with poor glycemic control and more complications^[54]. The prevalence of depression in T2DM patients in India shows considerable variation with estimates ranging from 8% to 84%^[55]. There is growing recognition that depressive symptomology needs to be identified and treated in primary care settings and diabetes educators can be trained to address diabetes-related distress^[56]. In countries with limited mental health infrastructure like India^[57], the implementation of such an approach can be particularly valuable.

Enabling smoking and tobacco cessation

Smoking cessation counseling for diabetes patients who are also tobacco smokers is an integral component of diabetes care^[31]. Approximately 19% of Indian men are current tobacco smokers of whom less than half (48.8%) were advised to quit smoking by a health-care provider^[58]. Promotion of smoking cessation as part of diabetes care should involve sensitization and training of all health-care providers involved in diabetes care. Mobile-health text-message or smartphone applications like mCessation should be evaluated for their effectiveness in improving quit-rates among diabetes patients^[59].

WAY FORWARD

Primary care facilities in the public sector cater to millions of diabetes patients but are deficient in characteristics compatible with a functioning, chronic care model. Team-based DSME/S for patients in primary care need high-quality training of nurses, multipurpose workers and other paramedical staff like pharmacists as diabetes educators. Decision support like when to initiate insulin to avoid clinical inertia can be provided to medical doctors working in rural and suburban areas through telemedicine-based consultation with specialists^[60]. Peer-support based community linkages may help patients cope with stress and reduce physical inactivity through health promotion activities like yoga and meditation.

Strengthening public primary health facilities for the provision of comprehensive outpatient care requires sustained political commitment and adequate funding. The government of India has already initiated a national program to push for upgrading 150000 sub-centers^[21], the bottom-most primary care facility to health and wellness centers with additional staffing through mid-level service providers and capable of the provision of comprehensive non-communicable disease management.

The feasibility of medication adherence support through patient counseling, peer education, and mHealth interventions also need exploration in Indian primary care settings. The provision of an uninterrupted supply of drugs and diagnostics is imperative in this regard as medicinal costs constitute the highest proportion of out of pocket spending in diabetes-related outpatient care in India^[5,37]. To promote refill adherence, a national program is currently operational that promotes ubiquitous availability of high-quality generic drugs at significantly lower costs compared to branded medicines to all patients^[61].

Future Indian diabetes care research should also focus quality of diabetes care accorded in primary care facilities especially those in resource-constrained settings. A national audit on diabetes care standards and patient health outcomes in primary care is also urgently needed to understand the measures needed to limit the continuously escalating costs for managing complicated diabetes patients (Table 1).

Table 1 Major challenges and potential solutions for promoting effective diabetes self-management through enhanced primary care

Major challenges	Potential solutions
Strengthening primary-care facilities for providing comprehensive outpatient care	Sustained political will and financial commitment towards NPCDCS Expand basket of laboratory investigations for maintaining the continuum of care Upgrading and maintaining registers by the introduction of data indicators and data standards with timely centralized data collection for the performance of clinical audits
Patient-centered care	Training paramedical staff as certified diabetes educators for enhanced DMSE/R m-Health applications for health education and behavior change communication Community outreach
Patient isolation and stress	Peer support Community linkages: yoga, meditation
Suboptimal adherence	
Drug availability and refill adherence	Ensuring uninterrupted supply of drugs, promotion of generic drugs to reduce out of pocket expenses, expansion of drug types and availability of insulin with the development of proper storage facilities
Correct adherence assessment	Development of culturally valid adherence scales
Adherence Support	Health education, mHealth
Avoiding clinical inertia	Training health workers for the correct dispensation of insulin therapy Regular availability of insulin and syringes to eliminate any out of pocket expenses
Implementation research	Intervention studies to improve patient-centered care, adherence support, and DMSE/R Cohort studies to evaluate the quality of care and assess long-term health outcomes
Operational research	Promotion of community linkage in primary care for diabetes patients
Economic evaluations	Cost-effectiveness of diabetes management with primary care compared to specialist treatment
Health technology assessment	Does provision of free glucometers and strips increase patient adherence to SMBG and improve treatment outcomes (improved glycemic control, fewer hypoglycemic episodes)

DMSE: Diabetes self-management education; NPCDCS: National program for prevention and control of cancer, diabetes, cardiovascular diseases and stroke.

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Observational Study

Different metabolic/obesity phenotypes are differentially associated with development of prediabetes in adults: Results from a 14-year cohort study

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Abstract

BACKGROUND

The risk of developing prediabetes based on the metabolic/obesity phenotypes has been poorly investigated.

AIM

To examine the association of baseline metabolic/obesity phenotypes and their changes over time with the risk of prediabetes development.

METHODS

In a population-based cohort study, 1741 adults (aged > 19 years) with normal blood glucose were followed for 14 years. Anthropometric and biochemical measures were evaluated regularly during the follow-up period. According to body mass index and metabolic health status, participants were categorized into four groups: Metabolically healthy normal weight (MHNW), metabolically healthy obese (MHO), metabolically unhealthy normal weight (MUNW) and metabolically unhealthy obese (MUO). Multivariable Cox regression analysis was used to measure the risk of prediabetes according to the baseline metabolic/obesity phenotype and their changes during the follow-up.

RESULTS

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In the whole population with a mean (95CCI for mean) follow up duration of 12.7 years (12.6-12.9), all three MUNW, MHO, MUO groups were at higher risk for developing prediabetes compared to the MHNW group ($P = 0.022$). The MUNW group had the highest risk for developing prediabetes (hazard ratio (HR): 3.84, 95%CI: 1.20, 12.27). In stratified analysis by sex, no significant association was found in men, while women in the MUNW group were at the greatest risk for prediabetes (HR: 6.74, 95%CI: 1.53, 29.66). Transforming from each phenotype to MHNW or MHO was not related to the risk of prediabetes development, whereas transforming from each phenotype to MUO was associated with an increased risk of prediabetes ($HR > 1$; $P < 0.05$).

CONCLUSION

Our findings indicate that MHO is not a high risk, unless it transforms into MUO over time. However, people in the MUNW group have the greatest risk for developing prediabetes, and therefore, they should be screened and treated.

Key words: Prediabetes; Obesity; Metabolic status; Cohort study

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Core tip: The risk of developing prediabetes based on metabolic/obesity phenotypes has been poorly investigated. In a 14-year follow-up cohort study, we observed that metabolically unhealthy normal weight, metabolically healthy obese (MHO), and metabolically unhealthy obese (MUO) were at higher risk for developing prediabetes compared to metabolically healthy normal weight (MHNW) subjects. The results stratified by sex demonstrated no significant association in men, while the risk of prediabetes development was significantly higher in all metabolic/obesity phenotypes in women compared to MHNW. Transforming from each phenotype to MHNW or MHO was not related to an increased risk of prediabetes development, whereas transforming from each phenotype to MUO was associated with an increased risk of prediabetes.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a public health concern worldwide^[1]. The prevalence and burden of diabetes has increased faster in low-income and developing countries than in high-income countries^[2]. Prediabetic subjects are at a 3-12 times higher risk for developing diabetes compared to the general population^[3]. In addition, the prevalence of cardiovascular and renal diseases has increased in American prediabetic patients over the last decades^[3]. Therefore, identification of effective measures to prevent prediabetes risk might be useful for reducing the risk of T2DM, cardiovascular and renal diseases.

Although body mass index (BMI), as a measure of obesity, is positively correlated with the risk of various non-communicable diseases^[4,5], approximately 35% of obese individuals are metabolically healthy^[6]. In contrast, many normal weight subjects may suffer from a variety of metabolic abnormalities, such as insulin resistance, hypertension, dyslipidemia and hyperglycemia^[7,8]. However, metabolic abnormalities are more common amongst metabolically healthy obese (MHO) than metabolically healthy normal weight (MHNW) individuals^[6]. Consistently, a recent meta-analysis showed that MHO subjects with or without fatty liver had a greater risk for developing T2DM compared to MHNW subjects without fatty liver^[9]. In a 10-year follow up study among Koreans, the incident diabetes risk was higher in both metabolically unhealthy normal weight (MUNW) and metabolically unhealthy obese (MUO) individuals than MHNW individuals. Nevertheless, in MHO subjects in this population, the incidence of T2DM was significantly higher in subjects younger than 45 years, but not in older adults^[10]. Although the association between metabolic/

obesity phenotypes and T2DM have been investigated in various populations^[10-14], few studies have been conducted to evaluate such association not only in Iran where diabetes mellitus is one of the main causes of years lived with disability, but also worldwide^[15]. In our previous publication, MHO and MUOW subjects were at considerably greater risk for developing T2DM compared to MHNW subjects^[12]. Nevertheless, the risk of developing prediabetes based on metabolic/obesity phenotypes has been poorly investigated. In a retrospective Japanese population cohort study, the prevalence of prediabetes was remarkably higher in obese individuals compared to normal weight subjects (60% *vs* 34%)^[16]. Another longitudinal study revealed no association between general adiposity and diabetes or prediabetes risk, while dysfunctional adiposity, determined by excess visceral fat and insulin resistance, was associated with the occurrence of diabetes or prediabetes^[17]. Due to our limited knowledge regarding prediabetes risk, in the current study, we aimed to: (1) Estimate the prevalence of different metabolic/obesity phenotypes in an Iranian population and (2) Determine the association of baseline metabolic/obesity phenotypes and their interchanges during follow-up with the risk of prediabetes development in a prospective cohort study.

MATERIALS AND METHODS

Study subjects

Subjects in the present study were from the Isfahan Diabetes Prevention Study (IDPS). Details regarding the IDPS population and study design have been described elsewhere^[18]. In brief, the IDPS is an ongoing prospective cohort study that began in 2003, and participants were selected from a consecutive sample of patients who attended the clinics of Isfahan Endocrine and Metabolism Research Center. This study was conducted to evaluate the role of lifestyle factors in developing prediabetes and T2DM in the immediate family of T2DM patients. A total of 1741 subjects (439 men and 1302 women) without prediabetes or T2DM aged from 30 to 70 years and with complete data were included in the current cohort study to identify metabolic status and metabolic/obesity phenotypes. Subjects were followed for 14 years (2003 to 2017). Information regarding health status and lifestyle risk factors for T2DM, like physical activity and dietary intakes and demographic variables were collected using validated questionnaires and updated according to a medical care standard in diabetes^[19]. Accordingly, participants were tested for the diagnosis of new-onset prediabetes or diabetes in at least at 3-year intervals. Informed written consent was obtained from each participant at baseline. The Ethical Committee of Isfahan University of Medical Sciences approved the study protocol.

Anthropometric assessment

All measurements were acquired by well-trained examiners at baseline. Weight was determined using a balanced scale while participants were minimally clothed and recorded to the nearest 0.1 kg. Height was measured using a wall-fixed tape measure while shoulders were in the normal position and participants were without footwear, and recorded to the nearest 0.5 cm. Waist circumference (WC) and hip circumference (HC) were measured using a metal tape measure without imposing any pressure to body surface and were recorded to the nearest 0.5 cm. WC was considered as the narrowest level between the lowest rib and iliac crest, and HC was considered as the largest level^[20]. BMI was calculated by dividing body weight in kg by height in m². Waist to hip ratio (WHR) was calculated as dividing WC by HC.

Laboratory measurements

A 10-h overnight fasting blood sample was gathered to measure serum lipids [total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) and fasting plasma glucose (FPG)]. Postprandial plasma glucose levels were determined in venous blood sample at 30, 60, and 120 min after oral glucose administration. Plasma glucose and lipid profile concentrations were measured using the oxidase method (Pars Azmoon, Tehran, Iran) adapted to a Selectra-2 auto-analyzer (Vital Scientific, Spankeren, The Netherlands). Serum LDL-C levels were calculated using the Friedwald equation when serum TG levels were < 400 mg/dL^[21]. Whole blood samples were used to determine HbA1c concentrations through the pink reagent kit on a DS5 analyzer. For all markers, intra- and inter-assay coefficients of variability (CVs) were < 2.2%.

Assessment of other variables

To measure blood pressure, subjects were asked to rest for 15 min, and then while subjects were sitting, blood pressure was measured twice with a 30 s interval between

the two measurements using a Mercury sphygmomanometer. The mean of the two measurements was recorded as the blood pressure value.

Definition of prediabetes and metabolic/obesity phenotypes

Prediabetes was defined according to the definition of the American Diabetes Association. Accordingly, subjects with $100 \leq \text{FPG} < 126 \text{ mg/dL}$ or $\text{HbA1C} \geq 6.5\%$ or 2-h oral glucose test tolerance (2h-OGTT) $\geq 200 \text{ mg/dL}$ were defined as being prediabetic^[19]. Normal weight and overweight/obese were defined as $\text{BMI} < 25$ and $\geq 25 \text{ kg/m}^2$, respectively. Metabolic unhealthy was defined as the presence of at least one component of the following criteria: (1) Elevated blood pressure (systolic blood pressure $\geq 130 \text{ mmHg}$ or diastolic blood pressure $\geq 85 \text{ mmHg}$); (2) Low HDL-C concentration ($< 50 \text{ mg/dL}$ in women and $< 40 \text{ mg/dL}$ in men); and (3) High serum TG ($\geq 150 \text{ mg/dL}$)^[22].

Statistical analysis

Participants were categorized into four metabolic/obesity phenotypes categories. Normal distribution of quantitative data was tested using the Kolmogorov-Smirnov test and Q-Q plot. Data were reported as mean \pm SE or percentage for continuous and categorical variables, respectively. The association between categorical variables was examined using the chi-square test. Between groups differences for quantitative variables were evaluated using Analysis of variance (ANOVA).

Event-free rates were estimated using the Kaplan-Meier method, and the differences between survival curves for all metabolic/obesity phenotypes at the end of follow-up were compared by using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) for developing prediabetes were calculated using univariate and multivariate Cox proportional hazards regression models. The crude model included only the metabolic/obesity phenotypes, and model 1 was adjusted for age, sex, smoking and physical activity as possible confounding factors. Statistical analyses were performed using statistical package for social science (SPSS version 16, SPSS, Inc., IL, United States).

RESULTS

Baseline characteristics of the study population across the metabolic/obesity phenotypes are shown in **Table 1**. Of the 1741 subjects with normal glucose tolerance at baseline, 274 persons (15.7%) were MHNW. The most and least prevalent phenotypes were MHO (48.4%) and MUNW (4.1%), respectively. Normal weight groups, either metabolically healthy or unhealthy, were more likely to be male and highly educated. In both normal weight and overweight/obese groups, the means of age, weight, BMI, WC, HC and WHR were higher in metabolically unhealthy subjects than metabolically healthy subjects. Similar results were also observed for biochemical tests, including blood sugar-30 -60 and-120 min, lipid profile (TG, TC, HDL-C, LDL-C), SBP and DBP. FPG and HbA1c were not significantly different across the metabolic/obesity phenotypes. Physical activity level and smoking were not significantly different across the metabolic/obesity phenotypes.

Person-years follow up (incidence rate per 1000-person years) for MHNW and overweight/obese subjects were 3007 (14.96) and 9501 (18), respectively. Corresponding values in MUNW subjects were 736 (29.89), and 6204 overweight/obese (20.9), respectively. In total, person-years follow up (incidence rate per 1000-person years) in metabolically healthy and unhealthy subjects were 12508 (17.34) and 6940 (22.33), respectively. The prevalence of different metabolic/obesity phenotypes at baseline and the end of study is illustrated in **Figure 1**. The most common phenotype at baseline or at the end of the study was related to MHO (baseline: 48.5% and end of follow-up: 46.9%), whilst the least common phenotype was MUNW (baseline: 4.1% and end of follow-up: 4.0%). At baseline, 24% of metabolically healthy subjects and 11% of metabolically unhealthy subjects had normal weights (**Figure 1**). Changing in the prevalence of metabolic/obesity phenotypes was statistically significant over the study follow-up (**Figure 1**). **Figure 2** shows how the prevalence of overweight and obesity based on metabolic health change changed during the follow-up. In all four groups, BMI status significantly changed (all P -values < 0.0001).

Figure 3A and **B** show the Kaplan-Meier survival curves of prediabetes incidence, comparing the two (metabolically healthy and unhealthy) and four groups (MHNW MUNW, MHO, MUO). The results of log rank tests showed significant difference between groups, indicating a significantly different probability of incidence rate of prediabetes between study groups. Metabolic unhealthy people had a higher probability (Chi-square = 5.71; $P = 0.023$), and the MUNW, MHO, MUO groups had higher event-rate rates compared to MHNW subjects (Chi-square = 12.49; $P = 0.006$).

Table 1 General characteristics of study population at baseline¹

	Metabolically healthy and normal weight	Metabolically healthy and overweight or obese	Metabolically unhealthy and normal weight	Metabolically unhealthy and overweight or obese	P value ²
Number (%)	274 (15.7)	843 (48.4)	71 (4.1)	553 (31.8)	
Age in yr	41.05 ± 0.42	42.33 ± 0.22	43.38 ± 0.76	43.31 ± 0.27	< 0.0001
Weight in kg	59.22 ± 0.46	74.77 ± 0.38	61.64 ± 0.99	77.92 ± 0.50	< 0.0001
BMI in kg/m ²	22.75 ± 0.11	29.47 ± 0.12	23.61 ± 0.16	30.35 ± 0.16	< 0.0001
WC in cm	76.79 ± 0.42	88.67 ± 0.29	81.19 ± 0.80	92.62 ± 0.38	< 0.0001
HC in cm	97.39 ± 0.30	108.71 ± 0.27	98.22 ± 0.57	109.51 ± 0.35	< 0.0001
WHR	0.79 ± 0.004	0.82 ± 0.002	0.83 ± 0.008	0.85 ± 0.003	< 0.0001
FBS in mg/dL	88.04 ± 0.42	88.33 ± 0.24	87.13 ± 1.12	88.54 ± 0.34	0.446
Blood sugar 30 min in mg/dL	124.91 ± 1.53	129.96 ± 0.94	132.30 ± 3.32	133.48 ± 1.13	< 0.0001
Blood sugar 60 min in mg/dL	118.09 ± 1.94	125.60 ± 1.12	127.76 ± 4.49	135.30 ± 1.38	< 0.0001
Blood sugar 120 min in mg/dL	95.74 ± 1.31	101.32 ± 0.74	102.16 ± 2.30	101.35 ± 0.95	0.001
HbA1c as %	4.92 ± 0.04	4.98 ± 0.03	4.84 ± 0.08	5.04 ± 0.04	0.070
Triglyceride, mg/dL	110.16 ± 3.14	124.47 ± 2.39	190.99 ± 7.46	218.15 ± 4.59	< 0.0001
Total cholesterol, mg/dL	182.54 ± 2.17	194.29 ± 1.39	192.34 ± 3.30	198.68 ± 1.69	< 0.0001
LDL-C, mg/dL	110.17 ± 1.92	120.04 ± 1.30	115.51 ± 3.28	118.31 ± 1.58	0.001
HDL-C, mg/dL	49.89 ± 0.83	49.93 ± 0.44	38.97 ± 1.0	39.03 ± 0.35	< 0.0001
Systolic blood pressure, mmHg	100.50 ± 0.08	110.01 ± 0.05	110.82 ± 0.17	120.22 ± 0.07	< 0.0001
Diastolic blood pressure in mmHg	60.79 ± 0.06	70.15 ± 0.04	70.96 ± 0.12	80.03 ± 0.05	< 0.0001
Physical activity , MET-hr/wk	19.73 ± 4.25	16.61 ± 3.03	26.63 ± 11.83	19.10 ± 3.11	0.742
Male as %	33.6	19.7	33.8	28.4	< 0.0001
Educational level as %					0.006
Illiterate	2.6	3.4	5.6	5.5	
< 12 yr	38.4	48.1	42.3	50.3	
= 12 yr	36.6	32.9	39.4	29.7	
> 12 yr	22.4	15.6	12.7	14.5	
Current smoker as %	16.8	6.9	13.0	11.2	0.073

¹Values are mean ± SE unless otherwise indicated.²By ANOVA or χ^2 test. BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WHR: Waist to hip ratio; FBS: Fasting blood sugar; LDL: Low density lipoprotein; HDL: High density lipoprotein; MET-h/Wk: Metabolic equivalent-hr/wk.

Table 2 shows the risk of prediabetes development across different metabolic/obesity phenotypes. In the whole population, all three MUNW, MHO, MUO groups were at higher risk for developing prediabetes compared to the MHNW group. Although this association was marginally significant in the crude model ($P = 0.058$), adjustment for potential confounders strengthened the associations ($P = 0.022$). In the crude model, the MUNW group was at the greatest risk for developing prediabetes compared to other groups (HR: 2.05, 95%CI: 1.05, 4.02), and this association became stronger after adjustment for confounders (HR: 3.84, 95%CI: 1.20, 12.27).

In stratified analysis by sex (**Table 2**), a non-statistically significant increase was observed in MUO men but attenuated after adjustment for potential confounders. However, consistent with the whole population, the risk of incident prediabetes in women was greater in the MHO, MUNW and MUO groups compared to the MHNW group. The greatest risk was found in the MUNW group (HR: 6.74, 95%CI: 1.53, 29.66; $P = 0.014$). When participants were categorized into six groups (metabolically healthy/unhealthy-normal weight/overweight/obese) and considering metabolically healthy-normal weight group as the reference, the greatest risk of developing prediabetes was observed in metabolically unhealthy-obese subjects in both the crude (HR: 2.09, 95%CI: 1.30, 3.38) and adjusted models (HR: 2.16, 95%CI: 1.32, 3.53) (data

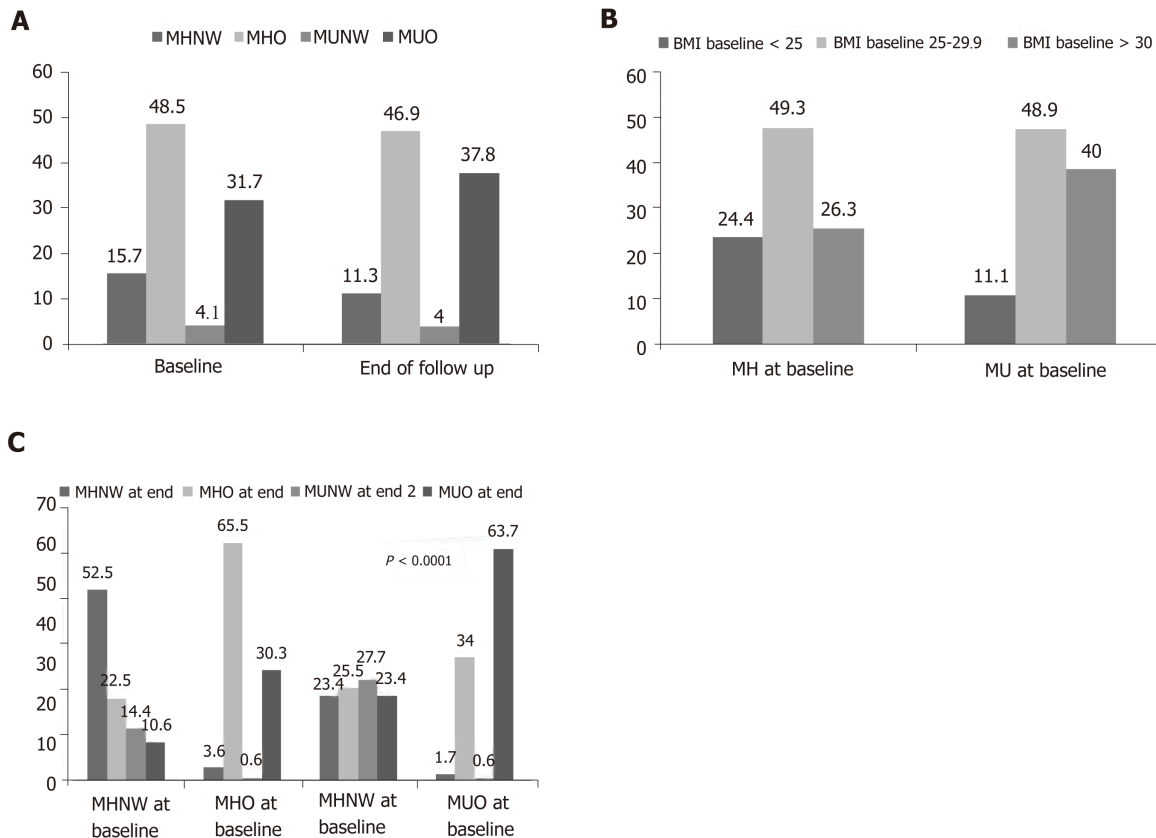


Figure 1 Prevalence of metabolic/obesity phenotypes at baseline and end of study (A), body mass index status at baseline and end of study based on baseline metabolic status (B), and prevalence of different metabolic/obesity phenotypes at the end of study based on baseline metabolic/obesity phenotype (C). MHNW: Metabolically healthy normal weight; MHO: Metabolically obese; MUNW: Metabolically unhealthy normal weight; MUO: Metabolically unhealthy obese; MH: Metabolically healthy; MU: Metabolically unhealthy.

not shown).

Table 3 shows the risk of developing prediabetes based on changing metabolic/obesity phenotypes during the follow-up. Transforming from each phenotype at baseline to MHNW or MHO was not significantly related to the risk of prediabetes incidence, whereas transforming from each phenotype to MUO was significantly associated with an increased risk of prediabetes compared with stable MHNW. Although there was no significant increment in the risk of prediabetes by transforming from MHNW and MHO to MUNW, stable MUNW was associated with a significantly higher risk for developing prediabetes (HR: 5.22, 95%CI: 1.53, 17.86; $P < 0.0001$).

DISCUSSION

In this prospective cohort study on the immediate family of patients with T2DM, we found that MHO was the most prevalent metabolic/obesity phenotype in this population. Although the risk of prediabetes increased in all individuals who were MUNW, MHO and MUO at baseline, individuals in the MUNW group had the greatest risk compared with other phenotypes. Moreover, transition from any phenotype into MUO and stable MUNW were associated with significantly increased risk of prediabetes by the end of follow-up. In the stratified analysis by sex, the effect of metabolic/obesity phenotype on prediabetes incidence was significant in females but not males and in line with the findings in the whole population, as the greatest risk was found in MUNW category. In the whole population and women, metabolic status was a strong predictor for prediabetes incidence rather than obesity status.

Thus far, several studies have examined the effect of metabolic/obesity phenotypes on diabetes incidence. In a 6-yr follow-up study among Chinese, Wang *et al*^[11] found that MUNW, MHO and MUO were at increased risk for developing T2DM. They also observed that transition from the MHO category at baseline into the MUO category at the end of follow-up was associated with an increased risk of T2DM compared to

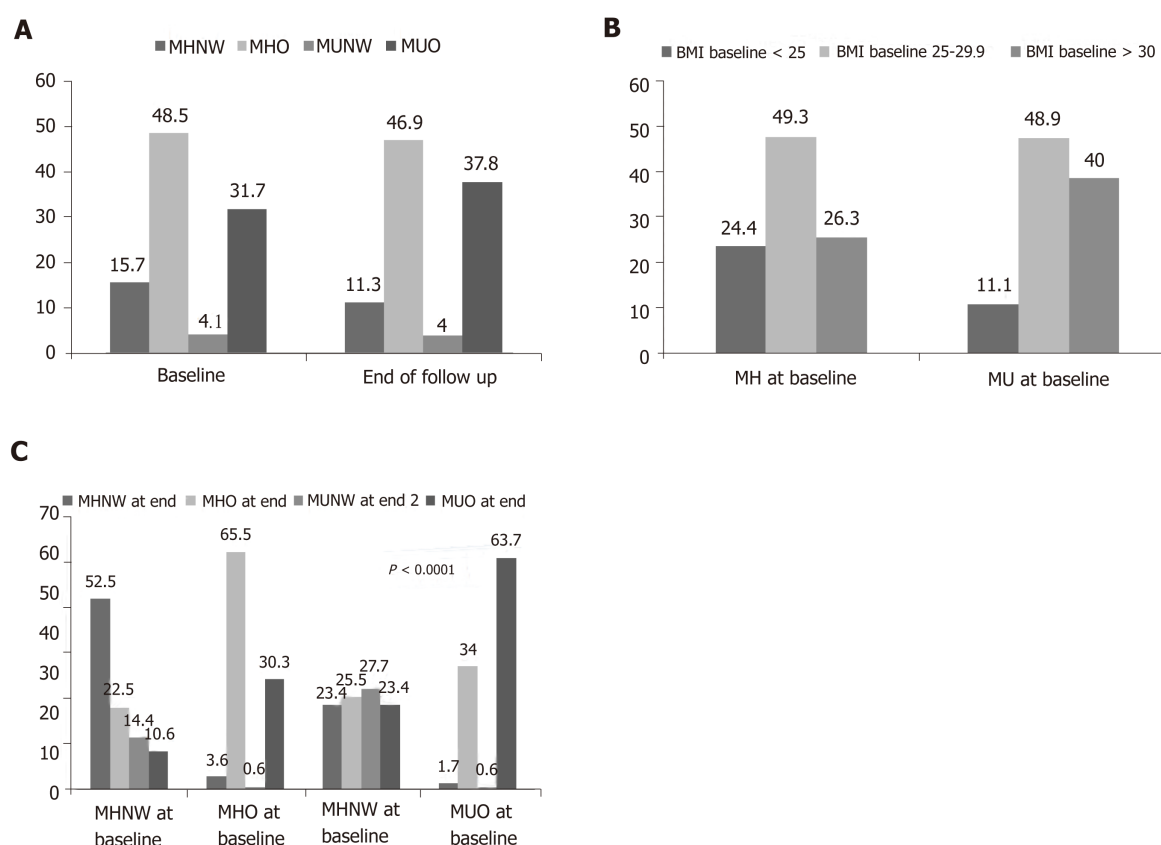


Figure 2 Changing in the prevalence of overweight and obesity based on metabolic health change during the follow-up. A: Metabolically healthy at both baseline and end; B: Metabolically healthy at baseline and metabolically unhealthy at the end; C: Metabolically unhealthy at baseline and metabolically healthy at the end; and D: Metabolically unhealthy at both baseline and end. BMI: Body mass index.

stable MHNW, but not compared to stable MHO. On the other hand, obesity at baseline, regardless of changes in metabolic status, increased the risk of incident T2DM. Nevertheless, in MUNW, transformation to MHNW was not associated with an increased risk of T2DM compared with stable MHNW^[11]. Similar results were found in a 10-year follow-up study among Korean subjects^[10]. They found that MUNW and MUO were at higher risk for developing diabetes and cardiovascular diseases compared to MHNW subjects, whilst the association in MHO was statistically significant only in younger individuals. Compared to stable MHNW, those with persistent MHO had a higher risk of incident T2DM after ten years follow-up^[10]. In our earlier study, we found that regardless of BMI, metabolically unhealthy subjects were more likely to develop T2DM. In spite of an increased risk of T2DM in MHO, it was considerably lower than MUO, suggesting that metabolic abnormality is a more relevant risk factor for developing T2DM than obesity^[12]. This finding is consistent with results of the present study showing that metabolically unhealthy subjects, even those with normal weight, are more likely to develop prediabetes compared to metabolically healthy counterparts. Further analysis according to changes in metabolic/obesity phenotypes also confirmed that metabolic health status is a better predictor of prediabetes incidence than BMI status. We observed that transition from MUNW or MUO into metabolically healthy status, regardless of changes in BMI, was not associated with an increased risk of prediabetes incidence. However, in participants with baseline metabolically healthy status, risk of prediabetes only increased when they were affected by both metabolic abnormality and obesity during the follow-up period.

The finding that MUNW subjects had the highest risk for prediabetes development is in line with the results of the English Longitudinal Study of Ageing (ELSA)^[13]. They found that despite the increased risk of T2DM in MHO individuals, they are at lower risk for T2DM compared to metabolically unhealthy subjects in any BMI category. For example, the risk of developing T2DM in MHO was 8.6 times higher than MHNW subjects whilst the corresponding value in MUNW subjects was 9.9 times higher^[13]. In an Iranian population-based cohort study among the elderly, Mirbolouk *et al*^[23] demonstrated that the MUNW phenotype was associated with the greatest risk of

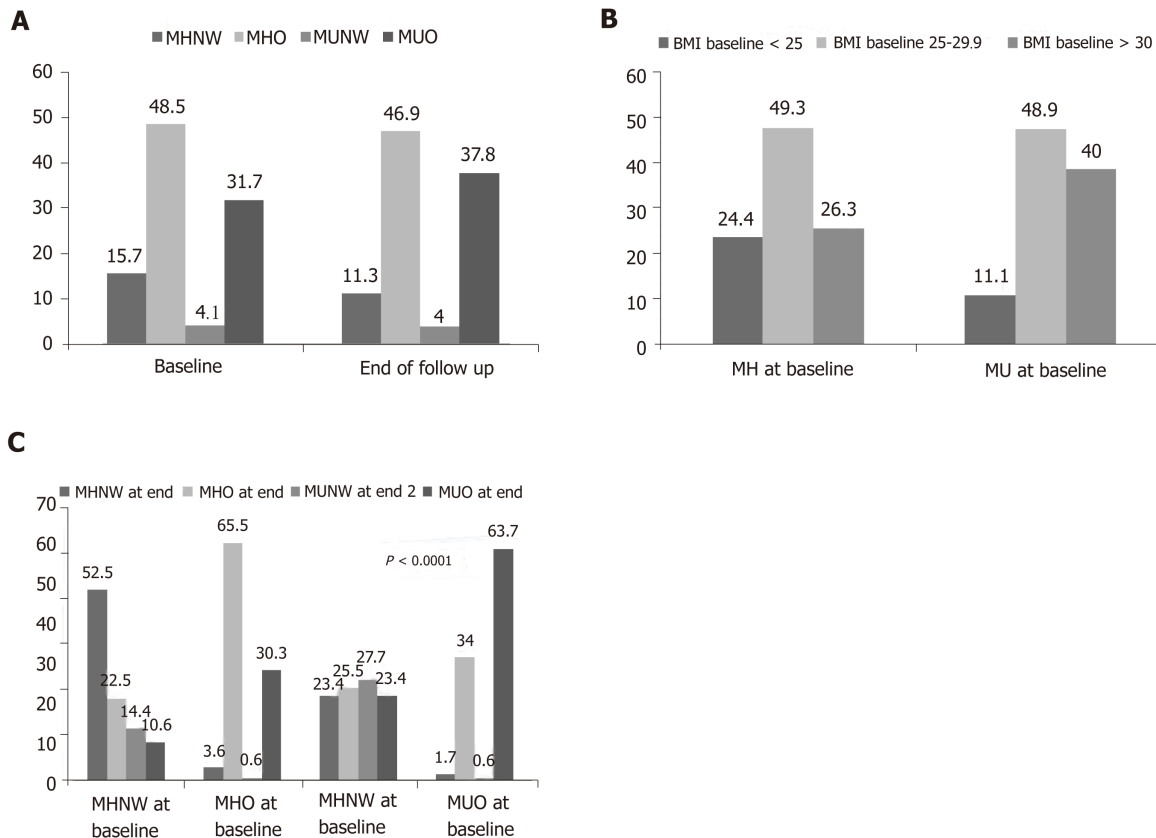


Figure 3 Kaplan-Meier curves. A: Kaplan-Meier curves comparing the estimated event-rate probability in metabolically healthy and unhealthy patients for prediabetes incidence; B: Kaplan-Meier curves comparing the estimated event-rate probability in metabolically healthy normal weight, metabolically unhealthy normal weight, metabolically unhealthy obese and metabolically unhealthy obese groups for prediabetes incidence. BMI: Body mass index.

developing cardiovascular disease (CVD), CVD mortality and all-causes mortality. However, the incident risk of CVD in MUNW and MUO was similar^[23]. Therefore, greater attention should be paid to MUNW subjects, as they may be less targeted for preventive interventions.

The reason for the greater risk of incident prediabetes among MUNW might be attributed to an aspect of the participants' body composition that was not measured. It has been shown that dysfunctional adiposity but not general adiposity is associated with an increased incidence of diabetes and prediabetes in obese adults^[17]. Moreover, in general, normal weight diabetic subjects have greater abdominal and total fat compared to obese diabetic individuals, which adversely affect insulin sensitivity^[24]. Sarcopenic obesity, a medical condition determined by low muscle mass accompanied by high fat mass, frequently occurs in older ages^[25] and is significantly correlated with insulin resistance^[26]. Therefore, BMI as an obesity index, which only consists of body weight and height, cannot reflect fat distribution. It is possible that, MUNW in our study population, who were older than other metabolic/obesity phenotypes, had more fat but less muscle masses compared to other categories. However, WC as a central adiposity measure was not greater in MUNW compared to MHO and MUO. Therefore, abdominal fat distribution might not explain our findings *per se*. Given that higher gluteofemoral fat mass is associated with lower risk of insulin resistance and diabetes^[27,28], it is possible that increased HC in MHO and MUO led to a lower risk of incident prediabetes compared to MUNW.

Several studies have suggested that reductions in visceral fat mass increase insulin sensitivity in MHO subjects, and consequently decrease diabetes risk^[29]. However, standard weight-reduction interventions may adversely affect appetite, mood and energy expenditure^[30] without any favorable effect on metabolic status in MHO subjects^[31-33]. These changes may promote weight regain. Therefore, regarding the relevance of favorable fat distribution in MHO, which is determined by lower visceral fat and higher subcutaneous fat, interventions targeting fat-loss rather than weight-loss might be more successful in reducing T2DM risk in MHO.

Although this is the first longitudinal study to predict the risk of prediabetes incidence according to metabolic/obesity phenotypes, it has several limitations that

Table 2 Multivariable-adjusted hazard ratios and 95% confidence intervals for prediabetes in the whole population and stratified by sex

	Metabolically healthy and normal weight	Metabolically healthy and overweight or obese	Metabolically unhealthy and normal weight	Metabolically unhealthy and overweight or obese	P value ¹
Whole population					
Crude	1	1.38 (0.92, 2.05)	2.05 (1.05, 4.02)	1.67 (1.10, 2.52)	0.058
Model 1	1	1.43 (0.69, 2.94)	3.84 (1.20, 12.27)	2.50 (1.19, 5.25)	0.022
Male					
Crude	1	1.06 (0.54, 2.08)	0.95 (0.30, 3.00)	1.39 (0.71, 2.73)	0.715
Model 1	1	0.94 (0.27, 3.27)	0.69 (0.08, 6.25)	1.10 (0.32, 3.75)	0.976
Female					
Crude	1	1.74 (1.04, 2.93)	3.25 (1.39, 7.58)	2.0 (1.16, 3.44)	0.026
Model 1	1	1.67 (0.63, 4.49)	6.74 (1.53, 29.66)	3.45 (1.23, 9.68)	0.014

¹By the Mantel-Haenszel extension χ^2 test. Model 1: Adjusted for age, sex, physical activity and smoking.

must be kept in mind. The study population was not a representative sample of Iranians, and therefore, our findings might not be generalizable to other populations of Iranians. Moreover, we used BMI as an anthropometric measure to determine obesity status, which does not consider fat distribution and does not differentiate between fat mass and lean mass. Finally, our study population mainly consisted of females, and therefore, the limited number of males may not allow us to identify true associations.

The strengths of our study are long-term follow-up and enough incident prediabetic cases that enhance the statistical power of analyses. To our knowledge, this is the first study that evaluated the association of metabolic/obesity phenotype with the development of prediabetes among Iranians. For diagnosing new cases of prediabetes, HbA1c, oral glucose test tolerance and fasting blood sugar were available so that new cases were not missed. Moreover, sex-specific associations were reported in the current analysis, and the confounding effects of various factors were controlled.

In conclusion, our study showed that MHO is not a high risk unless it progresses to MUO. However, the MUNW group has the greatest risk for developing prediabetes, and they should therefore be screened and treated. During the follow-up, changes to the phenotype status were significantly related to the risk of prediabetes development. In stratified analysis by sex, this association was evident among females but not males. Given that various metabolic/obesity phenotypes can increase the risk of prediabetes incidence, developing appropriate guidelines to care for various metabolic/obesity phenotypes to reduce prediabetes occurrence is necessary.

Table 3 Multivariable-adjusted hazard ratios and 95% confidence intervals for prediabetes incidence based on changes in metabolic/obesity phenotype at follow-up

Baseline metabolic/obesity phenotype	Last metabolic/obesity phenotype	HR (95%CI)
MHNW	MHNW	1 (reference)
	MHO	1.04 (0.40, 2.69)
	MUNW	2.67 (0.96, 7.40)
	MUO	5.87 (1.75, 19.66)
MHO	MHNW	1.25 (0.40, 3.97)
	MHO	1.10 (0.62, 1.95)
	MUNW	1.63 (0.14, 19.00)
	MUO	6.68 (3.56, 12.54)
MUNW	MHNW	2.17 (0.55, 8.52)
	MHO	0.65 (0.13, 3.24)
	MUNW	5.22 (1.53, 17.86)
	MUO	5.71 (1.51, 21.63)
MUO	MHNW	1.63 (0.28, 9.61)
	MHO	0.82 (0.41, 1.63)
	MUNW	0.00 (0.0, 0.0)
	MUO	3.98 (2.21, 7.15)

MHNW: Metabolically healthy normal weight; MHO: Metabolically obese; MUNW: Metabolically unhealthy normal weight; MUO: Metabolically unhealthy obese.

ARTICLE HIGHLIGHTS

Research background

The risk of developing prediabetes based on the metabolic/obesity phenotypes has been poorly investigated.

Research motivation

Due to the potential association between various metabolic/obesity phenotypes and the risk of prediabetes incidence, developing appropriate guidelines to care for various metabolic/obesity phenotypes to reduce prediabetes occurrence is necessary.

Research objectives

This study aimed to (1) estimate the prevalence of different metabolic/obesity phenotypes in an Iranian population and (2) determine the association of baseline metabolic/obesity phenotypes and their interchanges during follow-up with the risk of prediabetes development in a prospective cohort study.

Research methods

In a population-based cohort study, 1741 adults (aged > 19 years) with normal blood glucose were followed for 14 years. According to body mass index and metabolic health status, participants were categorized into four groups: metabolically healthy normal weight (MHNW), metabolically healthy obese (MHO), metabolically unhealthy normal weight (MUNW) and metabolically unhealthy obese (MUO). Multivariable Cox regression analysis was used to measure the risk of prediabetes according to the baseline metabolic/obesity phenotype and their changes during the follow-up.

Research results

In the whole population, all three MUNW, MHO, MUO groups were at higher risk for developing prediabetes compared to MHNW. The MUNW group was at the greatest risk for developing prediabetes (HR: 3.84). In stratified analysis by sex, no significant association was found in men, while women in the MUNW group were at the greatest risk for prediabetes (HR: 6.74). Transforming from each phenotype to MHNW or MHO was not related to the risk of prediabetes development, whereas transforming from each phenotype to MUO was associated with an increased risk of prediabetes.

Research conclusions

Our findings indicate that MHO is not a high risk unless it progresses to MUO. However, individuals in the MUNW group have the greatest risk for developing prediabetes, and therefore need to be screened and treated.

Research perspectives

Given that various metabolic/obesity phenotypes can boost the risk of prediabetes incidence, clinical trials need to be developed with appropriate guidelines to care for various metabolic/obesity phenotypes to reduce prediabetes occurrence.

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Prospective Study

Prospective study of total and various types of vegetables and the risk of metabolic syndrome among children and adolescents

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Abstract

BACKGROUND

Data available on the association between consumption of various types of vegetables and metabolic syndrome (MetS) remain inconsistent.

AIM

To investigate the association between the intake of various types of vegetables and MetS among children and adolescents and MetS.

METHODS

The Tehran Lipid and Glucose Study cohort included 424 children and adolescents initially free of MetS. At the 3.6 year follow-up, 47 new cases of MetS were identified. A 168-item semi-quantitative food-frequency questionnaire was used to collect information about total and various types of vegetables consumed, including allium-, green leafy-, fruity-, root-, stalk-, starchy-, potatoes, and cabbage. MetS was defined according to the Cook *et al*^[32] criteria.

RESULTS

The median (interquartile range) of total vegetable consumption was 217 (146-344) g/d. After adjustment for demographic characteristics and dietary intake, higher total- (≥ 350 g/d) and higher allium vegetable consumption (≥ 30 g/d) in the fourth quartile were significantly and inversely associated with risk of MetS

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compared to the first quartile. Consumption of green leafy vegetables in the third (21.4-38.3 g/d) *versus* the first quartile (≤ 13.5 g/d) demonstrated a significant inverse association with lower risk of MetS in children and adolescents; associations for other types of vegetables consumed were not significant.

CONCLUSION

Consumption of vegetables, especially allium and green leafy vegetables, in sufficient amounts may be beneficial in reducing the risk of MetS among children and adolescents.

Key words: Metabolic syndrome; Children and adolescents; Vegetable; Allium; Green leafy vegetables

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Core tip: Data on the association between vegetable intake as an individual dietary component and metabolic syndrome (MetS) remain inconsistent. This inconsistency of findings may probably be due to a difference in the amounts and specific subgroups of vegetables in different studies. This prospective study of Iranian children and adolescents reported an inverse association between total vegetable consumption and MetS risk. Among vegetable subgroups, consumption of green leafy- and allium vegetables was inversely associated with risk of MetS after adjustment for the main potential confounders.

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INTRODUCTION

Metabolic syndrome (MetS) is characterized by the clustering of metabolic abnormalities such as glucose intolerance, central obesity, hypertension, and dyslipidemia. Although there is no concurrence on a MetS definition for children and adolescents, the prevalence of MetS in obese children is documented to be approximately 30%^[1]. Since MetS components show stability from childhood to adulthood, children fulfilling diagnostic criteria for MetS may remain at high risk as they enter adulthood^[2]. MetS severity in childhood has been linked to future risk of type 2 diabetes and cardiovascular disease (CVD)^[3], indicating that implementing interventions for a healthy lifestyle early in childhood might be preventive against future development of MetS and its complications.

The primary goal for MetS management is to alleviate all metabolic risk factors through effective lifestyle changes^[4]. Research indicates healthy dietary patterns, such as the Mediterranean and Dietary approach to stop hypertension diets, all of which recommend high intakes of vegetables, improve MetS and type 2 diabetes^[5,6]; however, data on the associations between vegetable intakes as an individual dietary component and MetS, type 2 diabetes, and CVD remain inconsistent^[7-13]. Although one meta-analysis, including 26 observational studies, reported beneficial effects for consumption of vegetables on MetS^[7], another meta-analysis of randomized controlled trials found no effect on MetS components^[8]. Protective effects of vegetable consumption against other nutrition-related chronic diseases, such as type 2 diabetes, are also still not clear; some report an inverse association^[9], while others document none^[10,11]. Some others report a threshold of around two-three servings/day of vegetables, after which diabetes risk did not reduce further^[12,13]. This inconsistency of findings might probably be due to differences in the amounts and specific subgroups of vegetables in different studies. Biological effects of vegetables may vary due to their phytochemical profiles: Leafy vegetables as a source of nitrogen containing compounds, nitrate, and carotenoids, lutein, polyphenols, flavonoids, phenolic acids, and lignans^[14]; Cabbage as a source of polyphenols, flavonoids, phenolic acids, lignans; Allium vegetables as source of organosulfur compound, allyl cysteine, alliin,

allicin, and allyl disulfide, flavonoids, and phenolic compounds^[15]; and fruity vegetables as a source of carotenoids, lycopene and pro-vitamin A, beta-carotene^[7]. In addition, consuming a variety of vegetables, such as green leafy-, allium-, and cruciferous vegetables, has been inconsistently associated with CVD risk^[16-21] and type 2 diabetes^[9,11,22,23].

As mentioned, subgroups of vegetables may differ in nutritional content, energy, fiber, and phytochemicals^[24]. Considering the limited data available on the association of different types of vegetables with MetS, the aim of our study was to investigate the association between intake of various types of vegetables and MetS after 3.6 years of follow-up in Tehranian children and adolescents, aged 6-18 years.

MATERIALS AND METHODS

Subjects and methods

This prospective population-based study was conducted within the framework of the Tehran lipid and glucose study (TLGS), an ongoing, prospective community based study, aimed at preventing non-communicable disease and reducing its risk factors through promoting healthy lifestyles. Detailed characteristics of the TLGS have been described elsewhere^[25]. Briefly, phase I of the TLGS was initiated in March 1999. A multistage cluster sampling was used to randomly select > 15000 individuals, aged ≥ 3 years from residents of Tehran's urban district 13, a group representing the urban population of Tehran. Follow-up data are collected every 3 years to update participants, data on demographics, lifestyle, biochemical, clinical, and dietary assessments. Phases II, III, and IV are prospective follow-up studies conducted between 2002-2005, 2006-2008, and 2009-2011, respectively. The current prospective population-based study was conducted during a mean 3.6 years of follow-up (follow-up rate: 86%); baseline data were obtained from phase III of TLGS (2006-2008), and outcome examination data were from phase IV (2009-2011).

For the current study, of 12523 individuals who entered survey III of TLGS, based on age and gender, 4920 subjects were randomly selected for collection of nutritional data. This sample size was chosen owing to the cost, complexity, and time involved in collection of dietary data in a large population. Characteristics of participants who had complete dietary data were similar to those of the total population in phase III of the TLGS^[26]. Of the 4920 participants enrolled in the present study, 621 children and adolescents, aged 6-18 years, agreed to complete the food frequency questionnaire (FFQ). Those who had missing data on dietary intakes or MetS components ($n = 29$), those who had baseline MetS ($n = 69$), and participants who over- or under-reported ($n = 122$) were all excluded. According to the equation proposed by the institute of medicine^[27], by dividing the reported energy intake by the estimated energy requirement (EER), individuals who were not within the ± 3 SD range (those in the top and bottom 1% of the energy intake to EER ratio), were defined as under and over-reporters. Finally data of 424 participants was used for analysis (response rate 68% during the 3.6 years follow-up). Anthropometric and biochemical measurements of participants who provided follow-up assessments was similar to those lost to follow-up.

The study protocol was approved by the ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, and written informed consent was acquired from participants prior to their inclusion in the study.

Dietary assessment

Dietary intake information over the previous year was assessed using a 168-item, validated, semi-quantitative FFQ^[28]. Consumption frequency of each food item was documented on a daily, weekly, monthly basis, by a trained dietitian, during face to face interviews; portion sizes were reported in household measures and converted to gram using household measures^[29]. Percentages of carbohydrate, fat, and protein intake were calculated by multiplying the grams of consumption of each food by the content of carbohydrate, protein, and fat. The composition values were obtained from the US Department of Agriculture (USDA) Food Composition Table (FCT), as the Iranian FCT is incomplete (limited to only raw materials and a few nutrients). Vegetable consumption was assessed using 28 vegetables and reported as grams per day. General classification of our subgroups of vegetables (green leafy-, allium-, stalk-, fruity, root-, starchy-, cabbage, and potatoes) was based on Cooper *et al*^[9] and the classification used in the EPIC-InterAct study.

Reliability and validity of the FFQ for total vegetable consumption was acceptable (adjusted correlation coefficient between FFQ and multiple 24 recalls was 0.69 and 0.50, respectively; between the two FFQs it was 0.46 and 0.50 in males and females,

respectively)^[28]. Also, dietary patterns derived from the FFQ have shown reasonable reliability, validity, and stability over time^[30].

Biochemical measurement

Blood samples were drawn after 12-14 h of overnight fasting, between 7:00-9:00 a.m. from all participants, at baseline and follow-up. All blood analyses were done at the TLGS research laboratory on the day of sample collection. The enzymatic colorimetric method using glucose oxidase was used to measure fasting plasma glucose (FPG). High density lipoprotein cholesterol (HDL-C) was measured after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. Using the enzymatic colorimetric method with glycerol phosphate oxidase, triglycerides (TG) concentrations were measured. All analyses were performed using commercial kits (Pars Azmoon Inc., Tehran, Iran). Inter- and intra-assay coefficients of variations at baseline were both 2.2% for FPG, 2% and 0.5% for HDL-C, and 1.6% and 0.6% for TG, respectively.

Assessment of other variables

Blood pressure was measured manually twice using a standard mercury sphygmomanometer after a 15 min rest in supine position, and the mean of two measurements was considered as the participants' blood pressure. Height was measured without shoes using a stadiometer, while participants were standing with shoulders in normal alignment, and recorded to the nearest 0.1 cm. Weight was measured without shoes, with participants wearing light clothes, using a digital scale, and recorded to the nearest 0.1 kg (Seca 707; Seca Corporation, Hanover, MD, United States; range 0.1-150 kg). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). Waist circumference (WC) was measured at the midpoint between the iliac crest and lowest rib, recorded to 0.1 cm, in a standing position using an unstretched elastic tape. Physical activity level was calculated to metabolic equivalent task minutes per week, using the modifiable activity questionnaire, the high reliability (97%) and moderate validity (49%) of which have been verified previously for the Persian translated modifiable activity questionnaire in adolescents^[31]. Levels of physical activity were expressed as metabolic equivalent hours per week. Smoking history was collected using a questionnaire and categorized as smokers (smoked > 1 cigarette per day) and non-smoker/ex-smoker. Medical history and data on current use of medications, family history of diabetes, age, and gender were obtained using a questionnaire, as reported previously^[25].

Definition of MetS

Since there is no consensus regarding the criteria and definition of MetS for children and adolescents, the Cook *et al.*^[32] proposed definition, which defines MetS as ≥ 3 of the following criteria, was used: (1) TG ≥ 110 mg/dL or; (2) HDL-C < 40 mg/dL; (3) FPG ≥ 100 mg/dL, according to the recent recommendations of the American Diabetes Association^[33]; (4) Systolic blood pressure or diastolic blood pressure ≥ 90 th percentile for sex, age, and height, from the cut off points recommended by the National Heart, Lung, and Blood Institute^[34]; and (5) WC ≥ 90 th percentile for age and sex, according to national reference curves^[35]. Pre-MetS has been defined as having two components of these criteria^[36].

For individuals aged > 18 years after follow-up, MetS was defined as the presence of three or more of five components, as recommended by the Joint Interim Statement of the International Diabetes Federation as follows: (1) Low serum HDL cholesterol (< 40 mg/dL in men and < 50 mg/dL in women); (2) Abnormal glucose homeostasis (FPG ≥ 100 mg/dL or use of hyperglycemic medication); (3) Elevated blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg or use of antihypertensive medication); (4) High serum triglyceride concentration (≥ 150 mg/dL or use of antihypertriglyceridemia medication); and (5) Enlarged WC (≥ 95 cm according to the newly introduced cut-off points for Iranian adults for both genders)^[37,38].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (v. 15.0; SPSS, Chicago, IL, United States), with statistical significance set at as $P < 0.05$. Consumption of total- and various types of vegetables was categorized into quartiles according to their baseline intakes. One-way analysis of variance and chi-squared test were used to report baseline characteristics and dietary intakes (adjusted for energy intakes) in total vegetable categories; data are presented as means \pm standard error and median [interquartile range (IQR)] for continuous variables, and percentages for categorical variables. Odds ratio (ORs) and 95% confidence intervals (CIs) of incident MetS were estimated across quartiles of total- and different types of vegetable consumption using

the logistic regression model, with the first quartile being used as a reference. Model 1 was crude. Model 2 was adjusted for age, gender, physical activity, family history of diabetes, total energy-, and cholesterol intake at baseline. Model 3 was additionally adjusted for BMI at baseline. Tests for trend of ORs across quartiles of total and various types of vegetables were conducted by assigning the median value to each quintile as a continuous variable in the regression models. By multivariable regression models, we further performed stratified analysis by categories of number of components of MetS (0, 1, or 2 components of MetS) at baseline to estimate ORs of MetS based intake of total and various types of vegetable consumption (above/below the medians).

RESULTS

Among 424 children and adolescents free of MetS at baseline, 47 (11%) were diagnosed with MetS during a median follow-up period of 3.6 years. Participants (58% girls) were aged 13.5 (SD 3.7) years at baseline with BMI 20.0 (SD 3.8) kg/m². Median \pm IQR of total vegetables consumption was 217 (146-344) g/d. At baseline, the median (IQR) from highest to lowest range of various types of vegetable consumption was: Fruity vegetables 115 (69.5-211.0), leafy vegetables 21.4 (13.4-38.4), allium vegetables 17.6 (6.3-30.5), potatoes 10.3 (4.8-20.7), root vegetables 9.9 (4.2-22.3), other starchy vegetables 5.8 (3.3-12.6), cabbage 3.1 (0.0-6.2), and stalk vegetables 0.45 (0.0-1.1).

Table 1 presents the characteristics of participants according to quartiles of total vegetable consumption. Participants in the highest quartile had low systolic blood pressure, low fasting blood glucose, and high HDL cholesterol concentrations at baseline. After 3.6 years of follow-up, FBS was significantly lower and HDL cholesterol significantly higher among those in the highest quartile of total vegetable consumption, compared with those in the lowest. No statistically significant associations were found for age, gender, physical activity levels, family history of diabetes, parental education level and occupational status, and BMI. The prevalence of subjects with 0, 1, and 2 components of MetS did not differ across quartiles of vegetable consumption. There was no significant difference in total vegetable consumption [median (IQR)] between participants with 0 [206 (145-322 g/d)], 1 [217 (144-351 g/d)], and 2 components of MetS [226 (161-345 g/d)] at baseline.

Table 2 presents energy adjusted means for dietary components across quartiles of total vegetable consumption. Participants who consumed more vegetables, also consumed more of types of vegetables. Consequently, those with a high intake of vegetables consumed more total fiber, cholesterol, magnesium, potassium, fruit, nuts, and dairy products.

Table 3 presents ORs of MetS associated with the total and various types of vegetables among children and adolescents. Compared to the first quartile, a higher consumption of total- (≥ 350 g/d) and allium (≥ 30 g/d) vegetables in the fourth quartile was significantly and inversely associated with risk of MetS in unadjusted model (model 1), the adjusted model for demographic characteristics and dietary intake (model 2), and BMI (model 3 in allium vegetables). Consumption of green leafy vegetables in the third (21.4-38.3 g/d) *versus* the first quartile (≤ 13.5 g/d) was significantly and inversely associated with risk of MetS in the unadjusted (model 1) and the model adjusted for demographic characteristics and dietary intakes (model 2); further adjustment for BMI attenuated these associations. Among vegetables, fruity-, root-, stalk-, starchy-, cabbage, and potatoes, were not associated with MetS among children and adolescents.

Table 4 presents ORs for MetS based intake of total and various types of vegetable consumption (above/below the medians) among participants with 0, 1, or 2 components of MetS at baseline. Among participants with 1 component of MetS, green leafy- and allium vegetables reduced the risk of MetS by 71% (0.23, 95%CI: 0.07-0.71) and 77% (0.29, 95%CI: 0.07-0.71), after adjustment for confounding factors. No association was found between total-, fruity-, root-, stalk-, starchy vegetables, cabbage, and potatoes and risk of MetS among participant with ≥ 2 components at baseline.

DISCUSSION

This prospective study of Iranian children and adolescents reported an inverse association between total vegetable consumption and MetS risk. Among vegetable subgroups, consumption of green leafy- and allium vegetables was inversely

Table 1 Baseline characteristics of participants by quartiles of total vegetable consumption: Tehran lipid and glucose study

	Quartiles of total vegetable consumption				P ¹ value
	1	2	3	4	
Participants, <i>n</i>	106	106	106	106	
Number of components of MetS at baseline, <i>n</i>					
No component	42	45	45	49	0.91
1 component	40	41	40	47	
2 components, pre-MetS	24	20	21	10	
Metabolic syndrome after 3.6 yr, <i>n</i>	17	12	10	8	
Age, yr	13.1 ± 0.3	13.3 ± 0.3	13.7 ± 0.3	13.9 ± 0.3	0.31
Female, %	53.8	65.1	54.7	56.6	0.32
Low physical activity, %	61	73	69	52	0.74
Family history of diabetes, %	4.7	8.5	3.8	3.8	0.37
BMI, kg/m ²	19.6 ± 0.4	20.7 ± 0.3	20.0 ± 0.3	19.8 ± 0.3	0.18
Parental academic degrees, %	25.2	26.9	22.8	25.2	0.48
Parental occupational status, employed, %	83.6	87.4	81.7	82.2	0.39
Systolic blood pressure, mmHg					
At baseline	99.1 ± 1.1	98.1 ± 1.1	96.2 ± 1.1	95.0 ± 1.1	0.04
After 3.6 yr	101 ± 1.2	100.2 ± 2	101.2 ± 1.2	98.8 ± 1.2	0.44
Diastolic Blood pressure, mmHg					
At baseline	65.1 ± 0.9	65.0 ± 0.9	63.9 ± 0.9	64.3 ± 0.9	0.76
After 3.6 yr	66.5 ± 0.9	68.4 ± 0.9	68.2 ± 0.9	69.0 ± 0.9	0.27
Fasting blood glucose, mg/dL					
At baseline	86.6 ± 0.6	84.3 ± 0.6	85.1 ± 0.6	84.2 ± 0.6	0.036
After 3.6 yr	91.8 ± 0.7	89.8 ± 0.7	89.3 ± 0.7	88.9 ± 0.7	0.037
Serum triglycerides, mg/dL					
At baseline	78.0 (60.0 – 95.0)	83.0 (63.0 – 100.0)	83.0 (64.0 – 103.0)	78.5 (64.0 – 105.0)	0.64
After 3.6 yr	79.0 (60.7 – 109.0)	73.0 (60.0 – 101.0)	74.0 (60.0 – 96.0)	77.0 (62.5 – 98.5)	0.58
Serum HDL-C, mg/dL					
At baseline	45.2 ± 1.1	44.8 ± 1.1	44.8 ± 1.1	48.5 ± 1.1	0.030
After 3.6 yr	50.5 ± 1.2	50.0 ± 1.2	51.3 ± 1.2	55.1 ± 1.2	0.014
Waist circumference, cm					
At baseline	67.3 ± 1.0	68.2 ± 1.0	69.5 ± 1.0	70.3 ± 1.0	0.16
After 3.6 yr	76.1 ± 1.0	77.0 ± 1.0	77.9 ± 1.0	77.6 ± 1.0	0.62

¹mean ± SE for all these values, except for variables was determined. Serum triglycerides reported as median (interquartile range). *P* values determined using ANOVA for continuous variables and chi-square test for categorical variables. BMI: Body mass index; MetS: Metabolic syndrome; HDL-C: High density lipoprotein cholesterol.

associated with risk of MetS after adjustment for the main potential confounders. In addition, we found that among participants with 1 component of MetS, consumption of allium- and green leafy vegetables reduce risk of MetS. However limited sample size in participants with 0 and 2 components of MetS may influence the association between consumption of vegetables and MetS.

Although the effects of vegetable intake on MetS have been investigated by numerous epidemiological and interventional studies, most findings remain inconclusive^[7,8]. Furthermore, in relation to type 2 diabetes, the efficacy of vegetable consumption is still not clear; some studies report inverse associations^[9], while others report none^[10,11]. Another documented a threshold of around two-three servings/day of vegetables, after which diabetes and CVD risk did not reduce further^[12,13,16]. In the current study, we found that consumption of vegetables, median intake of 440 g/wk, reduced risk of MetS by 60% among children and adolescents, findings consistent with dietary pattern studies in which healthy diets rich in vegetables are associated with a reduced risk of MetS and its components in children and adolescents^[39,40]. However, in a comprehensive systematic review of studies addressing fruit and vegetable consumption and cardiovascular risk indicators in adolescents, only one-third of the studies showed significant inverse associations of fruit and vegetable

Table 2 Baseline dietary intakes of participants by quartiles of total vegetable consumption: Tehran lipid and glucose study

	Quartiles of total vegetable consumption				P value
	1	2	3	4	
Fruity vegetables, g/d	57.5 ± 9.2	99.2 ± 9.2	156 ± 9.2	293 ± 9.2	< 0.001
Root vegetables, g/d	7.9 ± 1.9	14.8 ± 1.9	18.7 ± 1.9	27.3 ± 1.9	< 0.001
Stalk vegetables, g/d	0.7 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.7 ± 0.2	0.002
Leafy vegetables, g/d	19.1 ± 2.3	24.2 ± 2.3	30.8 ± 2.2	44.3 ± 2.4	< 0.001
Potatoes, g/d	9.7 ± 2.8	13.6 ± 2.7	17.7 ± 2.7	28.8 ± 2.9	< 0.001
Starchy vegetables, g/d	7.6 ± 1.3	8.2 ± 1.2	13.4 ± 1.2	11.3 ± 1.3	0.003
Cabbage, g/d	2.4 ± 1.5	5.1 ± 1.4	8.6 ± 1.4	12.8 ± 1.5	< 0.001
Allium vegetables, g/d	13.7 ± 2.4	18.0 ± 2.3	25.3 ± 2.3	41.4 ± 2.4	< 0.001
Total energy, Kcal/d	1981 ± 113	2171 ± 113	2703 ± 113	3383 ± 113	< 0.001
Carbohydrate, % of total energy	56.5 ± 0.7	56.7 ± 0.7	57.2 ± 0.7	57.3 ± 0.7	0.82
Protein, % of total energy	13.2 ± 0.2	12.8 ± 0.2	13.1 ± 0.2	13.4 ± 0.2	0.33
Fat, % of total energy	32.4 ± 0.7	32.5 ± 0.7	32.3 ± 0.7	32.1 ± 0.7	0.98
SFA, % of total energy	11.4 ± 0.3	11.1 ± 0.3	10.9 ± 0.3	11.1 ± 0.3	0.64
MUFA, % of total energy	11.2 ± 0.3	11.0 ± 0.3	11.2 ± 0.3	11.1 ± 0.3	0.92
PUFA, % of total energy	6.5 ± 0.2	6.6 ± 0.2	6.7 ± 0.2	6.5 ± 0.2	0.90
Total fiber, g/d	27.4 ± 2.4	30.5 ± 2.4	42.0 ± 2.4	49.6 ± 2.4	< 0.001
Cholesterol, g/d	190 ± 14	205 ± 14	267 ± 14	316 ± 14	< 0.001
Magnesium, mg/d	301 ± 20	324 ± 20	433 ± 20	547 ± 20	< 0.001
Potassium, mg/d	2835 ± 171	3127 ± 171	4228 ± 171	5588 ± 171	< 0.001
Whole grain, g/d	90 ± 16	92 ± 16	94 ± 16	92 ± 16	0.251
Refined grain, g/d	356 ± 26	354 ± 26	359 ± 26	351 ± 26	0.632
Fruit, g/d	272 ± 31.6	308 ± 31.6	453 ± 31.6	609 ± 31.6	< 0.001
Nuts	6.9 ± 1.4	7.9 ± 1.3	9.8 ± 1.3	14.5 ± 1.3	0.001
Legumes	11.9 ± 2.2	12.0 ± 2.2	13.9 ± 2.2	14.5 ± 2.2	0.879
Dairy products	146 ± 39.7	517 ± 39.7	609 ± 39.7	704 ± 39.7	0.005
Meat, poultry, fish, g/d	53.8 ± 10.1	56.1 ± 10.1	59.1 ± 10.1	52.7 ± 10.1	0.548

Data are mean and standard error, adjusted for energy intakes. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

intake and MetS and its components^[41]. This inconsistency may be because of differences in the amounts and specific subgroups of vegetables intake in different studies. More prospective and interventional studies are needed to specify the effects of various types of vegetables.

The association between green leafy vegetables and nutrition-related chronic disease was less consistent in prospective studies. In the “CHANCES” project, results from NIH-AARP and EPIC Elderly (All, Greece) cohorts reported consumption of green leafy vegetables is associated with a reduced [OR: 0.87 (0.84–0.90)] and increased [OR: 1.23 (1.01–1.50), OR: 1.52 (1.13–2.04)] risk of type 2 diabetes, respectively. The pooled analysis indicated no overall association between intake of green leafy vegetables, type 2 diabetes^[42], and CVD risk^[16]. Zhang *et al*^[7], in a meta-analysis of observational studies, concluded that consumption of green leafy vegetables might not be associated with MetS risk. Despite the results given above, several different meta-analyses suggest that green leafy vegetable consumption significantly reduced risk of type 2 diabetes and CVD^[9,11,16,18]; an increase of 0.2 serving/d of green leafy vegetables was associated with 13% reduction in type 2 diabetes^[23]. Our study showed that although consumption of green leafy vegetables significantly reduced risk of MetS to a median intake of 27 g/wk, there was a threshold of around 30 g/d, after which this inverse association disappeared; similar reductions of associated risk until the last median intake was similarly reported in two previous cohort studies^[22,23]. In the Women’s Health Study (median intake 0.92 serving/d), and the Shanghai Women’s Health Study (median intake 94.1 g/d), type 2 diabetes risk reduced up to the fourth quartile of intake but not further^[22,23]. Green leafy vegetables contain a maximum amount of nitrate, thus a risk-benefit effect of

Table 3 Multivariate adjusted odds ratio (95%CI) for metabolic syndrome across quartiles of total and various types of vegetable consumption: Tehran lipid and glucose study

	Quartiles of total- and various types of vegetable consumption				P value forTren- d ¹
	1	2	3	4	
Total vegetables					
Median intake, g/d	104	179	265	441	
Range of intake, g/d	≤ 146	147-217	218-343	≥ 350	
Model 1	1	0.66 (0.30-1.47)	0.54 (0.23-1.25)	0.42 (0.17-0.92)	0.06
Model 2	1	0.54 (0.21-1.46)	0.42 (0.16-1.07)	0.36 (0.14-0.94)	0.04
Model 3	1	0.53 (0.20-1.46)	0.41 (0.15-1.10)	0.35 (0.13-0.95)	0.04
Allium vegetables					
Median intake, g/d	2.7	10.8	22.8	51.5	
Range of intake, g/d	≤6.2	6.3-17.3	17.4-30.5	≥ 30.6	
Model 1	1	0.68 (0.31-1.47)	0.44 (0.19-1.05)	0.34 (0.13-0.87)	0.024
Model 2	1	0.55 (0.23-1.31)	0.27 (0.11-0.74)	0.21 (0.11-0.64)	0.006
Model 3	1	0.56 (0.24-1.32)	0.35 (0.13-0.89)	0.27 (0.14-0.75)	0.012
Green leafy vegetables					
Median intake, g/d	9.1	17.6	27.3	54.4	
Range of intake, g/d	≤13.5	13.6-21.3	21.4-38.3	≥ 38.4	
Model 1	1	0.42 (0.18-0.98)	0.32 (0.13- 0.80)	0.58 (0.26-1.27)	0.37
Model 2	1	0.40 (0.16-1.02)	0.32 (0.12-0.89)	0.81 (0.32-2.01)	0.98
Model 3	1	0.42 (0.16-1.11)	0.40 (0.13-0.91)	1.12 (0.39-3.19)	0.51
Fruity vegetables					
Median intake, g/d	42	93	145	263	
Range of intake, g/d	≤ 69	70-115	116-211	≥ 212	
Model 1	1	0.61 (0.25-1.47)	0.61 (0.25-1.47)	1.08 (0.49-2.37)	0.69
Model 2	1	0.65 (0.25-1.65)	0.64 (0.25-1.65)	1.01 (0.38-2.68)	0.34
Model 3	1	0.71 (0.27-1.87)	0.84 (0.32-2.23)	1.65 (0.57-4.80)	0.44
Root vegetables					
Median intake, g/d	2.1	7.3	10.0	34.4	
Range of intake, g/d	≤ 4.2	4.3-9.9	10.0-21.9	≥ 22.0	
Model 1	1	0.57 (0.20-1.64)	2.23 (0.99-5.03)	1.11 (0.45-2.74)	0.89
Model 2	1	0.72 (0.24-2.12)	1.74 (0.72-4.17)	1.13 (0.43-2.97)	0.87
Model 3	1	0.68 (0.22-2.07)	1.88 (0.75-4.72)	1.45 (0.50-4.13)	0.51
Stalk vegetables					
Median intake, g/d	0.0	0.2	1.0	2.5	
Range of intake, g/d	≤ 0.00	0.1-0.5	0.6-1.1	≥ 1.2	
Model 1	1	0.57 (0.24-1.36)	0.54 (0.23-1.29)	0.88 (0.40-1.94)	0.86
Model 2	1	0.52 (0.20-1.36)	0.51 (0.20-1.31)	1.09 (0.46-2.58)	0.51
Model 3	1	0.61 (0.23-1.62)	0.57 (0.21-1.51)	1.32 (0.52-3.34)	0.34
Potatoes					
Median intake, g/d	2.4	10.3	20.7	36.3	
Range of intake, g/d	≤4.8	4.9-10.4	10.5-20.8	≥ 20.9	
Model 1	1	0.69 (0.32-1.50)	0.97 (0.41- 2.28)	0.80 (0.32-1.99)	0.84
Model 2	1	0.54 (0.23- 1.27)	0.86 (0.34-2.21)	0.68 (0.25-1.85)	0.72
Model 3	1	0.58 (0.23-1.44)	1.03 (0.38- 2.76)	0.55 (0.17-1.68)	0.54
Starchy vegetables					
Median intake, g/d	1.9	4.7	8.2	18.2	
Range of intake, g/d	≤ 3.3	3.4-5.8	5-12.6	≥ 12.7	
Model 1	1	0.91 (0.39-2.11)	1.0 (0.44-2.27)	0.66 (0.27-1.62)	0.36
Model 2	1	0.92 (0.37-2.31)	0.93 (0.38-2.26)	0.60 (0.22-1.62)	0.29
Model 3	1	0.99 (0.38-2.56)	0.80 (0.30-2.13)	0.80 (0.26-2.45)	0.41
Cabbage					

Median intake, g/d	0.0	1.9	6.2	15.3	
Range of intake, g/d	≤ 0.0	0.1 – 3.1	3.2-6.2	≥ 6.3	
Model 1	1	0.74 (0.36-1.52)	1.12 (0.40-3.14)	0.54 (0.21-1.38)	0.18
Model 2	1	0.79 (0.36-1.71)	1.25 (0.42-3.79)	0.51 (0.18-1.45)	0.07
Model 3	1	0.94 (0.42-2.10)	1.50 (0.48-4.69)	0.56 (0.18-1.71)	0.09

¹The median intake of each quartile category was assigned, and these quartile median variables were included as a continuous variable in logistic regression. Model 1 was crude. Model 2 was adjusted for age at baseline, gender, physical activity at baseline, family history of diabetes, total energy intake at baseline, and cholesterol intake at baseline. Model 3 was additionally adjusted for body mass index at baseline. CI: Confidence interval.

nitrate should be considered. The adequate intake for nitrate (3.7 mg/kg body weight/day equivalent to 222 mg nitrate per day for a 60 kg adult) is constantly exceeded in several dietary patterns. This overconsumption of dietary nitrate could hence be responsible for the disappearance of an inverse association between green leafy vegetable intake and MetS and the risk of type 2 diabetes after the third quartile of intake in our study and those of others^[43].

In the current study, allium vegetables were inversely associated with risk for MetS among children and adolescents. Positive effects of *A. sativum* on control of MetS and its components, especially dyslipidemia and diabetes, have been reported previously^[44]. Moreover, several prospective studies have been conducted regarding the positive effect of allium vegetables on CVD outcomes, chronic kidney disease, hypertension, ischemic heart disease mortality, cerebral vascular disease mortality, and myocardial infarction^[17,19,20] but not all^[21,45]. Allium is a rich source of phytonutrients, including organosulfur and phenolic compounds; their consumption two to three times per day improves the cardio-metabolic risk factors among individuals with diabetes and MetS, reduces inflammation and oxidative stress, and has a vasodilator effect^[46-49].

Few studies have investigated the association between cruciferous vegetables and MetS risk, and most of these report that dietary patterns rich in vegetables, including cruciferous, reduce the occurrence of MetS^[50,51]. Although our findings and also those of some previous studies found no protective effects of cruciferous vegetables^[21,45], others have reported a potential reduction in the risk of ischemic heart disease mortality, coronary heart disease, and subclinical atherosclerosis, which may be due to the lower amount of cruciferous vegetable intake in our study compared to others^[16,17].

In the current study, no association between fruity-, root-, stalk-, starchy vegetables, and potatoes and MetS was found. Results of research on the consumption of these vegetables and the risk of MetS is controversial, which may be due to the differences in the amounts and preparation methods used in these studies^[16,17,21,23,45,52-55]. Further investigations of various types of vegetable (*e.g.*, fruity-, starchy-, and root vegetables) consumption on MetS are needed.

Strengths and limitations

Major strengths of this study include the population-based prospective design of the study, participants of which were representative of Tehran's population, the large sample size, and use of a valid and reliable FFQ administered by a trained nutritionist (reducing any potential measurement errors), and the minimized loss to follow-up bias (response rate 86%). Nevertheless, the present study has a few limitations. First, MetS is heterogeneous; despite carefully adjusting for a range of known confounding factors, other factors associated with MetS among children and adolescents, including heredity and puberty status, were not addressed because of a lack of information. In addition, regarding the different types of vegetables (other than allium and green leafy vegetables) included in the analysis of this study, the failure to detect associations between consumption of these types of vegetables and MetS may be due to the narrow range of dietary intake among our participants.

In conclusion, findings of this prospective study indicate that consumption of green leafy vegetables and allium vegetables were associated with lower risk of MetS during 3 years of follow-up in children and adolescents.

Table 4 Multivariable odds ratio (95%CI) for metabolic syndrome based intake of total and various types of vegetable consumption (above/below the medians) among participants with 0, 1, or 2 components of metabolic syndrome at baseline: Tehran lipid and glucose study

	Model 1		Model 2		Model 3	
	< median	≥ median	< median	≥ median	< median	≥ median
Total vegetables, g/d						
0 component	1	0.48 (0.12-1.94)	1	0.77 (0.14-4.05)	1	0.82 (0.15-4.29)
1 component	1	0.68 (0.27-1.71)	1	0.62 (0.21-1.83)	1	0.67 (0.23-1.99)
2 component	1	1.98 (0.61-6.42)	1	2.00 (0.49-8.18)	1	2.24 (0.53-9.39)
Allium vegetables						
0 component	1	0.77 (0.21-2.85)	1	0.69 (0.17-2.76)	1	0.54 (0.12-2.42)
1 component	1	0.25 (0.08-0.73)	1	0.25 (0.08-0.76)	1	0.23 (0.07-0.71)
2 component	1	0.49 (0.16-1.52)	1	0.51 (0.15-1.78)	1	0.48 (0.13-1.69)
Green leafy vegetables						
0 component	1	0.81 (0.22-2.98)	1	1.04 (0.25-4.26)	1	1.10 (0.25-4.72)
1 component	1	0.31 (0.11-0.84)	1	0.31 (0.09-0.97)	1	0.29 (0.09-0.95)
2 component	1	1.01 (0.33-3.08)	1	1.43 (0.39-5.18)	1	1.39 (0.38-5.06)
Fruity vegetables						
0 component	1	1.13 (0.31-4.07)	1	1.15 (0.28-4.71)	1	0.88 (0.20-3.84)
1 component		0.66 (0.26-1.67)	1	0.60 (0.21-1.69)	1	0.64 (0.22-1.82)
2 component		1.51 (0.48-4.67)	1	2.02 (0.52-7.87)	1	2.11 (0.53-8.32)
Root vegetables						
0 component	1	2.36 (0.59-9.43)	1	2.57 (0.58-11.3)	1	2.31 (0.49-10.7)
1 component	1	2.20 (0.84-5.76)	1	1.68 (0.61-4.75)	1	1.67 (0.58-4.73)
2 component	1	1.97 (0.63-6.14)	1	1.48 (0.43-5.08)	1	1.31 (0.36-4.69)
Stalk vegetables						
0 component	1	1.28 (0.35-4.72)	1	1.33 (0.33-5.30)	1	1.08 (0.26-4.52)
1 component	1	0.72 (0.28-1.81)	1	0.73 (0.27-2.00)	1	0.74 (0.27-2.02)
2 component	1	1.18 (0.39-3.58)	1	1.09 (0.32-3.76)	1	1.13 (0.32-3.97)
Potatoes						
0 component	1	1.36 (0.34-5.45)	1	1.97 (0.39-9.90)	1	2.12 (0.39-11.4)
1 component	1	0.56 (0.22-1.42)	1	0.45 (0.17-1.22)	1	0.45 (0.16-1.24)
2 component	1	1.91 (0.55-6.66)	1	1.35 (0.34-5.39)	1	1.55 (0.36-6.65)
Starchy vegetables						
0 component	1	0.64 (0.17-2.36)	1	0.92 (0.21-3.90)	1	1.06 (0.24-4.60)
1 component	1	0.78 (0.31-1.96)	1	0.80 (0.29-2.18)	1	0.84 (0.30-2.32)
2 component	1	1.08 (0.35-3.30)	1	0.57 (0.15-2.11)	1	0.56 (0.15-2.14)
Cabbage						
0 component	1	0.64 (0.13-3.12)	1	0.88 (0.16-4.92)	1	0.89 (0.15-5.08)
1 component	1	1.19 (0.46-3.07)	1	1.64 (0.59-4.59)	1	1.54 (0.55-4.32)
2 component	1	0.48 (0.12-1.91)	1	0.53 (0.18-2.43)	1	0.45 (0.09-2.22)

Median intake of: Model 1 was crude; Model 2 was adjusted for, age at baseline, gender, physical activity at baseline, family history of diabetes, total energy intake at baseline, and cholesterol intake at baseline; Model 3 was additionally adjusted for body mass index at baseline. CI: Confidence interval.

ARTICLE HIGHLIGHTS

Research background

Metabolic syndrome (MetS) is characterized by the clustering of metabolic abnormalities, such as glucose intolerance, central obesity, hypertension, and dyslipidemia. The primary goal for MetS management is to alleviate all metabolic risk factors through effective lifestyle changes. Research indicates healthy dietary patterns, such as the Mediterranean and Dietary approach to stop hypertension diets, all of which recommend high intakes of vegetables, improve MetS and type 2 diabetes; however, data on the associations between vegetable intake as an individual dietary component and MetS remain inconsistent.

Research motivation

This inconsistency of findings between chronic disease such as MetS and vegetable consumption might probably be due to differences in specific subgroups of vegetables in different studies. Various types of vegetables differ in nutritional content, energy, fiber, and phytochemicals.

Research objectives

The aim of our study was to investigate the association between intake of various types of vegetables and MetS after 3.6 years of follow-up in Tehranian children and adolescents, aged 6-18 years.

Research methods

This prospective population-based study was conducted within the framework of the Tehran lipid and glucose study, an ongoing, prospective community based study, aimed at preventing non-communicable disease and reducing its risk factors through promoting healthy lifestyles. Of the 4920 participants enrolled in the present study, 621 children and adolescents, aged 6-18 years agreed to complete the food frequency questionnaire. Those who had missing data on dietary intake or MetS components, those who had baseline MetS, and participants who over- or under-reported, were all excluded. Finally, data of 424 participants were used for analysis. Dietary intake information over the previous year was assessed using a 168-item, validated, semi-quantitative food frequency questionnaire. Vegetable consumption was assessed using 28 vegetables and reported as grams per day. General classification of our subgroups of vegetables (green leafy-, allium-, stalk-, fruity-, root-, starchy-, cabbage, and potatoes) was based on EPIC-InterAct study. Blood samples were drawn after 12-14 h of overnight fasting and glucose, triglyceride and HDL-C concentrations were measured. Blood pressure and waist circumference were assessed using standard tools. MetS was defined using Cool *et al* criteria for individual <18 year. For participant aged ≥ 18 years, Joint Interim Statement of the International Diabetes Federation criterial was used to define the MetS.

Research results

Among 424 children and adolescents free of MetS at baseline, 47 (11%) were diagnosed with MetS during a median follow-up period of 3.6 years. Higher consumption of total- (≥ 350 g/d) and allium (≥ 30 g/d) vegetables were significantly and inversely associated with risk of MetS after adjustment for confounding factors. Consumption of green leafy vegetables in the third (21.4-38.3 g/d) *versus* the first quartile (≤ 13.5 g/d) was significantly and inversely associated with risk of MetS in the unadjusted (model 1) and the model adjusted for demographic characteristics and dietary intakes (model 2); further adjustment for BMI attenuated these associations. Among vegetables, fruity-, root-, stalk-, starchy-, cabbage, and potatoes were not associated with MetS among children and adolescents.

Research conclusions

Consumption of green leafy vegetables and allium vegetables were associated with lower risk of MetS during 3 years of follow-up in children and adolescents.

Research perspectives

Future studies addressing the underlying mechanisms of allium and green leafy vegetables in reducing the risk of MetS are needed.

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Recent advances and perspectives in next generation sequencing application to the genetic research of type 2 diabetes

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Abstract

Type 2 diabetes (T2D) mellitus is a common complex disease that currently affects more than 400 million people worldwide and has become a global health problem. High-throughput sequencing technologies such as whole-genome and whole-exome sequencing approaches have provided numerous new insights into the molecular bases of T2D. Recent advances in the application of sequencing technologies to T2D research include, but are not limited to: (1) Fine mapping of causal rare and common genetic variants; (2) Identification of confident gene-level associations; (3) Identification of novel candidate genes by specific scoring approaches; (4) Interrogation of disease-relevant genes and pathways by transcriptional profiling and epigenome mapping techniques; and (5) Investigation of microbial community alterations in patients with T2D. In this work we review these advances in application of next-generation sequencing methods for elucidation of T2D pathogenesis, as well as progress and challenges in implementation of this new knowledge about T2D genetics in diagnosis, prevention, and treatment of the disease.

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Core tip: Next-generation sequencing (NGS) technologies have a broad range of applications in studying the genetic causes of type 2 diabetes (T2D), such as: (1) Identification of rare and common genetic variants, associated with disease; (2) Functional studies for describing role of genes in disease pathogenesis; and (3) Evaluation of environmental contribution to the disease by using microbiome profiling methods. This review of NGS application to the genetic research of T2D presents the advances and challenges related with sequencing analysis-based studies and implementation of this knowledge in clinical practice.

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INTRODUCTION

Type 2 diabetes (T2D) mellitus is a common complex disease that currently affects more than 400 million people throughout the world, and it is projected 552 million cases of T2D by the year 2030^[1]. The disease is characterized by insulin resistance and beta-cell dysfunction and can seriously impair overall quality of life^[2]. T2D may lead to increased risk of cardiovascular disease, stroke, kidney failure and can result in lower life expectancy by 5-10 years^[3-5]. T2D etiology is known to have a significant genetic component that is confirmed by family- and twin-based studies. The risk of the disease developing is approximately 70% when both parents have T2D and approximately 40% when one parent has disease^[6]. Twin studies have shown that the heritability of T2D ranges from 26% to 73%, and the concordance rate for T2D in monozygotic twins can reach 76%^[7]. Early identification of individuals at high T2D risk enables delay or prevention of T2D onset through effective lifestyle and/or pharma-cological interventions and has been shown to reduce costs of healthcare that causes continuing strong interest in revealing risk markers of T2D^[8,9].

The development of high-throughput and affordable genotyping technologies, statistical tools and computational software has allowed remarkable progress over the past decade in the search for genetic associations. Since the first genome-wide association study (GWAS) for T2D identified novel susceptibility loci in 2007, more than 100 T2D susceptibility loci have been discovered^[10]. Next-generation sequencing (NGS) technologies have a broad range of applications in studying the genetic causes of T2D, such as: (1) Identification of rare and common genetic variants, associated with disease; (2) Functional studies for describing role of genes in disease pathogenesis; and (3) Evaluation of environmental contribution to the disease by using microbiome profiling methods. However, it remains uncertain if and to what extent our increasing knowledge of genetic and epigenetic T2D risk factors gained by NGS methods will translate into clinical practice.

The aim of this article is to summarize recent progress and discoveries for T2D genetics focusing on the sequencing analysis-based studies and review the challenges in studying the genetic basis of T2D in order to improve diagnosis, prevention, and treatment.

T2D SUSCEPTIBILITY LOCI IDENTIFIED BEFORE THE ERA OF GWAS

The earliest genetic studies of T2D susceptibility focused on family-based linkage analysis and analysis of candidate genes in small-size groups of patients. This approach was successful in identifying familial genetic variants with large effects such as those involved in monogenic forms of the disease. In the past two decades,

numerous candidate gene studies have been performed to identify genetic variants for T2D. However only 4 genetic markers identified in these studies have been confirmed later by GWAS. The first genetic variant for T2D was the P12A polymorphism (rs1801282) in peroxisomal proliferator activated-receptor gamma gene (*PPARG*)^[11]. Then, in 2003, in a large-scale association study the previously identified association between the E23K (rs5219) polymorphism in a gene encoding inwardly rectifying potassium channel subfamily J, member 11 (*KCNJ11*) and T2D was replicated^[12]. E23K can alter function by inducing spontaneous over-activity of pancreatic β -cells, thus increasing the threshold ATP concentration for insulin release^[13]. In previous studies a polymorphism in this genes (*KCNJ11* E23K) has been reported to be associated with T2D in several populations, although the data was inconsistent^[14-18]. Transcription factor 7-like 2 (T-cell specific, HMG-box) (*TCF7L2*) was shown to be associated with T2D^[19]. *TCF7L2* gene product is a member of the high mobility group box family of transcription factors, activated by the WNT signaling pathway and may play a master role in regulating insulin biosynthesis, secretion, and processing. Subsequently, two single nucleotide polymorphisms (SNPs) within intron 3 of *TCF7L2*, rs7903146 and rs12255372, were confirmed to be strongly associated with T2D risk^[20-22]. Wolfram syndrome 1 gene (wolframin) (*WFS1*) was reported to be associated with T2D on the basis of in-depth studies of candidate genes^[23]. The *WFS1* gene encodes wolframin, endoplasmic reticulum (ER) membrane protein with a role in ER calcium homeostasis. Mutations in *WFS1* are known to be associated with Wolfram syndrome^[24].

GENOME-WIDE ASSOCIATION STUDIES ON T2D

Advances in technology of SNP genotyping, implementation of recent genetic knowledge gained from the Human Genome Project, and development of robust statistical methods have allowed GWAS to become the basic method for identification of common genetic variants associated with complex diseases such as T2D. Since the application of GWAS technology the discovery of genetic variants associated with T2D has developed dramatically.

In 2007, the first GWAS performed for T2D has identified three novel susceptibility loci related to pancreatic β -cells: (1) Solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*), which is expressed exclusively in insulin-producing β -cells; (2) Insulin-degrading enzyme (*IDE*)-kinesin-interacting factor 11 (*KIF11*)-hematopoietically expressed homeobox (*HHEX*); and (3) Exostosin glycosyltransferase 2 (*EXT2*)-ALX homeobox 4 (*ALX4*)^[25]. Subsequent GWAS revealed four additional loci associated with T2D, namely CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), cyclin-dependent kinase inhibitor 2A (*CDKN2A/B*), insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), and fat mass and obesity associated (*FTO*)^[26-30]. In addition, HNF1 homeobox B (*HNF1B/TCF2*), a gene related to maturity-onset diabetes of the young type 5 (MODY5), was shown to be associated with T2D^[31]. One important finding from the initial GWAS results was that effect sizes for common variants involved in T2D were likely to be modest. The statistical power to detect associations between genetic variants and a trait depends on the sample size, the distribution of effect sizes of (unknown) causal genetic variants, the frequency of those variants, and the linkage disequilibrium (LD) between observed genotyped DNA variants and the unknown causal variants^[32]. This led to an innovative data merging strategy now known as GWAS meta-analysis and resulted in multiple waves of GWAS studies for T2D.

In 2008, six new T2D loci including *JAZF1*, *CDC123/calcium/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, and *NOTCH2* were reported by a meta-analysis combining three previous GWAS [Diabetes Genetic Initiative (DGI), Finland-United States Investigation of NIDDM Genetics (FUSION), and Wellcome Trust Case Control Consortium (WTCCC)]^[33]. In 2009, two loci, namely insulin receptor substrate 1 (*IRS1*) and melatonin receptor 1B (*MTNR1B*) were identified to be associated with T2D by GWAS^[34-36]. The *IRS1* gene is related to insulin resistance and hyperinsulinemia, whereas *MTNR1B* is involved in impaired early insulin response to glucose^[35].

In 2010 the second wave of the GWAS identified 17 new loci associated with T2D which was made possible because of improved efficiency of GWAS genotyping technology, enabling interrogation of larger numbers of SNPs that better cover common genetic variation across populations in increased sample sizes, as well as because of methodological innovations, such as imputation (described below), which allows prediction of genotypes at SNPs not typed on GWAS arrays^[37].

In the past year a leap forward has occurred from smaller, cumulative advances to the description of up to around 250 genome-wide significant loci of T2D^[10]. In this work, a large meta-analysis of GWAS in sample of T2D including 62892 cases and

596424 controls was performed by combining 3 GWAS data sets of European ancestry: DIABetes Genetics Replication and Meta-analysis (DIAGRAM), Genetic Epidemiology Research on Aging, and the full cohort release of the UK Biobank 39 previously unknown loci have been identified^[38]. This study highlighted the benefits of integrating multiple omics data to identify functional genes and putative regulatory mechanisms caused by genetic variation. Future applications of integrative omics data analyses are expected to improve our understanding of the biological mechanisms underlying common diseases such as T2D^[38].

MAPPING OF CAUSAL VARIANTS AND DISEASE GENES BY NGS METHODS

While conventional genome-wide association studies allow to identify associated loci, GWAS alone cannot be used to map causal variants (many of which are expectedly rare in population), as the method strictly focuses on pre-selected common variants identified by the HapMap project in the beginning of the century^[39]. On the other hand, NGS presents a reasonable alternative to the chip-based methods. For genotyping purposes, NGS reads are aligned to a reference genome, and a set of statistical procedures is performed to identify variant sites^[40]. Thus, NGS directly identifies most of the genetic variants present in an individual's genome irrespective of their frequency, which enables testing of all variants' association. In this section, we will focus on how NGS datasets might be used for identification of novel causal variants for T2D, and which loci have been identified by these methods.

Fine mapping of GWAS signal using NGS-based reference panels

Large genome and exome sequencing and aggregation consortia, such as the 1000 Genomes project or UK10K provide valuable insights into linkage disequilibrium, *i.e.*, co-occurrence rates, between different variants, enabling probabilistic reconstruction of individual genome sequences from fixed number of genotyped loci (such as in traditional GWAS). This in turn enables testing for the role of rare variants without sequencing *per se*^[37,41,42]. Large reference panels for such genotype imputation have been constructed from sequencing data^[43]. Genotype imputation has been widely used in the studies of the genetic architecture of T2D^[44]. An interesting example is a 2014 study of Icelandic population^[45]. In this work, whole-genome sequencing study of a cohort of 2630 Icelanders was performed; and the identified SNPs and indels were imputed into 98721 controls and T2D patients genotyped with Illumina SNP chips. As a result of this study a rare variant in *HNFI1A* gene, encoding for a transcription factor required for the expression of several liver-specific genes was identified. Moreover, a new signal with association $P < 1 \times 10^{-8}$ at rs76895963, located within the first intron of cyclin D2 (*CCND2*) was observed^[45]. Two of the most recent and comprehensive research efforts aimed at fine mapping of association signal using imputation and islet-specific epigenome maps identified multiple previously unreported loci for T2D, including *PNPLA3*, *LPL*, *TPCN2*, *DENND2C*, and *KIF2B*^[46,47]. Apart from using NGS datasets for rare variant imputation, different approaches based on combined SNP and exome chip methods have been developed, enhancing the power of imputation-based analyses^[48].

Association of single rare variants with T2D in NGS-based studies

As previously stated, many new genetic associations relevant to T2D have been revealed by GWASs, but these findings represent common and mid-frequency genetic variants with small effect sizes and explain only a small proportion of heritability of the disease. Sequencing approach enables more complete assessments of low-frequency and rare genetic variants that can be promising in investigation of complex traits.

Many published studies have focused on identification of T2D susceptibility loci from NGS data. In Danish study, the exomes of 1974 Danes were sequenced to a depth of $8 \times$ and subsequently a two-stage follow-up in 15989 Danes and in a further 63896 Europeans were performed. A low-frequency coding variant in CD300LG associated with fasting HDL-cholesterol and two common coding variants in COBLL1 and MACF1 have been shown to be associated with T2D^[49]. CD300LG encodes a protein proposed to serve multiple functions, including endocytosis of various immunoglobulins and mediation of L-selectin-dependent lymphocyte rolling^[50,51]. Non-coding SNPs in COBLL1 and MACF1 have previously been associated with other metabolic phenotypes^[52-54].

To investigate the hypothesis of "missing heritability", the Genetics of Type 2 Diabetes and Type 2 Diabetes Genetic Exploration by Next-generation sequencing in

multi-Ethnic Samples Consortium (GoT2D/T2D-GENES Consortium) undertook whole genome sequencing in 2657 Europeans with and without diabetes, and exome sequencing in a total of 12940 subjects from five ancestral groups. Results of this study showed that the variants associated with T2D were overwhelmingly common and most located within regions previously identified by GWAS. A few coding variant associations outside established common variant GWAS regions have been identified (rs41278853 in MTMR3 gene; rs11549795, rs28265, rs36571 in ASCC2 gene). A coding variant reached genome-wide significance that was common in East Asian ancestry population (*PAX4* Arg192His, rs2233580)^[55]. *PAX4* gene encodes a transcription factor involved in islet differentiation and function. Some *PAX4* variants have been associated with early-onset monogenic diabetes^[56,57].

Specific statistical approaches for rare variant associations on NGS data

Despite decreasing costs of NGS-based analyses, there still remain certain notable limitations of such studies. The most evident limitation of all the rare variant-based tests on both whole-genome and imputed SNP array datasets is the difficulty of obtaining enough observations to make confident statistical inference. For example, if a causal variant occurs at a rate of 10^{-4} in a population, one would require many hundreds of thousands of individuals to test its association with the disease. To allow testing for the association of rare variants, especially in smaller samples, a group of techniques were developed, called Rare Variant Association tests. Most of rare-variant tests are designed to identify candidate disease genes through aggregation of all rare variants inside the coding sequence of each gene. Numerous strategies for gene-level testing of rare-variant association have been developed^[58]. The two main groups of such methods test either the imbalance of rare allele counts between cases and controls (burden tests) or the proportion of phenotypic variance explained by rare variant genotypes (variance-component tests). However, for T2D almost few significant gene-level associations have been found even in the largest NGS-based population cohorts^[55,59]. Only the largest study performed by whole-exome sequencing to date, which included 20791 T2D cases and 24440 controls of multiple ancestries (Hispanic/Latino, European, African-American, East-Asian, South-Asian), identified several gene-level associations: in 3 genes at exome-wide significance, including a T2D protective series of > 30 *SLC30A8* alleles, and within 12 gene sets, including those corresponding to T2D drug targets and candidate genes from knockout mice. The strongest T2D rare variant gene-level signals was shown to explain at most 25% of the heritability of the strongest common single variant signals^[60].

Several alternative techniques have been developed to overcome the limitations of rare variant testing. In samples of limited size based on exome sequencing or targeted resequencing, contribution of rare variants might be assessed using tests for case-specificity conditioned on true population minor allele frequency^[61]. Such strategy may help to identify variants that serve as the candidate causal markers for the pathology. In a recent study by our group, we identified potential association for the *VAV3*, *ADAMTS13*, *HBQ1*, and *DBH* genes with T2D and obesity. While these genes have not been previously implicated in the disease, they are reasonable targets for further clinical investigation.

Another approach to counteract statistical power limitation of rare-variant based tests in small NGS-based datasets is the usage of pedigrees. The biggest advantage of familial studies is that cohorts of related individuals would have higher frequency of alleles that are rare in the general population. One recent example of pedigree-based analysis is a study of 20 Mexican-American families comprising 1034 highly related individuals^[62]. While this study still did not identify any significant associations for individual rare variants, it has shown gene-level association for the *CYP3A4* and *OR2T11* genes with glycemic traits, such as fasting glucose levels and 2h insulin levels.

Overall, there are several ways in which NGS might be used to assist identification of causal genes and variants for T2D pathology. These associations are of ultimate relevance for genomic risk prediction of T2D and clinical decision making^[63]. Some of the inherent limitation of the technology, however, still do not allow thorough analysis of chromosome- and genome-level genetic variation and/or complex genome regions that are poorly accessible to short read sequencing^[64-66]. The spread of third-generation sequencing technologies, such as the Oxford Nanopore Technologies single-molecule sequencing, as well as modifications to the existing laboratory and/or bioinformatic practices would shed light on the roles of higher-level genetic variants in T2D pathology.

NGS IN FUNCTIONAL GENOMIC STUDIES OF T2D

Apart from methods aimed at genotyping, NGS can also be used to dissect functional genome elements rather than sequence variants. NGS techniques for these purposes include transcriptional profiling approaches (RNA-Seq), epigenome mapping techniques (positional methods), and other^[67,68]. These methods are commonly used to both identify candidate disease genes and understand pathological mechanisms behind the observed phenotype. Below, we will provide several recent examples of application of these methods to the research of T2D (Figure 1).

Transcriptional profiling of whole tissues and single cells by RNA-Seq

Transcriptional profiling methods, such as RNA-Seq, are used to study activity patterns of genes. In the recent decade, transcriptomic technologies were frequently used to decipher the molecular pathology behind human disease^[69]. T2D, being one of the most common pathologies, has also been extensively studied by transcriptional profiling techniques in the recent decade^[70]. Traditional way to analyze RNA-Seq data is to align the reads to a reference genome and count the numbers of reads or fragments mapped to each gene or transcript. These counts are then used to search for genes which significantly change their expression in case vs controls (differentially expressed genes, DEGs) using conventional statistical tests or linear regression models, and identify biological processes which are dysregulated in one of the conditions. The latter task is solved by a family of gene set enrichment tests that analyze overrepresentation of genes from a certain pathway among the identified DEGs. Multiple downstream analyses can be performed to identify disease genes and pathways from both bulk and single-cell RNA-Seq data^[71]. Below, we will focus on several notable examples of how both bulk and single-cell technologies can be used to identify genes involved in pathological mechanisms of T2D.

One example of a conventional bulk RNA-Seq approach used to identify disease-relevant pathways can be found in a recent work that studied transcriptional profiles of diabetic keratinocytes^[72]. This study showed extensive dysregulation of immunity-related genes in these cells compared to controls, with as many as 420 differentially expressed genes identified in total. Moreover, this study has suggested a causal role of miR-340-3p-*DTX3L* interaction in the pathological processes occurring in diabetic skin.

Multiple studies have also focused on the roles of microRNA (miRNA) in the pathology of T2D^[73]. microRNAs are a separate class of RNA molecules which play an important role in gene regulation via post-transcriptional gene silencing. One of the most recent studies aiming at systematic analysis of microRNA involvement in T2D by aggregation of published data identified as many 158 microRNAs reported to be differentially expressed in T2D. One example of an important microRNA identified in this study is the miR-375 RNA which affects expression of several disease-relevant genes in islets and other tissues.

Many studies suggest that the alterations in miRNA levels are associated with T2D development and its complications. miRNA may play a key role in regulation of the processes of carbohydrate and lipid metabolisms, adipocytokine and insulin signaling pathways involved in T2D development. It was shown that the dysregulated in the islets miR-7-5p, -129-3p, -136-5p, -187-3p, -224-5p, -369-5p, -375-495-3p, -589-5p, -655-3p affect the expression of important genes involved in insulin signaling pathway. The altered level of miRNA miR-17-5p, -155-5p, -125b-5p, -30e-5p, -27a-5p, -221-3p, -199a-5p, -130b-3p, -181a-5p, -29a, -29b can cause the dysregulation of lipid and glucose metabolisms. For miR-130b-3p, -140-5p, -147a, -199a-5p, -27b, -221-3p and -30e-5p) their involvement in the regulation of adipogenesis was identified^[74]. Stability of miRNAs, their presence in various body fluids and significant changes of specific circulating miRNAs' concentrations associated with diseases allow studying them as potential reliable biomarkers for complex diseases such as T2D and related complications. However, there are some obstacles for straightforward clinical application of circulating miRNAs. The biggest difficulty is due to the composition of circulating miRNA that are sum of many different tissues and cell types in the body. At the same time, it is well known that the expression of miRNAs varies considerably between different tissues.

Another important branch of NGS-based transcriptional profiling techniques is the single-cell RNA sequencing (scRNA-Seq) which allows researchers to study transcriptional responses of individual cells and cell-types. scRNA-Seq techniques are also being extensively used to identify key disease genes for T2D in pancreas cells. For example, scRNA-Seq of pancreatic islets suggested a role of *FXRD2* and *GPD2* genes in pathological processes behind T2D in certain islet cell types, with as many as 245 dysregulated genes in total^[75,76].

Identification of epigenetic disease markers

Another widely used group of NGS methods is aimed at understanding the language

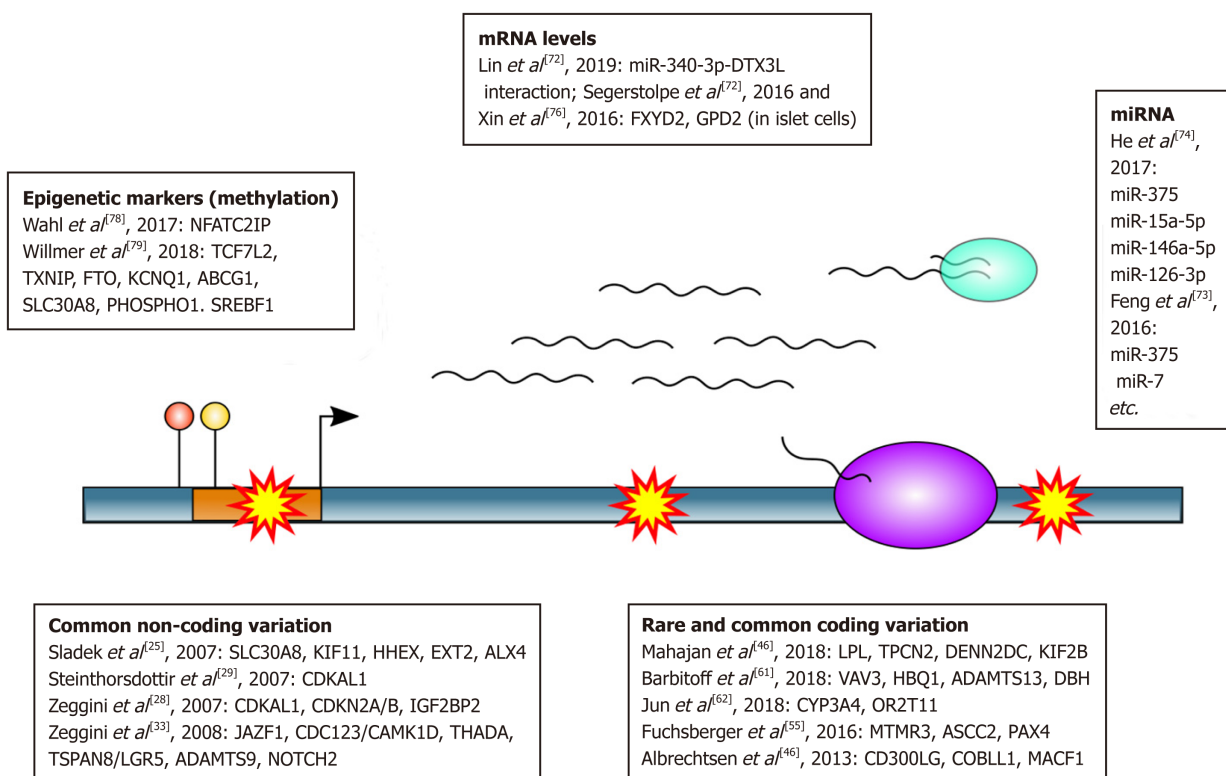


Figure 1 Schematic representation of main type 2 diabetes loci identified recently by high-throughput (mostly, next-generation sequencing-based) technologies. Each box represents certain type of genetic and epigenetic markers of type 2 diabetes.

of epigenetic marks, *i.e.*, non-DNA based units of genetic information. NGS technologies for epigenome studies include but are not limited to: (1) Methods for detection of specific DNA-protein interaction (*e.g.*, Chromatin Immuno Precipitation followed by Sequencing); (2) Methods for identification of DNA methylation sites (such as reduced representation bisulfite sequencing); and (3) Open chromatin mapping technologies (*e.g.*, DNase-Seq or ATAC-Seq)^[67]. All of these methods provide valuable insights into dysregulation of cellular processes, which is of ultimate importance for T2D pathology^[77]. Epigenetic marks, as the dynamic features of the cell, are frequently considered as convenient biomarkers for disease risk prediction and prognosis in the clinic. A large-scale survey on the adverse outcome of adiposity showed that methylation pattern at certain loci predicts development of T2D in overweight people^[78]. A recent analysis of published data identified 8 differentially methylated genes as potential blood biomarkers of T2D (*TCF7L2*, *KCNQ1*, *ABCG1*, *TXNIP*, *PHOSPHO1*, *SREBF1*, *SLC30A8*, and *FTO*)^[79]. Epigenome profiles might also be used to enhance identification of causal variants at complex GWAS loci^[80].

Overall, RNA-Seq and positional NGS techniques provide a very useful framework to investigate cellular processes that are affected during disease pathogenesis. These data may in turn be used for both prediction of diabetes risk and for designing clinical treatment of the disease; furthermore, simultaneous consideration of genotype, expression profile and epigenetic factors might assist efficient personalized treatment of T2D. Further integration of multiple omics datasets would allow researchers and clinicians to have a comprehensive look into the molecular pathology behind T2D.

NGS STUDIES OF HUMAN GUT MICROBIOME AND T2D ASSOCIATIONS

Rapid progress of NGS technologies and bioinformatic data processing methods led to the advent of metagenome studies, *i.e.*, investigation of the microbial composition of natural inhabitants. A decade of advances in the field of intestinal microbiome analysis demonstrated that alterations of gut bacteria composition is implicated in a few medical conditions, including diabetes and obesity^[81-83]. Such progress can be attributed to a number of factors, for example, stable decrease of price per single run for NGS platforms, continuous development of bioinformatic tool/pipelines^[84-87], creation of specialized gut microbiome 16s rRNA databases and use of metaproteo-

mics, metabolomics and metatranscriptomics in conjuncture with genetic profiling^[88-92]. Still, there is no consensus concerning optimal conditions for conducting microbiome research. Choice between 16S RNA profiling/shotgun sequencing methods, differences in effective coverage between V1-V9 hypervariable regions, more precise quantitative analysis for microbiota constituents^[93], and generalized protocols for sample acquisition are still in discussion, with main emphasis often being put on low reproducibility of results, partly due to the unstable nature of samples' bacterial composition^[81,86,87,92,94]. Overall, intestinal microbiome genetic profiling may find use in clinical practice with development of presently elusive "golden standard" for this research field, leading to better understanding of gut microbiota's role in human homeostasis and associations with diseases^[95].

General overview of microorganisms involved at least partially with T2D

As of 2014, microbial community of human gut was estimated to contain at least 957 bacterial genera with phyla Actinobacteria, Bacteroides, Firmicutes, Proteobacteria and Verrucomicrobia demonstrating most diversity and abundance^[96]. While both types of diabetes mellitus are known to cause significant changes in gastrointestinal microbial composition, underlying mechanisms for dysbiosis and roles of all microbiome constituents, including bacteria, archaea, eukaryota and fungi, are still not fully understood. *Roseburia intestinalis*, *Faecalibacterium prausnitzii*, and families *Ruminococcaceae/Lachnospiraceae*, all known as butyrate producers, were detected to be lower during T2D^[97,98]. Abundance of *Akkermansia muciniphila*, a mucin-degrading primarily mucosal bacteria, had been connected to lower insulin resistance, while their low concentrations were associated with obesity, diabetes, IBD, ulcerative colitis and appendicitis, suggesting future use of this bacteria as a biomarker^[99]. However, such broad spectrum of diseases makes effective clinical usage questionable. *Prevotellacopri* and *Bacteroides vulgatus* were mentioned as possible promoters for insulin resistance due to active branched chain amino acids (BCAA) production^[100]. Data on general *Firmicutes/Bacteroidetes* ratio changes during prediabetes and T2D are contradicting, which may be explained by differences in sequencing methods and bioinformatics approaches^[100,101]. Recent 16S/18S/ITS microbiome profiling study of T2D with 49 adult participants in India showed interesting correlation for archaea, where concentration of *Methanobrevibacter* increased in direction from healthy subjects to fully developed T2D, while *Methanosphaera* concentration gradually decreased. Fungal component demonstrated overall abundance growth with inclusion of pathogenic *Aspergillus* and *Candida* phyla^[98]. Most of aforementioned microorganisms were proposed as possible indicators for prediabetes, T1D (type 1 diabetes) and T2D, but their use in clinical practice is not recommended at the moment due to low amount of data and contradictory nature of results between studies, which may be solved in the future^[86].

Linkage of microbiome to diabetes through obesity and metabolic syndrome.

Both T2D and obesity demonstrate a growing trend across the globe, with subjects suffering from the latter being often viewed as possible T2D risk group^[102,103]. Recent findings in the field of microbiome variation during diabetes and obesity had reaffirmed earlier theories concerning microbiota's participation in adipose tissue function and insulin resistance. Network-based gene expression association studies of host's genome underline digestive metabolism, immunization, and signal transduction as the most prominent mechanisms in development of obesity/T2D^[104], while the data on gastrointestinal microbiome role is yet to be unified in coherent system. Gut microbiota had been shown to regulate body mass in a set of fecal transplantation experiments conducted on lean, obese and germ-free mice. Transplantation of gastrointestinal microbiota from lean to obese mice led to lower insulin resistance, while transfer of microbiota from obese to lean mice led to body mass increase by 60% and higher insulin resistance^[83,104,105]. Low grade inflammation, acquired through activation of TLR4/MyD88/NF- κ B pathway by lipopolysaccharides from gram-negative bacterial walls, had been connected to insulin resistance through insulin receptor substrate serine phosphorylation by participants of inflammatory cascade^[106]. Inhibition of NF- κ B led to increase of *Akkermansia/Lactobacillus*, reduced body mass and lower insulin tolerance^[100,107]. Short chain fatty acids (SCFA), obtained by bacteria through fermentation of non-digestible fibers, serve as signaling molecules in a broad list of processes, including proliferation of pancreatic β cells and insulin biosynthesis. This partially explains prebiotic treatment effectiveness and changes in abundance of *Roseburia intestinalis* and *Faecalibacterium prausnitzii*, but further research is required, as results from different studies often contradict each other^[100,108,109]. High serum levels of BCAA are attributed to both obesity and T2D with steady increase of *Prevotella copri* and *Bacteroides vulgatus* during the onset of the diseases^[110]. Both probiotics and prebiotics tend to increase insulin sensitivity and lower body mass, although studies

have small sample sizes and require longitudinal research^[111,112].

Metformin effects on microbiome composition

Recent findings demonstrate that effectiveness of metformin, most prescribed antidiabetic drug whose pharmacodynamics mainly involve activation of hepatic AMP-activated protein kinase in liver, may be partially attributed to mediation of diabetic dysbiosis. Increase of *Akkermansia muciniphila* abundance after metformin treatment was detected in both human and animal studies, while in vitro conditions in gut simulator demonstrated metformin as a growth factor for both *Akkermansia muciniphila* and *Bifidobacterium adolescentis*^[113,114]. Metformin therapy was found to promote growth of SCFA-producing bacteria in rats (*Allobaculum*, *Bacteroides*, *Blautia*, *Butyricoccus*, *Lactobacillus*, *Akkermansia* and *Phascolarctobacterium*) and humans (*Akkermansia*, *Lactobacillus*, *Bifidobacterium*, *Prevotella*, *Megasphaera*, *Shewanella*, *Blautia* or *Butyrivibrio*)^[113].

NEW APPROACHES FOR CLINICIAN INTERPRETATIONS OF NGS DATA

The identification of multiple loci by GWAS and sequencing technologies has given a considerable impetus to the disclosure of pathogenesis of T2D and provides a tempting opportunity to translate genetic information to clinical practice. This knowledge may have potential role in disease risk prediction including identification of subjects at risk of developing disease at an early-stage, and in clinical management of individuals to modify treatment regimens so that affected individuals would benefit most by their therapy and avoid the occurrence of complications^[63]. The emerging availability of genomic and electronic health data in large populations is a powerful tool for research that has drawn interest in bringing precision medicine to diabetes^[115].

Can a genetic test motivate lifestyle changes?

According to the latest polls people are interested in genetic testing for T2D risk since this allows them to evaluate the individual feature of pathology state^[116]. However, several studies have shown that some factors contribute to the failure of individuals to conduct a genetic test. The main factors that influence refusal include distrust of medical researchers, religious prejudices and lower levels of education^[117,118]. Some have argued that the clinical significance of genetic markers of T2D have only a minor role in predicting the risk with careful clinical risk assessment, the predictive value increases^[116,119].

Until recently, it has been assumed that genetic predisposition awareness can motivate healthy behavior^[120]. According to some authors, it is considered that the patient does not appear motivated to a healthy lifestyle after identifying his genetic predisposition^[121-123]. At the same time, research on the molecular basis of the development of T2D is absolutely necessary when making a diagnosis, since young individuals with T1D can also be obese^[124,125]. Misdiagnosis of diabetes can lead to misuse of medical treatment^[126].

Studies of genetic biomarkers: Prediction, and diagnosis of T2D

Many studies have analyzed the utility of genetic variants in T2D risk prediction for undiagnosed individuals with T2D using cross-sectional studies and incident T2D using longitudinal studies. Early studies provided much optimism and showed that common variants at the *TCF7L2* locus predict the progression to diabetes in subjects with impaired glucose tolerance^[63,127]. Unfortunately, diabetes mellitus is diagnosed on the basis of its biochemical effects (increased glucose), and the absence of detection of the main defect, which indicates the absence of the disease^[128]. However, at present, aggregated available data do not provide robust evidence to support the utility of genetic testing for T2D predictions and indicate a modest contribution of genetic variants^[129-131]. Several large population-based follow-up studies have been published aiming to investigate the predictive power of common genetic variants on the risk of incident T2D. The results of these studies were similar to those from cross-sectional case-control studies. It was shown that risk variants did not essentially increase the AUC to predict T2D when combined with clinical risk factors^[132]. However, it seems possible to improve T2D risk prediction and overcome factors limiting predictive power, such as: (1) Modest effect sizes of common variants, (2) Insufficient knowledge of rare and coding variants missed by GWAS; (3) Heterogeneous nature of the disease; and (4) Genetic diversity between ethnic groups (detailed below).

The limitations related with modest effect sizes of common alleles and necessity of

further investigation aimed to identify rare and coding variants involved in T2D pathogenesis have been reviewed above. T2D seemingly encompasses a group of several subtypes of diseases, which makes it rather difficult to distinguish it from other types, as it may be the result of defects in various metabolic pathways. The accuracy of prediction models may be affected by the fact that latent autoimmune diabetes in adults has been identified and the number of monogenic forms of diabetes is increasing, which can also indicate the level of misclassification^[133].

In different populations, heterogeneity in association of genetic variants with the disease was demonstrated, apparently related to the design of the study, in particular the results of a large meta-analysis that combines cases of T2D with different origins or signs and evaluates them with a generalized intermediate hyperglycemia phenotype, despite the fact that the phenotype may differ due to a multitude of unrelated causes within the physiology of the body or the environment^[134]. In recent years, a large number of projects have been carried out to study the causes of diabetes, large-scale studies have been created and huge biobanks of samples of these patients have been collected. In addition, some variants were found that are important in the prevention and treatment of T2D, found in individual population isolates, demonstrating the value of studying genetically isolated populations^[128]. Because of genetic drift, deleterious variants with large phenotypic effects could rise randomly to higher allele frequencies. Which makes investigation of such variants' association easier in isolated populations compared to the admixed ones, in which these variants might not be present or might be very rare^[10].

Circulating miRNAs in plasma or serum have several features that make them ideal candidate biomarkers of complex diseases such as T2D^[135]. Hundreds of miRNAs are actively or passively released to the blood circulation to regulate specific gene function^[136]. Current studies demonstrate that changes in expression miRNAs involve in dysfunction of insulin and progression of T2D. Many studies confirmed that some miRNAs have been identified and found to be associated with T2D^[137]. miR-21, miR-126 and miR146a have been shown to have potential to be biomarkers of early diagnosis of T2D disease^[138-140]. Thus, the above mentioned miRNAs and a number of other miRNAs may be candidates for testing the effectiveness of therapy but further studies are needed to identify them^[137].

Genetic tests of T2D: Implications for therapy

T2D commonly develops with insulin resistance, a disorder in which cells located primarily within the muscles, liver, and fat tissue do not use insulin properly, and progresses to pancreatic beta-cell failure. T2D trigger are insulin resistance and inadequate insulin secretion^[141].

Selection of drug therapy based on the genetic features of the individual can be a huge breakthrough because there are individual drug idiosyncrasy and many patients eventually fail to achieve recommended levels of glycemic control due to their genetic characteristics^[142,143]. Currently, only half of patients initiating therapy with metformin or sulfonylurea, reached a level of hemoglobin A1c in 7%^[144]. It should be emphasized that sulfonylureas and metformin are the most studied classes of drugs used to treat T2D^[115].

Sulphonylureas (SUs) are widely used drugs in the clinical practice however, different side effects, such as weight gain and increased risk of hypoglycemia, have been frequently^[145]. Studies have shown that these drugs can act effectively in response to a defect induced by variants in *KCNJ11* (rs5219, rs5215) and *ABCC8* (rs757110) in patients with T2D^[146,147]. Also important in the selection of SUs play role *CYP2C9* (rs1799853, rs1057910), *TCF7L2* (rs12255372, rs7903146), *IRS1* (rs2943641, rs1801278) and *CAPN10* (rs3842570, rs3792267, rs5030952)^[148-151]. It should also be noted rs7754840 in the gene *CDKAL1*, which is significantly associated with the response to treatment with sulfonylurea and in combination with other clinical and pathological data will help move to individual therapy of patients with T2D^[152].

Metformin is the most commonly used drug in the treatment of T2D, which is not metabolized in the liver, therefore, the effect of reducing the level of metformin is not affected by genetic variants in the genes encoding metabolizing enzymes^[153]. *SLC22A1* (rs12208357, rs34130495, rs35167514, rs34059508) is the most studied gene that is involved in the response to metformin^[154]. However, other genes involved in the metabolism of metformin have been identified, for example, *SLC22A2* (rs316019), *PPARG* (rs1801282)^[145,155]. It should also be noted that the T2D-associated variant rs7903146 in *TCF7L2* influences the acute response to both glipizide and metformin in persons free of overt diabetes^[156].

CONCLUSION

The growing power and reducing cost sparked an enormous range of applications of NGS technology that gave us the excellent instrument for solving various problems in molecular biology. Rational usage of this instrument, taking into account all of its benefits and limitations, is the next step on the way to elucidation of pathogenesis of complex diseases such as T2D. Results obtained in sequencing-based studies combined with earlier findings from GWAS and candidate genes studies allow ordering and improving our knowledge about T2D and give us an opportunity to translate genetic information to clinical practice. The increasing knowledge provides a fascinating opportunity to use this information to predict the occurrence of disease and to identify subgroups of patients for whom therapies will have the greatest efficacy or the least adverse effect. However, this new knowledge should be treated with caution. Unfortunately, the accuracy of risk prediction models based on genetic information of T2D is not remarkable to date. Hence, further research and technological improvement is needed in studying the individual and aggregate contribution of genetic markers for the development of diabetes for widespread use in clinical practice.

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Basic Study

Epidermal growth factor receptor rs17337023 polymorphism in hypertensive gestational diabetic women: A pilot study

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Abstract

BACKGROUND

Women with gestational diabetes mellitus have an increased risk of developing gestational hypertension, which can increase fetal and neonatal morbidity and mortality. In the past decade, single nucleotide polymorphisms in several genes have been identified as risk factors for development of gestational hypertension. The epidermal growth factor receptor activates tyrosine kinase mediated blood vessels contractility; and inflammatory cascades. Abnormalities in these mechanism are known to contribute towards hypertension. It is thus plausible that polymorphisms in the epidermal growth factor receptor gene would be associated with the development of hypertension in women with gestational diabetes.

AIM

To determine whether the epidermal growth factor receptor rs17337023 SNP is associated with the occurrence of hypertension in gestational diabetic women.

METHODS

This pilot case-control study was conducted at two tertiary care hospitals in Karachi, from January 2017-August 2018. Two hundred and two women at 28 week of gestation with gestational diabetes were recruited and classified into normotensive ($n = 80$) and hypertensive ($n = 122$) groups. Their blood samples were genotyped for epidermal growth factor receptor polymorphism rs17337023 using tetra-ARMS polymerase chain reaction. Descriptive analysis was applied on baseline data. Polymorphism data was analyzed for genotype and allele frequency determination using chi-squared statistics. In all cases, a P value of < 0.05 was considered significant.

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RESULTS

Subjects were age-matched and thus no difference was observed in relation to age of the study subjects ($P > 0.05$). Body fat percentage was significantly higher in hypertensive females as compared to normotensive subjects (35.138 ± 4.29 Case *vs* 25.01 ± 8.28 Control; $P < 0.05$). Similarly, systolic and diastolic blood pressures among groups were significantly higher in hypertensive group than the normotensive group ($P < 0.05$). Overall epidermal growth factor receptor rs17337023 polymorphism genotype frequency was similar in both groups, with the heterozygous AT genotype (56 in Case *vs* 48 in Control; $P = 0.079$) showing predominance in both groups. Furthermore, the odds ratio for A allele was 1.282 ($P = 0.219$) and for T allele was 0.780 ($P = 0.221$) in this study.

CONCLUSION

This pilot study indicates that polymorphisms in rs17337023 may not be involved in the pathophysiology of gestational hypertension in gestational diabetes *via* inflammatory cascade mechanism. Further large-scale studies should explore polymorphism in epidermal growth factor receptor and other genes in this regard.

Key words: Gestational diabetes mellitus; Gestational hypertension; Epidermal growth factor receptor; rs17337023; Single nucleotide polymorphism; Polymorphism; Case-control

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Core tip: Gestational Hypertension (GHTN) can increase risk of fetal and neonatal morbidity and mortality. Many environmental, nutritional and genetic factors are related to the development of GHTN. Among them, Epidermal Growth Factor Receptor (EGFR) has been found to contribute to arterial hypertension. It is thus plausible that Single nucleotide polymorphisms (SNPs) in EGFR gene would be associated with the development of GHTN in women with GDM. This pilot study indicated that EGFR rs17337023 polymorphism may not be involved in the pathophysiology of GHTN in GDM positive females in a local population. Further large-scale studies should explore SNPs in EGFR and other genes in this regard.

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INTRODUCTION

Gestational diabetes mellitus (GDM), defined as any degree of glucose intolerance with onset or first recognition during pregnancy^[1,2], is a significant risk factor for maternal development of a hypertensive pregnancy disorder (HPD)^[3-5]. Up to 10% of all pregnancies are complicated by HPDs^[6,7], especially in cases with pre-existing GDM^[8]. One type of HPD, Gestational Hypertension (GHTN) occurs in 1.8%-4.4% of pregnancies^[9]. GHTN is defined as blood pressure (BP) that reaches $\geq 140/90$ mmHg for the first-time during pregnancy (after 20 wk gestation), without proteinuria. BP normalizes by 12 week postpartum^[10]. Complications of GHTN include increased risk for fetal death and severe neonatal morbidity and mortality^[11]. Hypothesized mechanisms of HPD development include dysfunction of the placenta, endothelium or lipid metabolism, as well as inflammatory states^[12]. However, it is being increasingly established that genetic factors also contribute towards HPDs^[13].

Single nucleotide polymorphisms (SNPs) have been a particular focus in genetic mechanisms leading to HPD^[14]. SNPs such as NOS SNP rs2070744^[15], APM1 SNP rs1501299^[16], CYP19A1 SNP rs700158^[17], KDR SNP rs2071559^[18] and HSD11B1 rs846910^[19], have been found to be associated with HPDs. The epidermal growth factor receptor (EGFR) is a single chain transmembrane protein of the ErbB family of

receptor tyrosine kinases, which is activated following binding with peptide growth factors of the EGF-family of proteins^[20]. The functions of EGFR include inducing cell growth and differentiation^[21]. EGFR is abundantly expressed in the vascular wall and myocardium, and is thought to be linked to arterial hypertension, possibly by producing vasoconstriction and renal Na⁺ retention^[22]. Apart from its normal EGF Ligands, EGFR also undergoes transactivation by vasoactive substances such as catecholamines^[23] and aldosterone^[24]. The EGFR SNP rs17337023 (T > A), located on Exon 16 with a global variant allele frequency of 0.456^[25], is associated with chronic inflammation, which may lead to vascular damage and hypertension^[26,27]. Given the mechanistic link of EGFR to BP regulation, we decided to conduct a pilot study to explore any association of SNP rs17337023 with the development of GHTN in pregnant females with GDM.

MATERIALS AND METHODS

In a case-control study, $n = 202$ pregnant women at 28 wk of gestation with GDM were recruited. The study was conducted at Aga Khan University and Jinnah Postgraduate Medical Center during the period of January 2017 till August 2018. The sample size was calculated using the Open-Epi website^[28], with a confidence level of 95%, power of 80%, least extreme odds ratio (OR) of 2 and a pregnancy hypertension prevalence of 8% taken according to previously published data sources^[29]. The minimum sample size calculated for this research was $n = 106$. The institutional ethics committee approved the research protocol (Ref # 4523-BBS-ERC-16) (REF: No.F.2-81/GENL-2017-IRB/15107/JPMC). GDM was diagnosed by means of a 75-g 2-h oral glucose tolerance test, as per the criteria set by the IADPSG^[30]. All study subjects gave a written informed consent followed by weight and body mass index (BMI) assessment based on South Asian criteria for BMI values [normal weight (BMI 18-22.9 kg/m²), and obese (BMI ≥ 26 kg/m²)]^[31]. BP assessment was done following the latest European Society of Cardiology and the European Society of Hypertension task force guidelines^[32,33], (Normal BP < 139/85 mmHg and Hypertension > 139/85 mmHg). Subjects diagnosed with GHTN were subsequently being treated by antihypertensive medication. Any individual with a history of pre-existing diabetes, or any inflammatory condition, taking oral contraception or hormonal support, was not included in this study. Based on these measurements, grouping of study subjects was done as follow: (A) Normotensive ($n = 80$); (B) Hypertensive ($n = 122$) (on diet or medication).

Ten milliliters of venous blood were collected from each subject. DNA was extracted from whole blood by Qiagen DNA extraction kit (Cat. #51185, Valencia, CA, United States). The quantification of extracted DNA was performed by measuring the ultraviolet absorbance of the samples using a Nanodrop-ND1000 (Thermo Fisher Scientific, Waltham, MA). The absorbance ratio (A280/A260) was determined for 2 μ L samples using ND-1000 V3.8.1 software (Thermo Fisher Scientific, Waltham, MA). A ratio of approximately 1.8 was considered acceptable for confirming the purity of extracted DNA. Furthermore, around 10% of samples were confirmed on gel electrophoresis by running 1 μ L of sample in a 1% agarose gel against a 1 kb ladder. Tetra arms polymerase chain reaction (PCR) was performed using the Ruby Taq PCR Master mix 2X (Cat. #71191, Affymetrix, United States) as per the manufacturer's instructions. PCR products were electrophoresed in a 2% agarose gel. Genotyping quality control was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates was > 99 %). The following primer set was used for gene amplification: Statistical analyses were conducted using the IBM Statistical Package for the Social, Sciences (IBM SPSS version 21; IBM Corp Inc, Armonk, NY). Descriptive analysis was applied, and data was expressed either as mean \pm standard deviation or absolute number and percentage. SNP data was analysed for genotype and allele frequency determination by applying chi-squared statistics. In all situations a P value of < 0.05 was considered significant. The statistical analyses for this study were performed and reviewed by Syed Adnan Ali (PhD. Statistics) of the University of Karachi.

RESULTS

The detailed results are shown in Table 1, 2 and Figure 1. All study subjects were age-matched and therefore, no difference was observed in relation to age of the study subjects ($P > 0.05$). Body fat percentage was significantly higher in hypertensive females as compared to normotensive subjects ($P < 0.05$). Similarly, systolic and diastolic BP among were significantly higher in hypertensive group than the normo-

tensive group ($P < 0.05$). 90% of the hypertensive females practiced sedentary lifestyle versus 17.7% normotensive females ($P < 0.05$).

Overall EGFR rs17337023 polymorphism genotype frequency was similar in both the normotensive and hypertensive groups, with the heterozygous AT genotype showing predominance in both groups. Furthermore, the OR for A allele was 1.282 ($P = 0.219$) and for T allele was 0.780 ($P = 0.221$) in this study.

DISCUSSION

The developmental causes of GHTN in women with pre-existing GDM is poorly understood and it is possible that genetic factors such as SNPs may play a role. Many studies have demonstrated associations of certain SNPs with development of HPDs. Our objective was to investigate whether the EGFR SNP rs17337023 displayed any significant association with the occurrence of GHTN in pregnant women with GDM. However, the findings of our study showed that the frequency of the rs17337023 genotype was not significantly different in the two groups. Furthermore, the OR for the A and T alleles were also non-significant. These results suggest that the SNP rs17337023 does not play any major role in the pathophysiology of GHTN in GDM.

There are possible explanations for the lack of any significant association. The study proposing mechanisms linking EGFR to arterial hypertension does so primarily on the basis of results obtained from experimenting using animal models, and voices uncertainties about its applicability to humans^[22]. Moreover, apart from Rheumatoid Arthritis, the SNP rs17337023 has been shown to have no significant association with pathologies such as Gastric Carcinoma^[34] and Nasopharyngeal Carcinoma^[35]. This suggests that the function of EGFR is not altered significantly enough due to the SNP rs17337023 mutation to cause any major pathological state. It is possible, however, that other EGFR SNPs may indeed be associated with GHTN in women with GDM.

Additionally, since the sample for our pilot study consisted of 202 women from Pakistan, it is possible that the results may show greater significance if the study were replicated in another population with a larger sample size. The lack of association between the EGFR SNP rs17337023 served to suggest that the EGFR gene may not be involved in the pathophysiology of GHTN in the case of pre-existing GDM. Our study was limited by the inability to recruit a larger sample size due to cultural beliefs and barriers towards participation in genetic studies. Moreover, the group of women with GHTN were not managed uniformly in terms of diet and antihypertensive medications. Nevertheless, the rs17337023 polymorphism was in Hardy-Weinberg Equilibrium for cases and controls, suggesting the randomness of the sample as a strength of our study.

This pilot study indicates that polymorphisms in rs17337023 may not be involved in the pathophysiology of gestational hypertension in gestational diabetes *via* inflammatory cascade mechanism. Further large-scale studies should explore polymorphism in epidermal growth factor receptor and other genes in this regard.

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Table 1 Polymerase chain reaction primers details

Gene	Primers	Base pairs	PCR cycle	Amplicon size
Epidermal growth factor receptor (EGFR) rs17337023	Forward outer: ATTAACCACCAATCCAAC ATCCAGAC	26	67 °C; 30 s; 30 cycles	T allele 180; C allele 271; Control 406
	Reverse outer: CTTTCCTCCACTGAGGA CAAAGTT	25		
	Forward inner (A allele): TCTCTTCACTTCCTACAG ATGCTCA	26		
	Reverse inner (T allele): AGCCTTCAAGACCTGGCG CA	20		

PCR: Polymerase chain reaction.

Table 2 Descriptive statistics and genotype frequency of study subjects

	Hypertensive pregnant case (n = 122)	Normotensive pregnant control (n = 80)	P-value
Age (Yr)	30.55 ± 8.05	29.13 ± 10.19	0.054
Weight (kg)	77.56 ± 16.88	69.24 ± 11.07	0.025
Body Fat %	35.138 ± 4.29	25.01 ± 8.28	0.000
Waist circumference (cm)	104.50 ± 12.09	86.92 ± 12.03	0.000
Systolic blood pressure (mmHg)	131.76 ± 13.04	122.02 ± 8.27	0.000
Diastolic blood pressure (mmHg)	85.88 ± 8.45	73.31 ± 11.27	0.000
Walk			
None	90.1%	17.7%	0.000
30 min/3 days week	7.9%	74.2%	
30 min/5 days week	2.0%	8.1%	
Genotype frequency			
EGFR rs17337023 polymorphism			
AA	30	19	0.079
AT	56	48	
TT	36	13	
Allele Odds Ratio			
Allele A	1.282 [0.860-1.912]		0.219
Allele T	0.780 [0.523-1.163]		0.221

Data presented as Mean ± SD and percentages. In all cases a *P* value of < 0.05 was considered significant. Hardy-Weinberg Equilibrium (HWE) for case *P* = 0.378 and control *P* = 0.064, Where EGFR is epidermal growth factor receptor.

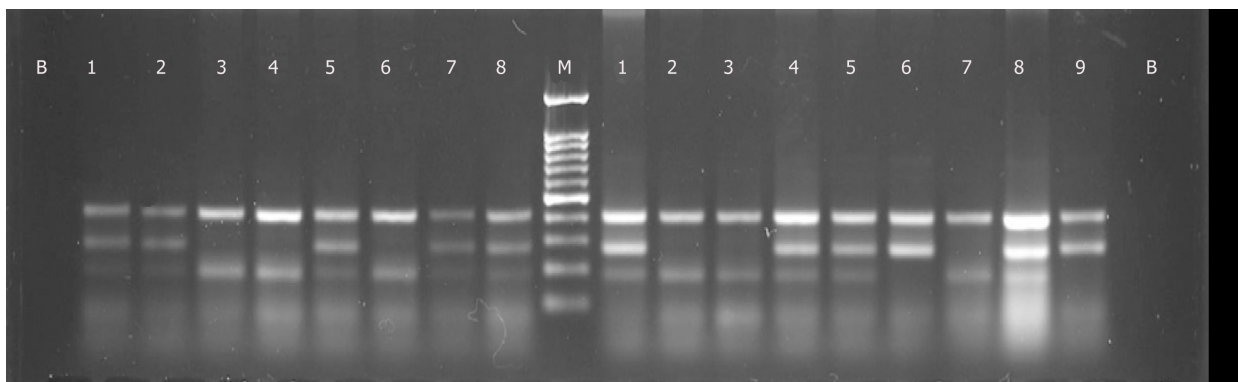


Figure 1 Gel Electrophoresis of epidermal growth factor receptor samples 1 to 8 cases and 1 to 9 Controls. B is blank, and M is the 100bp ladder. Tetra arms control band is visible at 406bp; C allele at 271bp and T allele at 180bp.

ARTICLE HIGHLIGHTS

Research background

Pregnancy induced hypertension and diabetes are an increasing threat to the wellbeing of both mother and the baby. The basic pathophysiological link to disease predisposition is attributed to the functionality of epidermis and angiogenesis. Several genetic studies have provided evidence that epidermal growth factor dysfunction can lead to hypertension and its complications in pregnancy.

Research motivation

Materno-fetal mortality is on the rise in lower middle income countries; predominantly due to lack of primary prevention of non-communicable diseases. This led us to investigate one of the route cause *i.e.* genetic modification as a risk for development of disease.

Research objectives

Explore any association of SNP rs17337023 with the development of gestational hypertension in pregnant females with gestational diabetes.

Research methods

A case-control study was conducted recruiting 202 pregnant women at 28 wk of gestation. Their blood pressure, blood glucose levels were measures and genotyping of EGFR SNP rs17337023 was performed via tetra arms PCR.

Research results

No difference was seen in the EGFR rs17337023 polymorphism genotype frequency among both normotensive and hypertensive groups in this study.

Research conclusions

This pilot study indicates that polymorphisms in rs17337023 may not be involved in the pathophysiology of gestational hypertension in gestational diabetes. Further large-scale studies should explore polymorphism in epidermal growth factor receptor and other genes in this regard.

Research perspectives

This study has shown some negative results linking a specific area of the gene EGFR; however, it should be noted that other factors may also be in play such as obesity and family history that can be a contributing factor along with genetic predisposition for hypertension. This opens up new avenues for researchers to perform prospective studies to identify the causal link between genetic and environmental factors.

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Observational Study

Diabetes empowerment scores among type 2 diabetes mellitus patients and its correlated factors: A cross-sectional study in a primary care setting in Malaysia

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Abstract**BACKGROUND**

There are limited studies on diabetes empowerment among type 2 diabetes patients, particularly in the primary care setting.

AIM

To assess the diabetes empowerment scores and its correlated factors among type 2 diabetes patients in a primary care clinic in Malaysia.

METHODS

This is a cross sectional study involving 322 patients with type 2 diabetes mellitus (DM) followed up in a primary care clinic. Systematic sampling method was used for patient recruitment. The Diabetes Empowerment Scale (DES) questionnaire was used to measure patient empowerment. It consists of three domains: (1) Managing the psychosocial aspect of diabetes (9 items); (2) Assessing dissatisfaction and readiness to change (9 items); and (3) Setting and achieving diabetes goal (10 items). A score was considered high if it ranged from 100 to 140. Data analysis was performed using SPSS version 25 and multiple linear regressions was used to identify the predictors of total diabetes empowerment scores.

RESULTS

The median age of the study population was 55 years old. 56% were male and the mean duration of diabetes was 4 years. The total median score of the DES was 110 [interquartile range (IQR) = 10]. The median scores of the three subscales

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were 40 with (IQR = 4) for “Managing the psychosocial aspect of diabetes”; 36 with (IQR = 3) for “Assessing dissatisfaction and readiness to change”; and 34 with (IQR = 5) for “Setting and achieving diabetes goal”. According to multiple linear regressions, factors that had significant correlation with higher empowerment scores among type 2 diabetes patients included an above secondary education level ($P < 0.001$), diabetes education exposure ($P = 0.003$), lack of ischemic heart disease ($P = 0.017$), and lower glycated hemoglobin (HbA1c) levels ($P < 0.001$).

CONCLUSION

Diabetes empowerment scores were high among type 2 diabetes patients in this study population. Predictors for high empowerment scores included above secondary education level, diabetes education exposure, lack of ischemic heart disease status and lower HbA1c.

Key words: Diabetes; Empowerment; Scores; Diabetes Empowerment Scale; Type 2 diabetes; Primary care; Malaysia

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Core tip: This study aims to assess the diabetes empowerment scores and its correlated factors among type 2 diabetes patients in a primary care clinic in Malaysia. Median age of the study population was 55 years old, 56% were male and mean duration of diabetes was 4 years. The total median score of the Diabetes Empowerment Scale was 110 (interquartile range = 10). Diabetes empowerment scores were high among type 2 diabetes patients in this study population. The predictors for high empowerment score were those who had above secondary education level, diabetes education exposure, no ischemic heart disease status and lower glycated hemoglobin.

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INTRODUCTION

Diabetes has become a global epidemic of the 21st century. Over 70% of known cases of diabetes occur in developing countries. Four hundred and fifteen million adults were estimated to have diabetes globally in 2015, or 1 in 11 adults. This is estimated to rise to 642 million by 2040^[1]. The Southeast Asian region has seen a recent dramatic increase in diabetes. An estimated 96 million people have diabetes in the region, 90% of whom have type 2, which is preventable^[2]. In Malaysia, the incidence rate has also significantly increased from 8.3% in 1996 to 17.5% (3.5million) in 2015^[3]. The Federal Territory of Putrajaya was noted to have the highest increment in the prevalence of diabetes in adults, from 2011 to 2015, that is 8.8% to 19.2%^[4]. Primary care was identified as the backbone in managing diabetes. A majority sought treatment at government health clinics (59.3%) and private clinics (15.1%)^[3]. Therefore, the population attending government health clinics would provide a better picture of overall diabetes management.

Evidence shows that self-empowerment is important in managing chronic diseases, especially diabetes^[5,6]. Self-empowerment is an approach that can improve the ability of the patients with diabetes to understand the disease process better, involve themselves actively in self-care, and practise healthy lifestyles for better disease control^[6,7]. The process of empowerment improves diabetes control by helping patients in making decisions in regards to diabetes care and self-realization of their responsibilities in managing type 2 DM^[8]. Tol *et al*^[9] and Liu *et al*^[10] showed that self-efficacy and self-esteem have a strong relationship with empowerment. However, there are few studies on diabetes empowerment among type 2 diabetes patients in Malaysia. Therefore, this study was conducted to examine diabetes empowerment and its correlated factors among patients with type 2 diabetes in a primary care

setting in Malaysia.

MATERIALS AND METHODS

Setting

This is a cross-sectional study of patients registered with a primary health care clinic located in Putrajaya, a Federal Territory and the administrative capital of Malaysia. It has a total population of 91900^[11]. The study was conducted over a 3-month period from January 2019 to March 2019.

Inclusion criteria

The inclusion criterion for this research included patients aged 18 years and above diagnosed with type 2 diabetes mellitus (DM) and following up for at least 6 months in the primary care clinic. The exclusion criteria included intellectual disability or dependence for activities of daily living, being bed ridden, requiring nursing care to carry out daily activities or being clinically unstable during the study period. The sample size was calculated using the Lemeshow formula based on the prevalence of high DES scores of 36.9% for married and 3.8% for unmarried. The calculated sample size was 322 after taking account of non-respondent rate of 30%, 80% power and significance level of 0.05.

Data collection

Face-to-face interviews were conducted using an adapted structured questionnaire. After obtaining ethical approval, we approached the participants and explained the nature of the study before obtaining written consent to participate in the study. Systematic sampling was used to recruit respondents. The estimated number of diabetic patients attending follow up in the primary care clinic is about 15 patients per day, and 900 patients over the three-month duration of data collection. The estimated sample size for this study was 322; therefore, a sampling interval of 3 was used as the constant during study recruitment. The starting number of 1 was selected randomly from the health clinic registration counter using a dice.

Data collection instrument

The questionnaire was initially prepared in English by the author. Then, forward and backward translations into Malay and English languages were performed by two certified translators. The questionnaire was a self-administered type divided into two sections. The first section includes the patients' sociodemographic information. The second section explores the clinical profiles, clinical outcome and total diabetes empowerment scores.

Diabetes Empowerment Scale

The Diabetes Empowerment Scale (DES-28) was developed by the University of Michigan Diabetes Research and Training Center. The questionnaires consist of 28 items with 3 subscales, with each item rated along a 5-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree). The range of score was divided in three subgroups as low (28-65 scores), middle (66-103) and high (104-140). Cronbach's alpha coefficient is a measure of internal consistency and can be interpreted as the mean of all possible split-half coefficients^[12]. By convention, if Cronbach's alpha is greater than or equal to 0.7 to 0.8, there is acceptable agreement^[13].

This DES-28 is a reliable tool with good internal consistency (Cronbach's alpha = 0.96)^[14]. The Cronbach's alpha of each subscale was 0.93 for "managing the psychosocial aspects of diabetes"; 0.81 for "assessing dissatisfaction and readiness to change"; and 0.91 for "setting and achieving diabetes goals". Each coefficient for the overall DES and three subscales was good^[14].

For the DES Malay version, the questionnaire was originally in English by the author from the University of Michigan Diabetes Research and Training Center, then forward and backward translated into Malay and English languages by two certified translators. The questionnaire was a self-administered questionnaire, which was pretested through a pilot study prior to the actual data collection. The Cronbach's alpha coefficient for the Malay version total DES was 0.92.

The pilot study included 30 patients, 10% of the actual sample size of 322. Recruitment was performed via the systematic sampling method, with every one in two patients registered at the health clinic counter for follow-up selected for the pilot study. About five to eight respondents were collected a day for five days. Question 5 had a spelling error, "realitik" which was corrected to "realistis". Two other questions were rephrased for easier understanding, namely questions 1 and 2 "apa bahagian" (What part) to "bahagian apa". The findings from this pilot study were not included

in the data analysis of the actual study.

Operational definitions

Ethnicity was defined as Malay, Chinese, Indian or others. Education level was according to the respondents' self-reported highest attained level of education: No formal education, primary school, secondary school or tertiary (diploma/university). Smoking status was defined as whether the patient is a smoker, non-smoker or ex-smoker who had quit smoking at least 6 months from the quit date^[13]. BMI was calculated as the weight in kg divided by the square of height in meter, and classified according to the Asian population^[14]. Diabetes duration was defined as the duration of diabetes in years. Compliance to treatment was defined as the patients' self-reported compliance to treatment. The clinical outcomes [systolic and diastolic blood pressure, low-density lipoprotein (LDL) level, high-density lipoprotein (HDL) level, triglycerides (TG) level, glycated hemoglobin (HbA1c) %] in this study were defined in terms of the latest levels measured.

Data analysis

Statistical Package for Social Sciences (SPSS) version 25.0 was used to analyze the data collected from the study. Descriptive analysis was used to describe the characteristics of the respondents in terms of frequencies, percentages, median, and interquartile range (IQR). In this study, we used Chi-square test for the categorical data, Spearson's test, Mann-Whitney *U* test and Kruskal Wallis test for the continuous data to identify the associations between the total diabetes empowerment scores with sociodemographic factors, clinical profiles and clinical outcomes. Multiple linear regressions were used to identify the predictors of total diabetes empowerment score. All variables with a *P* value < 0.25 in the univariate analysis, as well as clinically significant variables, were entered into the multiple linear regression. The dependent variable was total diabetes empowerment score among type 2 diabetes patients. The independent variables are sociodemographic factors (age, gender, ethnicity, level of education, marital status, smoking status) and clinical profiles (DM durations, DM education exposure, compliances to treatment, BMI, hypertension status, dyslipidemia status, ischemic heart disease status, asthma status systolic and diastolic blood pressure, HbA1c, HDL level, LDL level and TG level).

Ethical approval

Ethical approval was obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-17-3085-38099).

RESULTS

A total of 322 participants were recruited into this study, for a response rate of 93.7%. There were no missing data in our study. **Table 1** demonstrates the sociodemographic and clinical characteristics of the study population. Median age was 55 years old with IQR of 18. More than half of the participants were male (58.7%, *n* = 189). The majority of the study population were Malay (92.2%), married (92.2%) and had an education above the secondary school level (88.8%). Two-thirds of the participants were non-smokers (66.5%). With regards to the clinical profiles (**Table 2**), the median diabetic duration for the participants was 4 years (IQR = 7). The mean systolic and diastolic blood pressures are 133.2 ± 15.5 mmHg and 83.7 ± 10.0 mmHg respectively. More than half of respondents were obese (62.7%), had hypertension (64.3%) and dyslipidemia (76.4%). The median for HbA1c was 7.4% with IQR 2.6. The mean for LDL was 3.0 ± 1.0 mmol/L. The median for HDL was 1.1 mmol/L with IQR 0.3, and the median for TG was 1.4 mmol/L with IQR 0.9.

Most of the participants had received diabetes education (82%, *n* = 264). The total diabetes empowerment median score was 110 (IQR = 10) and classified as high. The median scores of the three subscales were 40 (IQR = 4) for "Managing the psychosocial aspect of diabetes"; 36 (IQR = 3) for "Assessing dissatisfaction and readiness to change"; and 34 (IQR = 5) for "Setting and achieving diabetes goal". Spearman Correlation coefficient showed a statistically significant relationship between HbA1c level (*r* = -0.132, *P* value 0.018) with the total empowerment score as shown in **Table 3**. Mann Whitney *U* test showed that those with diabetes education exposure (*P* = 0.004), received above secondary school level (*P* < 0.001), and those without ischemic heart disease (*P* = 0.004) were statistically significant correlated with total diabetes empowerment score as shown in **Tables 4** and **5**.

There is no significant correlation between total diabetes empowerment score with other variables like age, diabetes duration, systolic and diastolic blood pressure, gender, ethnicity, marital status, smoking status, hypertension status, dyslipidemia

Table 1 Sociodemographic profiles of the study participants in primary health care clinic in Putrajaya (*n* = 322), *n* (%)

Variables	Frequency	Median (IQR)
Age (yr)		55 (18)
Gender		
Male	189 (58.7)	
Female	133 (41.3)	
Ethnicity		
Malay	297 (92.2)	
Chinese	6 (1.9)	
Indian	14 (4.3)	
Others	5 (1.6)	
Education level		
No formal education	12 (3.7)	
Primary school	24 (7.5)	
Secondary school	132 (41.0)	
Diploma/University	154 (47.8)	
Marital status		
Single	25 (7.8)	
Married	297 (92.2)	
Smoking status		
Yes	46 (14.3)	
Never	214 (66.5)	
Ex-Smoker	62 (19.2)	

IQR: Interquartile range.

status, asthma status, compliance to treatment, LDL level, HDL level and TG level. According to multiple linear regressions, factors that had significant correlation with higher empowerment scores among type 2 diabetes patients included above secondary education level ($P < 0.001$), diabetes education exposure ($P = 0.003$), lack of ischemic heart disease ($P = 0.017$) and lower HbA1c ($P < 0.001$) as shown in **Table 6**.

DISCUSSION

In our study, the median score of the total diabetes empowerment was 110. We thus conclude that the empowerment of this study population is high based on the range for high empowerment score range in DES being 104 to 140. The total mean score found by Tol *et al*^[9] was 88.13 ± 30.3 , which indicated a middle score according to DES score range, lower than that of our study. This is probably due the difference of the education level between the two study populations, as less than half of their study population had a diploma or higher education, and the study was conducted in a diabetes research centre. A majority of our study population had an education above the secondary school level, the study was conducted in an urban primary care clinic setting. This may be due to socio-culture restrictions as well. For example, in Iran, quality diabetes care is not widely available, with a significant knowledge gap in handling diabetes. Diabetes diagnosis, prevention and management are suboptimal^[14].

The study findings showed that the subscale of "Setting and achieving diabetes goal" has highest median score among the three subscales. This finding is similar with two previous studies^[15,16]. The literature has shown that structured goal setting is the best way to aid diabetes patients to set behavior goals to practice healthy lifestyle and improve HbA1c level^[17,18].

This study shows that a higher than secondary school education level is significantly correlated with diabetes empowerment score. This result is similar with other studies. Tol *et al*^[18] showed that an education level of diploma or higher had higher empowerment score. Similarly, D'Souza *et al*^[19] showed that those with high school and diploma education level had higher diabetes empowerment scores^[15,16]. This indicates that patients with a higher education level possibly understand the disease process better and have more awareness towards self-care of diabetes management^[19].

Table 2 Clinical profiles of the type 2 diabetes mellitus patients with total diabetes empowerment scores, *n* (%)

Variables	Frequency	Median (IQR)
Diabetes duration (yr)		4.00 (7.0)
Compliance to diabetes treatment		
Yes	310 (96.3)	
No	12 (3.7)	
Diabetes education exposure		
Yes	264 (82.0)	
No	58 (18.0)	
BMI (kg/m ²)		28.70 (7.12)
Underweight (< 18.5)	3 (1.0)	
Normal (18.5-22.9)	29 (9.0)	
Overweight (23-27.4)	88 (27.3)	
Obese (> 27.5)	202 (62.7)	
Hypertension status		
Yes	207 (64.3)	
No	115 (35.7)	
Dyslipidaemia status		
Yes	246 (76.4)	
No	76 (23.6)	
Ischemic heart disease status		
Yes	42 (13.0)	
No	280 (87.0)	
Asthma status		
Yes	30 (9.3)	
No	292 (90.7)	

BMI: Body mass index; IQR: Interquartile range.

This study found that diabetes education exposure had a significant relationship with the total diabetes empowerment score. Those participants who had diabetes education exposure had better empowerment compared to those who had no diabetes education exposure. Diabetes education consists of structured programs, which cover basic information on diabetes, insulin therapy, blood glucose levels and targets, physical exercise, diet management and hypoglycemia^[20]. It incorporates practical skills especially using the home blood glucose monitoring and insulin therapy in diabetes management. The education program also emphasizes the importance of achieving targeted glycemic control to prevent complications and it includes foot care^[21]. Thus, those who received diabetes education exposure are better skilled in managing their disease, as reported in the literature. Enhancement of patient empowerment is achieved when patients are educated with adequate information on their health conditions^[22].

Our study showed a significant correlation between those without ischemic heart disease with total diabetes empowerment score. The majority of the patients without ischemic heart disease had secondary education and above (89.6%), which correlates to higher diabetes empowerment score.

Our study found that HbA1c was 7.4% with an IQR of 2.6. It would be better to compare this to the mean HbA1c for type 2 DM population in Malaysia^[23]. According to National Diabetes Registry, the mean HbA1c for type 2 DM from 2009 to 2012 was 8.1. Our study showed that a lower HbA1c level was significantly correlated with higher diabetes empowerment scores. This finding is consistent with those of a previous study^[16]. Patients with higher empowerment score were better in self-care and practicing healthy lifestyle contributing to a better HbA1c level^[8,24,25]. Age had no significant correlation with diabetes empowerment score in our study. Our study participants were aged between 26 to 84 years old with a median age of 55 (IQR = 18). This finding was not similar compared to the study done previously by D'Souza *et al*^[19] in a study in Oman, which reported that higher empowerment levels were seen among those 40-49 years old.

Table 3 The correlation between clinical outcome with total diabetes empowerment scores and subscales and among type 2 diabetes mellitus patients

Variables	Total diabetes empowerment score		Managing the psychosocial aspect of diabetes		Assessing dissatisfaction and readiness to change		Setting and achieving diabetes goal	
	Coefficient correlation	P value	Coefficient correlation	P value	Coefficient correlation	P value	Coefficient correlation	P value
Systolic blood pressure ¹ (mmHg)	0.046	0.411	0.073	0.192	0.014	0.798	0.013	0.821
Diastolic blood pressure ¹ (mmHg)	-0.009	0.867	-0.011	0.849	-0.055	0.323	0.024	0.674
HbA1c ¹ (%)	-0.132	0.018	-0.122	0.028	-0.11	0.049	-1.168	0.003
HDL level ¹ (mmol/L)	0.022	0.693	0.019	0.734	0.013	0.816	0.104	0.063
LDL level ¹ (mmol/L)	-0.087	0.12	-0.064	0.252	-0.044	0.435	-0.062	0.269
TG level ¹ (mmol/L)	-0.034	0.538	0.043	0.438	-0.015	0.788	-0.067	0.231

¹Indicates Spearman's test was used. HbA1c: Glycated hemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides.

There is no significant relationship between diabetes duration with total diabetes empowerment score in our study. This contradicts a study done in Oman in which the duration of diabetes was significantly correlated with total diabetes empowerment score^[16]. The median diabetes duration in our study was 4 years (with IQR = 7), similar to a study in Iran^[15]. However, in the study in Oman, 63% of participants had been diagnosed with diabetes for more than 10 years^[16]. Patients have better empowerment when they had diabetes for a longer duration, which translates into a longer duration of learning and adopting skills and knowledge through experience and exposure to diabetes education to make better decisions for self-care, set targets and achieve goals^[10].

In our study, there was no significant correlation between gender and empowerment score. Tol *et al*^[18] showed that females were more empowered than males, probably due to the distribution of their sample, in which more than half of their participants were female. The literature indicates that gender may influence lifestyle modification, as men are more proactive with their health, but women are more likely to change eating habits^[26].

Strengths and limitations

To date, this is the first study conducted among type 2 diabetes patients in the primary care setting in Malaysia. Furthermore, the sample size of this study is relatively larger than others in the literature^[15,16]. In addition, this study has not only identified socio-demographic factors, but also correlates clinical profiles and outcomes with total diabetes empowerment scores, which has not been reported by any local studies, especially in the primary care setting. The limitations are mainly due to the recruitment of participants at a single clinic, which may not be representative of the country's population. This is due to the short duration of the study and limitation of human resources. Therefore, similar future studies should consider multiple centers. This is a cross sectional study, and only an associational and not causal relation can be inferred in this study.

Our study reported high empowerment scores among type 2 diabetes patients. Potential predictors for total diabetes empowerment scores in our study included higher than secondary education level, diabetes education exposure, lack of ischemic heart disease and lower HbA1c levels.

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Table 4 The correlation between sociodemographic factors with total diabetes empowerment scores and subscales among type 2 diabetes mellitus patients

Variables	Total diabetes empowerment score			Managing the psychosocial aspect of diabetes			Assessing dissatisfaction and readiness to change			Setting and achieving diabetes goal		
	Coefficient correlation	Median rank	P value	Coefficient correlation	Median rank	P value	Coefficient correlation	Median rank	P value	Coefficient correlation	Median rank	P value
Age ¹	0.05		0.37	0.095		0.087	0.089		0.111	-0.06		0.919
Gender ²			0.629			0.692			0.173			0.528
Male		159.4			163.18			155.6			158.79	
Female		164.49			159.11			169.89			165.35	
Ethnicity ²			0.56			0.2			0.757			0.332
Malay		162.38			163.38			161.04			162.94	
Non-Malay		151.06			139.14			167			144.42	
Education level ²			< 0.001			< 0.001			< 0.001			< 0.001
Below Secondary		109.36			106.86			110.49			105.69	
Above Secondary		168.06			168.38			167.92			168.52	
Marital Status ²			0.478			0.119			0.346			0.564
Single		148.82			134.28			144.74			151.34	
Married		162.57			163.79			162.91			162.36	
Smoking status ²			0.18			0.704			0.008			0.105
Non-smoker		166.42			162.87			171.18			167.38	
Smoker		151.75			158.79			142.32			149.85	

¹Indicates Spearman's test was used.²Indicates Mann Whitney test was used.

Last but not least, we would like to thank the Director General of Health Malaysia for his permission to publish this article.

Table 5 The correlation between clinical profiles with total diabetes empowerment scores and subscales among type 2 diabetes mellitus patients

Variables	Total diabetes empowerment score			Managing the psychosocial aspect of diabetes			Assessing dissatisfaction and readiness to change			Setting and achieving diabetes goal		
	Coefficient correlation	Median rank	P value	Coefficient correlation	Median rank	P value	Coefficient correlation	Median rank	P value	Coefficient correlation	Median rank	P value
Diabetes Duration ¹ (yr)	-0.016		0.774	-0.1		0.857	0.011		0.847	-0.055		0.324
Diabetes education exposure ²			0.004			0.01			0.001			0.05
Yes		168.43			167.59			169.72			168.18	
No		129.97			133.8			126.38			131.1	
Compliance to treatment ²			0.326			0.538			0.284			0.241
Yes		162.5			162.11			162.59			162.68	
No		135.63			145.67			133.38			131.04	
BMI ³ (kg/m ²)			0.568			0.96			0.605			0.938
Underweight												
Normal												
Overweight												
Obese												
Hypertension status ²			0.11			0.478			0.707			0.052
Yes		155.53			158.82			160.06			154.11	
No		172.6			166.32			165.1			174.81	
Dyslipidemia status ²			0.789			0.371			0.679			0.341
Yes		162.27			164.02			162.69			158.8	
No		159.01			153.35			157.66			170.24	
Ischemic heart disease status ²			0.004			0.011			0.104			0.001
Yes		122.83			128.32			139.83			118.65	
No		167.3			166.48			164.75			167.93	
Asthma status ²			0.69			0.265			0.829			0.4
Yes		167.95			179.08			158.02			174.92	
No		160.84			159.69			161.86			160.12	

¹Indicates Spearman's test was used.²Indicates Mann Whitney test was used.³Indicates Kruskal Wallis was used. BMI: Body mass index.

Table 6 Predictor of total empowerment scores among type 2 diabetes mellitus patients using multiple linear regressions

Variables	Unstandardized coefficients		t	Sig.	95%CI for B	
	Beta				Lower bound	Upper bound
Those without ischemic heart disease	5.621		2.409	0.017	1.03	10.212
Those with secondary education level and above	16.023		6.263	< 0.001	10.99	21.057
HbA1c level	-1.403		-3.668	< 0.001	-2.155	-0.65
Those received DM education exposure	6.301		3.026	0.003	2.204	10.399
Smoker status	-1.157		-0.685	0.494	-4.481	2.168
Hypertension status	1.866		1.098	0.273	-1.444	5.092

Dependent Variable: Total DES; Beta is coefficient of the gradient of the regression line and the strength of the relationship between a predictor and the outcome variable; *t* is t-statistic tests; Sig is *P* value. DM: Diabetes mellitus; DES: Diabetes empowerment scores; HbA1c: Glycated hemoglobin.

ARTICLE HIGHLIGHTS

Research background

There is a limited study on the diabetes empowerment among type 2 diabetes patients particularly in primary care settings. This study aims to assess the diabetes empowerment scores and its correlated factors among type 2 diabetes patients in a primary care clinic in Malaysia.

Research motivation

Diabetes is becoming a global epidemic of the 21st century and over 70% of known cases of diabetes occur in the developing countries. Evidence shows that self-empowerment is important in managing chronic diseases, especially diabetes. Self-empowerment is an approach that can improve the ability of the patients with diabetes to understand the disease process better, involve actively in self-care and practice healthy lifestyles for better disease control. Therefore, it is very crucial to identify the predictors for diabetes empowerment score among type 2 diabetes patients.

Research objectives

Our objective was to access the diabetes empowerment score among type 2 diabetes patients, also to identify correlated factors with diabetes empowerment scores among type 2 diabetes mellitus (DM) patients in primary care clinic. In addition, we aimed to identify the predictors for diabetes empowerment score among type 2 diabetes patients.

Research methods

This is a cross sectional study involving 322 adults with type 2 DM patients followed up in a primary clinic. Systematic sampling method was used for patients' recruitment. The Diabetes Empowerment Scale (DES) questionnaire was used to measure patients' empowerment. Data analysis was done using SPSS version 25 and multiple linear regressions was used to identify the predictors of total diabetes empowerment scores.

Research results

Median age of the study population was 55 years old, 56% were male and mean duration of diabetes was 4 years. The total median score of the DES was 110 [interquartile range (IQR) = 10]. The median scores of the three subscales were 40 with (IQR = 4) for "Managing the psychosocial aspect of diabetes", 36 with (IQR = 3) for "Assessing dissatisfaction and readiness to change" and 34 with (IQR = 5) for "Setting and achieving diabetes goal". According to multiple linear regressions, factors that had significant correlation with higher empowerment scores among type 2 diabetes patients were those who had above secondary education level ($P < 0.001$), those who had diabetes education exposure ($P = 0.003$), those who had no ischemic heart disease ($P = 0.017$) and those who had lower glycated hemoglobin (HbA1c) level ($P < 0.001$).

Research conclusions

Diabetes empowerment scores were high among type 2 diabetes patients in this study population. The predictors for high empowerment score were those who had above secondary education level, diabetes education exposure, no ischemic heart disease status and lower HbA1c.

Research perspectives

Given the high empowerment score were those who had above secondary education level, diabetes education exposure, no ischemic heart disease status and lower HbA1c, hence all the diabetes patients should be educate and empower on self-care for long-term diabetes management.

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HNF1A mutation in a Thai patient with maturity-onset diabetes of the young: A case report

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Abstract

BACKGROUND

Maturity-onset diabetes of the young (MODY) is the most common form of monogenic diabetes. The disease is transmitted in autosomal dominant mode and diabetes is usually diagnosed before age 25 year. MODY 3 is caused by mutation of hepatocyte nuclear factor (*HNF*) 1A genes and is the most common MODY subtype. Diagnosis of MODY 3 is crucial since glycemic control can be accomplished by very low dose of sulfonylurea. In this report we described a Thai MODY 3 patient who had excellence plasma glucose control by treating with glicazide 20 mg per day and insulin therapy can be discontinued.

CASE SUMMARY

A 31-year-old woman was diagnosed diabetes mellitus at 14 years old. The disease was transmitted from her grandmother and mother compatible with autosomal dominant inheritance. Sanger sequencing of proband's DNA

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identified mutation of *HNF1A* at codon 203 which changed amino acid from arginine to cysteine (R203C). This mutation was carried only by family members who have diabetes. The patient has been treated effectively with a combination of oral hypoglycemic agents and must include a very low dose of glicazide (20 mg/d). Insulin therapy was successfully discontinued.

CONCLUSION

We demonstrated a first case of pharmacogenetics in Thai MODY 3 patient. Our findings underscore the essential role of molecular genetics in diagnosis and guidance of appropriate treatment of diabetes mellitus in particular patient.

Key words: Oral sulfonylureas; Maturity-onset diabetes of the young; *HNF1A*; Case report

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Core tip: Maturity-onset diabetes of the young (MODY) is the most common form of diabetes in patients diagnosed under the age of 25. In addition, MODY is characterized by autosomal dominant inheritance. We report a R203C mutation in the *HNF1A* causing MODY type 3. The genetic diagnosis is implicated to alter SU treatment. This study revealed that excellent glycemic control in this patient could be achieved by very low dose SU. Furthermore, this is the first report of exceptional response to treatment with SU in Thai MODY3.

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INTRODUCTION

Maturity-onset diabetes of the young (MODY) is the most common type of monogenic diabetes, it is inherited in an autosomal dominant manner, and it is normally diagnosed before 25 years of age. To date, at least 15 subtypes of MODY caused by mutations of 15 different genes have been identified^[1,2]. Thus, the clinical heterogeneity of MODY is explained by its genetic heterogeneity^[3]. MODY3 is caused by mutation of hepatocyte nuclear factor 1A (*HNF1A*), which encodes a transcription factor that regulates functions of several proteins, including amylin, insulin, GLUT2, and L-pyruvate kinase, that are important for glucose metabolism and insulin secretion. *HNF1A* dysfunction are leading to Diabetes development and imbalance of insulin in patients. More than 350 mutations of *HNF1A* have been identified, and MODY3 is the most common MODY subtype among Caucasians^[4]. In contrast, Asians most commonly have MODY-X or MODY subtype without identified genetic cause^[5-10]. Identification of MODY3 is very important, because pancreatic β -cells exhibited hyperexcitability in this subtype in response to treatment with sulfonylurea (SU)^[11]. The Siriraj Center of Research Excellence for Diabetes and Obesity (SiCORE-DO) discovered 3 different *HNF1A* mutations, including R203C, G554fsX556, and P475L, in 3 unrelated MODY pedigree^[12-14]. Here, we report a Thai MODY3 patient carrying *HNF1A* R203C mutation that exhibited outstanding diabetes control with low-dose glicazide, which is a short-acting second-generation SU. Rapid deterioration of her glycemic control was observed after withdrawal of SU. The purpose of this report is to present alteration of drug treatment in patient by genetic diagnosis.

CASE PRESENTATION

Chief complaints

A 31-year-old Thai woman came to Siriraj Diabetes Center, Siriraj Hospital, Bangkok, Thailand for her diabetes management.

History of present illness

The patient has been following up every 3 months at the Siriraj Diabetes Center, Siriraj Hospital, Bangkok, Thailand. Currently, she was 44 years old and treated with glicazide 20 mg/d. She has excellent glycemic control without diabetic complications. Laboratory assessment included fasting plasma glucose (FPG) 78 mg/dL, hemoglobin A1c (HbA1c) 6.7%, serum creatinine (0.56 mg/dL), total cholesterol (TC) 173 mg/dL, high-density lipoprotein (HDL) 99 mg/dL, low-density lipoprotein (LDL) 62.6 mg/dL, and triglycerides (TG) 57 mg/dL.

History of past illness

The patient was first seen at Siriraj Diabetes Center when she was 31 years old and diabetes was diagnosed at age 14.

Personal and family history

Her mother and brother were diagnosed with diabetes at age 17 and 13, respectively. There was no history of diabetic ketoacidosis, and glycemic control could be achieved without insulin treatment for more than 5 years after diabetes diagnosis in all 3 patients.

Physical examination upon admission

The patient's body mass index (BMI), waist-to-hip ratio, and blood pressure was 19.43 kg/m², 0.83, and 120/70 mmHg, respectively (Table 1).

Laboratory examinations

Laboratory assessments at her first visit to Siriraj Diabetes Center included FPG 126 mg/dL, HbA1c 9.5%, serum creatinine (0.6 mg/dL), TC 156 mg/dL, HDL 71 mg/dL, LDL 90 mg/dL, and total TG 55 mg/dL.

Sequencing profile and timeline of patient's glycemic control with and without SU

Sanger sequencing of her DNA revealed heterozygous mutation of *HNF1A* at codon 203 in exon 3 that caused substitution of cysteine for arginine (R203C) (Figure 1). This mutation was also identified in all diabetic family members, but not in non-diabetic family members whose DNA were available for sequencing (Figure 2). The patient's glycemic control profile (with and without SU) is shown in Figure 3. The results of our analysis revealed that excellent glycemic control could only be achieved when our patient was taking SU. Interestingly – when SU treatment was withdrawn, severe hyperglycemia eventually developed, even when insulin was given. The optimal dose of glicazide in this case was 20 mg per day. This patient continues to do well with no observed diabetic complications.

FINAL DIAGNOSIS

SU hyperresponsiveness in MODY subtype 3 due to *HNF1A* mutation.

TREATMENT

The patient has been successfully treated with glicazide 20 mg/d, metformin 2000 mg/d and sitagliptin 100 mg/d.

OUTCOME AND FOLLOW-UP

The patient's glycemic control has been excellent and without hypoglycemic episodes during the last 4 years of follow up. No diabetic complications have developed.

DISCUSSION

MODY3 is one of the best examples of precision medicine in diabetes. Studies in animal models showed that total deletion of *HNF1A* resulted in decreased SU uptake by hepatocytes and decreased excretion^[2,12,15,16]. Clinical studies in humans demonstrated that MODY3 patients treated with SU exhibited excellent glycemic control, and withdrawal of SU led to severe hyperglycemia – even with insulin treatment. However, dosage adjustment is essential since inappropriate SU dose can lead to hypoglycemia^[11]. The current recommendation for treatment of MODY3 patients is to

Table 1 Demographic, anthropometric, and clinical characteristics of the case profiled in this report

Characteristics	Values
Age (yr)	31
Age at onset (yr)	14
Duration (yr)	17
BMI (kg/m ²)	19.43
Waist circumference (cm)	77
Waist-to-hip ratio	0.83
Systolic BP (mmHg)	120
FPG (mg/dL)	126
HbA1c (%)	9.5
Serum creatinine (mg/dL)	0.6
Total cholesterol (mg/dL)	156
Total triglycerides (mg/dL)	55
LDL (mg/dL)	90
HDL (mg/dL)	71.0

BMI: Body mass index; BP: Blood pressure; FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin; LDL: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein cholesterol.

use a very low dose of SU. Caution should be exercised if SU is to be withdrawn from the treatment plan since a deterioration in the patient's glycemic status can be anticipated. Our MODY3 patient exhibited exceptional plasma glucose control using a very low dose of glicazide, and severe hyperglycemia developed after glicazide was discontinued, even though she was treated with metformin, sitagliptin, and insulin glargine. Moreover, her glicazide dosage was titrated to 20 mg/d to avoid hypoglycemia, even though the usual dose is up to 80 mg/d for treatment of type 2 diabetes. Upon reaching her maintenance dosage and after stabilization of her blood sugar, insulin therapy could be discontinued and the durability of glycemic control has been almost 4 years (Figure 3). To our knowledge, this is the first report of exceptional response to treatment with SU in Thai MODY3. Our findings are in agreement with those from previous reports in MODY3 patients from different ethnicities, including Caucasian, Saudi Arabian, and Tunisian^[17-19]. A study from the United Kingdom reported lower HbA_{1c} and lower BMI at genetic diagnosis, and shorter duration of diabetes to be factors that significantly influence treatment success after treatment with SU in MODY3 patients^[20]. However, this finding has not yet been investigated or confirmed in Asian population due to the relatively lower prevalence of MODY3 in this ethnicity.

CONCLUSION

In this report, we presented and described a 31-year-old Thai MODY3 patient with a heterozygous mutation of *HNF1A* at R203C who demonstrated excellent diabetic control with a very low dose of SU. To our knowledge, this is the first report of exceptional response to treatment with SU in Thai MODY3. Our findings emphasize the critical role of correct genetic diagnosis, especially in patients with early-onset diabetes.

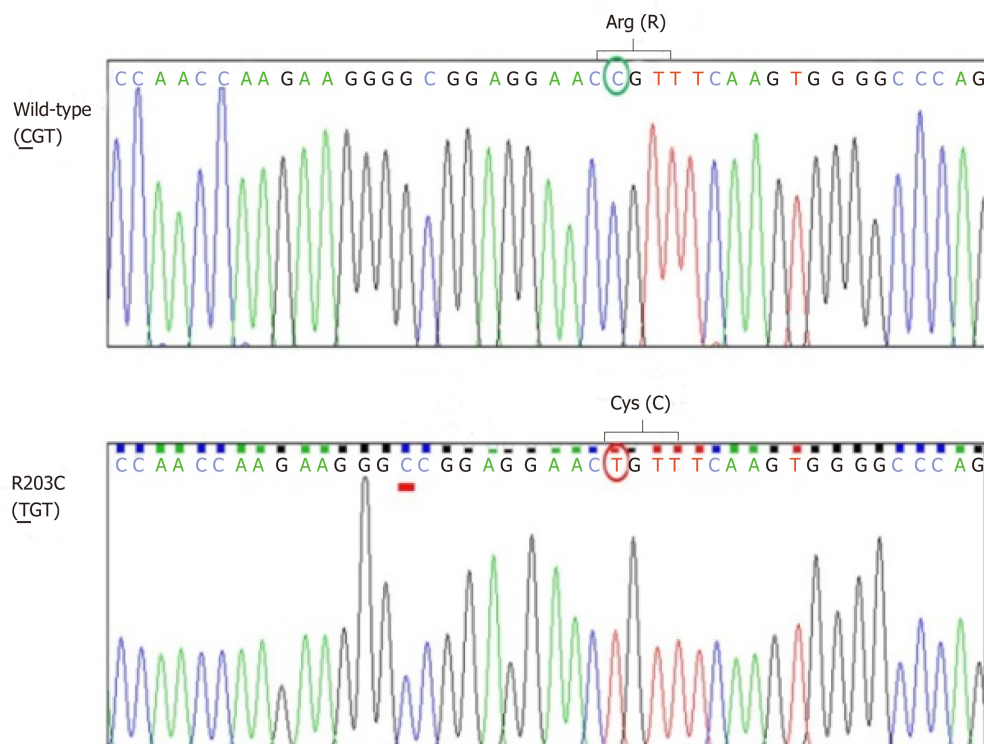


Figure 1 Sequencing profile of exon 3 of *HNF1A* in the mutation region (R203C). The green circle indicates the location of C in wild-type, and the red circle indicates the location of T substitution in heterozygous.

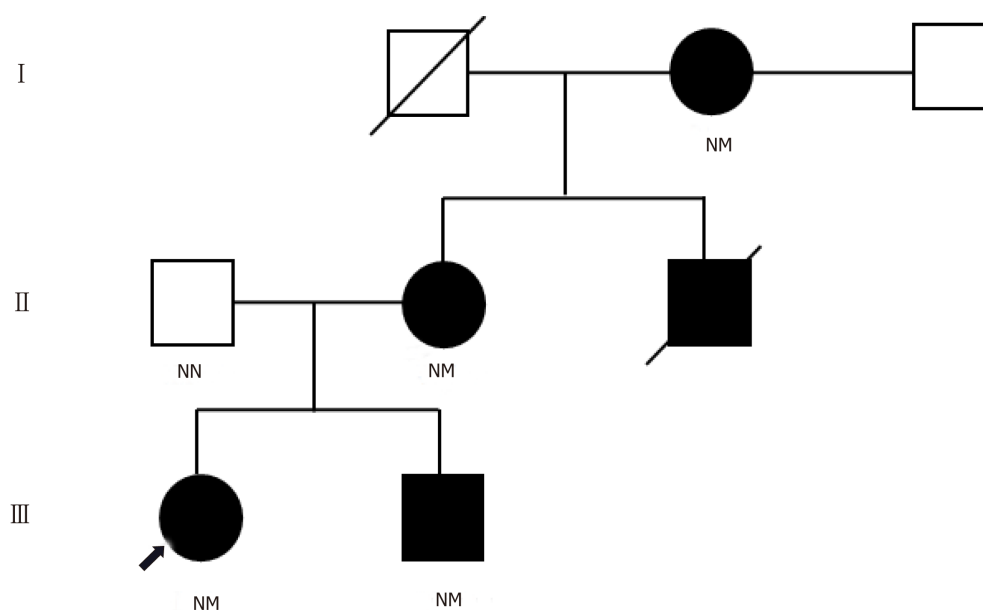


Figure 2 Pedigree showing autosomal dominant inheritance of diabetes associated with a hepatocyte nuclear factor-1-alpha mutation. Symbols and abbreviations: Circles: Females; squares: Males; Darkened circles or squares: Diabetes; NM: Heterozygous *HNF1A* R203C; NN: *HNF1A* wild-type genotype.

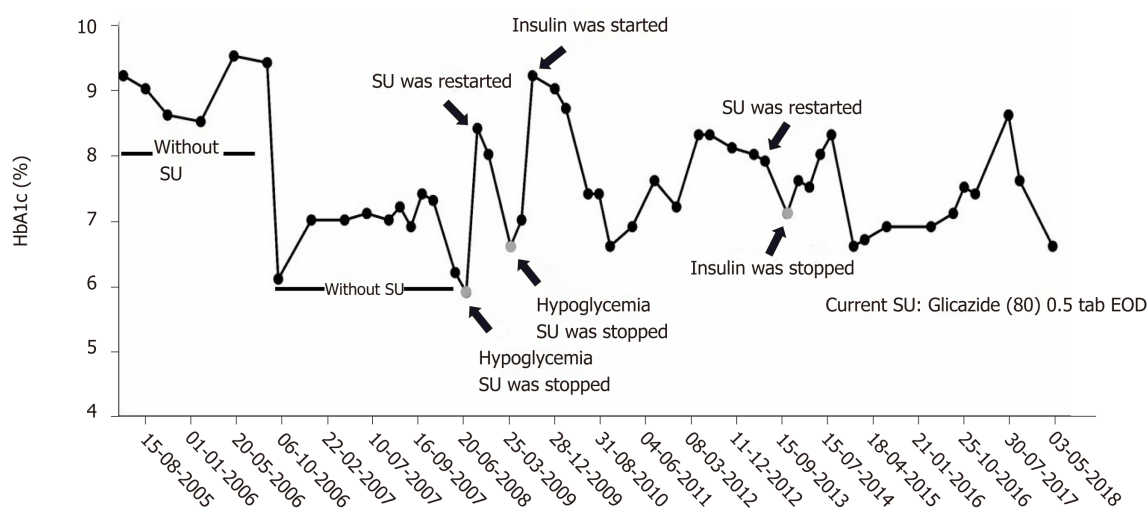


Figure 3 Timeline of patient's glycemic control with and without sulfonylurea.

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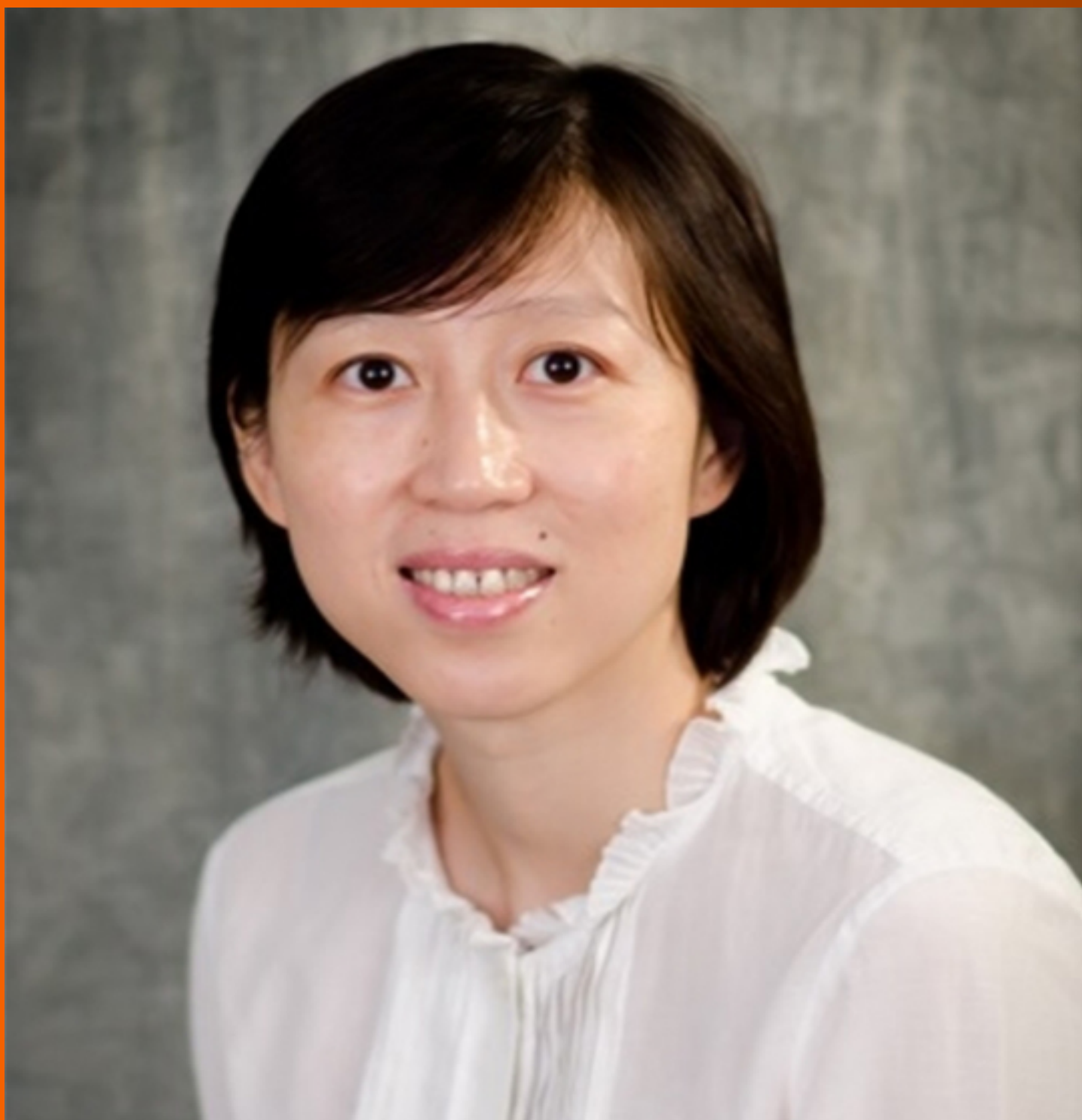


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Bone health in diabetes and prediabetes

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Abstract

Bone fragility has been recognized as a complication of diabetes, both type 1 diabetes (T1D) and type 2 diabetes (T2D), whereas the relationship between prediabetes and fracture risk is less clear. Fractures can deeply impact a diabetic patient's quality of life. However, the mechanisms underlying bone fragility in diabetes are complex and have not been fully elucidated. Patients with T1D generally exhibit low bone mineral density (BMD), although the relatively small reduction in BMD does not entirely explain the increase in fracture risk. On the contrary, patients with T2D or prediabetes have normal or even higher BMD as compared with healthy subjects. These observations suggest that factors other than bone mass may influence fracture risk. Some of these factors have been identified, including disease duration, poor glycemic control, presence of diabetes complications, and certain antidiabetic drugs. Nevertheless, currently available tools for the prediction of risk inadequately capture diabetic patients at increased risk of fracture. Aim of this review is to provide a comprehensive overview of bone health and the mechanisms responsible for increased susceptibility to fracture across the spectrum of glycemic status, spanning from insulin resistance to overt forms of diabetes. The management of bone fragility in diabetic patient is also discussed.

Key words: Bone; Fractures; Type 1 diabetes; Type 2 diabetes; Prediabetes; Diabetes complications; Bone density; Hypoglycemic agents

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Core tip: Diabetes mellitus, either type 1 or type 2, is associated with increased fracture risk. Diabetic hyperglycemia and insulin resistance underlie functional alterations of bone cells and bone marrow fat that affect several determinants of bone strength, including bone matrix proteins and bone mass, geometry and microarchitecture.

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Diabetes-related microvascular complications and certain antidiabetic drugs appear to further increase fracture risk, both directly and indirectly. The prevention and management of bone fragility in diabetes includes identification of patients at risk, correction of modifiable risk factors including appropriate choice of antidiabetic drugs and use of anti-fracture drugs with proven efficacy.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia leading to serious microvascular and macrovascular complications. In recent years, bone fragility has emerged as a further complication of DM, both Type 1 diabetes (T1D) and type 2 diabetes (T2D). Aim of this review is to provide a comprehensive overview of bone health across the spectrum of glycemic status, spanning from insulin resistance to overt forms of diabetes.

Insulin and bone

Insulin is an anabolic hormone central to the regulation of substrate metabolism in key organs and tissues such as skeletal muscle, the liver and adipose tissue^[1]. Both osteoblasts and osteoclasts express the insulin receptor. Insulin stimulates osteoclast formation and promotes proliferation, differentiation and survival of osteoblasts, with an overall balance in favor of bone formation^[2]. Studies on insulin receptor knockout mice indicate that insulin signaling is necessary for normal bone acquisition^[3,4], likely due to the role of insulin in the regulation of bone energy metabolism. In fact, insulin administration increases ¹⁸F-fluorodeoxyglucose ([¹⁸F]FDG) uptake by bone in mice, which is markedly reduced in mice lacking the insulin receptor in osteoblasts^[5]. Furthermore, activation of the insulin receptor in the growth plate of mice fed with a hypercaloric diet stimulates skeletal growth and growth plate chondrogenesis^[6]. Osteoblasts also express the Insulin-like growth factor 1 (IGF-1) receptor^[7]. IGF-1 binds both to the IGF-1 receptor and, with lower binding affinity, to the insulin receptor, thus triggering the insulin signaling pathway and exerting osteoanabolic actions.

DM

Depending on the pathogenic mechanism(s) causing chronic hyperglycemia, DM is classified into few main general categories. T1D is distinguished by absolute insulin deficiency due to destruction of pancreatic beta-cells on an autoimmune or idiopathic base. Latent autoimmune diabetes in adults (LADA) is a less common form of autoimmune diabetes that arises in the adult age and is characterized by circulating islet autoantibodies and insulin independence at diagnosis. In T2D, insulin resistance leading to compensatory increase of insulin secretion causes progressive worsening of beta cell function that eventually results in relative insulin deficiency and hyperglycemia. Other forms of DM include monogenic forms (*e.g.*, maturity onset diabetes of the young, MODY), gestational diabetes, and secondary forms either associated with conditions that affect insulin secretion (*e.g.*, pancreatic diseases) or certain drugs (*e.g.*, glucocorticoids and immunosuppressants after organ transplantation). This review will focus on the main diabetes categories, *i.e.* T1D and T2D, as well as on those alterations of glucose metabolism collectively identified as prediabetes^[8].

Diabetes and prediabetes: clinical impact on bone

Fracture risk in T1D

Fracture risk is increased in T1D, with a 2- to 6-fold higher risk of fracture as compared with non-diabetic subjects, the risk being greatest in T1D women^[9,10]. In a recent analysis that assessed the determinants of fracture risk in T1D adult patients, nearly half of the subjects reported at least one fracture after diabetes diagnosis^[11].

Older age, longer T1D duration, age < 20 years at diagnosis and family history of osteoporosis or osteopenia were associated with fracture occurrence.

Fracture risk in T2D and prediabetes

Individuals with T2D have a 1.2- to 3-fold higher risk of fracture as compared with non-diabetic subjects, particularly for hip fractures^[9,12], but also for upper arm and ankle fractures^[13]. Fracture risk appears to be greater in those with a body mass index (BMI) < 30 kg/m² as compared with obese individuals^[14], and not to significantly differ by gender^[9,15]. Diabetes duration longer than 10 years, low levels of physical activity, use of insulin and systemic corticosteroids and increasing age are also associated with higher fracture risk in T2D^[14]. Falls represent another risk factor for fractures, especially in diabetic women^[14,16,17]. The association between diabetes, especially T2D, and increased risk of falls is well recognized^[18,19] and mainly attributed to diabetes related complications such as therapy-induced hypoglycemic episodes, impaired muscle strength due to sarcopenia, retinopathy-related impaired vision, peripheral artery disease and neuropathy^[20,21]. As in a vicious circle, fractures may lead to imbalance, alterations in posture and decreased muscle strength, eventually reducing physical performance and further increasing the risk of falls^[22]. Predictive factors of falls and their contribution to fracture risk in T1D patients have not been clearly identified^[23].

Despite a clear association between T2D and increased fracture risk^[9,19,24], evidence supporting an association between prediabetes and fracture risk is inconsistent. Observations in adolescents suggest that insulin resistance may be detrimental for bone development through puberty, independent of body composition and the level of physical activity^[25]. However, no association between insulin resistance and fracture risk was evident after adjustment for BMI and bone mineral density (BMD) in a large cohort of elderly subjects^[26]. These findings are consistent with studies that found no statistically significant difference in fracture risk between subjects with or without prediabetes^[27,28], but are in contrast with those reporting an association between prediabetes, adjusted for BMI and/or BMD, and lower fracture risk^[29].

Assessment of fracture risk in diabetes

Schwartz and colleagues analyzed data from nearly 17,000 older community-dwelling men and women, and found that, for a given T-score and age or FRAX[®] score (the most widely used fracture risk index), subjects with diabetes had a higher fracture risk than those without diabetes^[30]. Similarly, Giangregorio *et al*^[31] found that FRAX underestimates the risk of major osteoporotic and hip fractures in individuals with diabetes. Recently, four options have been assessed to enhance the performance of FRAX in patients with DM (using rheumatoid arthritis as a proxy for the effects of DM, trabecular bone score [TBS]-adjustment, reducing the femoral neck T-score input by 0.5 SD, increasing the age input by 10 years)^[32]. Although each correction improved the performance of the FRAX tool in predicting fracture risk, no single method was optimal for all fracture outcomes and durations of diabetes.

DIABETIC BONE DISEASE-PATHOPHYSIOLOGY

Several factors might be responsible for the increased fracture risk in diabetic patients. Diabetes-related changes affect bone strength, which in turn depends on different and complex components, *i.e.* BMD, bone microarchitecture and its microenvironment and material properties.

Bone cells

Cellular and molecular components cross-talk to maintain skeletal integrity in an intricate balance that can be altered in DM. It is important to understand alterations in these components, as they have also direct clinical consequences and may represent targets for clinical interventions. Structural elements with a role in physiologic bone formation include support cells like osteoblasts and osteocytes, remodeling cells known as osteoclasts, and non-cellular components like osteoid (hydroxyapatite, collagen, non-collagen-structural proteins) and mineral salts deposited within the matrix. Mesenchymal stem cells (MSC), *i.e.*, the osteoblast precursors, may also differentiate into adipocytes. The fate of MSCs depends on a fine balance between the WNT signaling pathway, which promotes osteogenesis, and the peroxisome proliferator-activated receptor- γ (PPAR- γ) pathway, which promotes adipogenesis^[33]. An imbalance between these pathways may result in one cell type predominating over the other. Along with the bone-resorbing osteoclasts, osteoblasts are involved in a fundamental process that lasts the whole human life, bone remodeling, wherein old bone is substituted with new bone to maintain bone strength and mineral

homeostasis, and to repair microdamage^[34].

Osteoblasts in T1D

Preclinical studies^[25] documented alterations in transcription of osteoblasts promoting genes, in particular Runx2, which is involved in MSC differentiation into pre-OBs and in the regulation of bone matrix protein genes. Some preclinical studies suggested that Runx2 is downregulated by hyperglycemia^[35,36], although other studies reported no modification^[37,38]. The *Wnt/beta catenin* gene, which is known to promote OB differentiation, is also downregulated^[39]. In T1D, low levels of IGF-1, which promotes differentiation of MSCs into OBs^[40,41] and bone mineralization^[42], may also contribute to reduced bone formation. It is also known that serum from T1D patients decreases collagen production in human OBs when used as a culture medium^[43]. Moreover, individuals with T1D have low levels of parathyroid hormone (PTH)^[44], which in normal conditions prevents OB apoptosis^[45], improves bone density and increases mineralization and enhances, synergistically with IGF-1, osteoblast differentiation into osteocytes^[46]. An increase in circulating levels of proinflammatory cytokines such as TNF- α , IL1 and IL6 due to hyperglycemia^[47,48], may impair OB proliferation and differentiation *in vitro*^[49-53], or even stimulate OB apoptosis^[54,55], while inhibiting bone healing *in vivo*^[56]. Overall, the evidence suggests that an impairment on OB function and survival may be responsible for reduced bone formation in T1D.

Osteoblasts in T2D

Few studies on OBs from T2D subjects are available. Postmenopausal women with T2D were reported to have higher levels of OB precursor cells than BMI-matched non-diabetic controls. OBs were more immature compared with controls, and Dickkopf-related protein 1 (DKK-1), a regulator produced by bone marrow stromal cells that inhibits OB maturation, was increased^[57]. Thus, it appears that individuals with T2D have increased levels of immature OBs, which may explain lower bone quality and higher BMD.

Osteocytes in T1D

In mouse models of T1D, a reduction in osteocyte density and number, and an increase in apoptosis have been reported^[58-60]. Sclerostin, an osteocyte-derived protein that inhibits bone formation^[61,62] and stimulates OB apoptosis^[63], is elevated in adults with long-standing T1D^[64] prediabetes^[65], or T2D^[66]. Surprisingly, however, a large Danish retrospective study of T1D patients found that T1D patients with higher serum levels of sclerostin had a lower incidence of bone fractures^[67].

Osteocytes in T2D and prediabetes

As mentioned, osteocyte-derived sclerostin is elevated in adults with T2D and prediabetes^[65,66]. In T2D, there is a direct correlation between sclerostin levels, disease duration and glycemic control, and an inverse correlation with bone turnover markers^[66,68]. Anti-sclerostin antibodies increased bone mass in diabetic rats^[69]. This finding is of particular interest, as an anti-sclerostin monoclonal antibody (romosozumab) is now available for the treatment of osteoporosis in humans^[70].

Osteoclasts in T1D

In physiological conditions, the OB-derived receptor activator of nuclear factor kappa-B ligand (RANKL), promotes the differentiation and activation of osteoclasts through the receptor RANK on osteoclast surface. This process is inhibited by osteoprotegerin (OPG), also produced by OBs, which binds to RANKL thereby preventing its interaction with RANK. Patients with T1D and poor glycemic control exhibit more active bone resorption. Consistently, the analysis of peripherally detected osteoclasts in patients with T1D showed a lower sensitivity to inhibitory factors such as OPG^[71]. An increased *OPG* gene expression compared to healthy controls has also been reported^[72], possibly to compensate for the lower sensitivity to OPG. Other *in vitro* studies, however, showed a reduction in RANKL and its cellular actions in hyperglycemic environments^[73], which could indicate a limited role of RANKL and OPG in the pathogenesis of bone alterations in DM. Finally, a higher concentration of markers of osteoclastic activity (cathepsin K, tartrate-resistant acid phosphatase [TRAP], C terminal telopeptide) has been observed in insulinopenic mice^[74,75], although this increase was significant only in the case of severe or long-lasting diabetes. This variability in osteoclastic activation suggests that disease severity and duration may influence the degree of diabetes-induced bone resorption^[76,77].

Osteoclasts in T2D

High glucose levels inhibit osteoclast differentiation and suppress matrix degradation by osteoclasts in animal models of T2D^[78]. Accordingly, circulating osteoclast

precursors were found to be increased and more immature in T2D postmenopausal women compared with BMI-matched healthy controls, possibly due to lower RANKL levels^[57]. It may be speculated that a lower level of maturation compromises OC activity, leading to decreased bone resorption resulting in higher BMD in T2D.

BMD

BMD in T1D: Low BMD is reported in nearly all studies involving T1D patients of any age compared to non-diabetic controls^[79]. The reduction in BMD worsens with longer disease duration^[80], poor glycemic control, early age of onset of T1D, and higher insulin dosage^[81]. Furthermore, T1D adult patients with microvascular complications have lower BMD than those without microvascular disease^[81-86], suggesting a role for bone vascularization in the pathogenesis of diabetic bone disease. Children and adolescents with T1D have smaller cross-sectional areas and weaker bones despite an increase in bone formation markers, suggesting impaired osteoblast activity during growth^[87]. It is likely that an inadequate peak bone mass is reached at the end of the skeletal maturation due to low levels of IGF-1 and the catabolic effects of uncontrolled hyperglycemia during critical growth period^[88,89]. Consistently, patients with onset of diabetes before age 10 years reach a lower than average mean near-adult height, adult height being inversely correlated with glycemic control^[90].

Altered vitamin D and calcium metabolism due to hyperglycemia may further contribute to reduced BMD in T1D^[91]. Reduced BMD, however, might not be the only factor contributing to increased fracture risk. Recent observations suggest that, opposite to what one would expect, BMD does not worsen over time in patients with T1D as compared with nondiabetic individuals^[92].

BMD in T2D and prediabetes: Subjects with T2D generally have higher BMD as compared with healthy controls, with significant differences of 0.04 (95%CI: 0.02, 0.05) at the femoral neck, 0.06 (95%CI: 0.04, 0.08) at the hip and 0.06 (95%CI: 0.04, 0.07) at the spine^[93]. As insulin is known to exert anabolic effects on bone, high circulating insulin levels may explain the observed increase in BMD in T2D^[94]. Accordingly, some studies indicate a positive association between circulating insulin levels and BMD, independent of BMI^[95-97]. However, in most studies the positive association between insulin levels or indices of insulin resistance and BMD was lost after adjusting for BMI^[26,98-101], implying that the increase in BMD observed in insulin resistant states is mediated by body mass. In fact, obesity has long been considered to be protective towards osteoporosis and osteoporotic fractures, being associated with increased mechanical load stimulating bone formation^[102], androgens-to-estrogens conversion in adipose tissue, lower serum levels of sex hormone binding globulin (SHBG)^[103], increased circulating leptin^[104] and insulin growth factor, and hyperinsulinemia^[99]. Recent findings challenge this belief, suggesting that even though BMD increases with body weight, this cannot compensate for obesity-associated greater impact forces during falls. Data from a multiethnic cohort of nearly 2000 pre- or perimenopausal women indicate that higher BMI is associated with higher BMD, but also with lower composite strength indexes^[105]. Conflicting data on the association between obesity and fracture risk, with earlier studies demonstrating a protective effect^[106-109] and more recent studies indicating an increase in risk^[110-114], suggest that BMI is not the only relevant factor in this context, and that body composition and fat distribution may also play a role^[115]. Elevated waist circumference and waist-to-hip ratio have been associated with an increased hip fracture risk in a large prospective cohort study^[116]. In obese Chinese women, increased fat mass and percent body fat were positively associated with BMD, whereas increased central fat was inversely associated with BMD^[117]. Accordingly, visceral adiposity has been associated with increased risk of both vertebral and non-vertebral fractures^[118,119]. Central adiposity reflects the amount of visceral adipose tissue (VAT), which is more cellular, vascular, innervated and characterized by the presence of more inflammatory and immune cells, lesser pre-adipocyte differentiating capacity and higher proportion of large adipocytes as compared with subcutaneous adipose tissue (SAT)^[120]. VAT is tightly correlated with insulin resistance^[121], which, together with low-grade chronic inflammation, possibly mediates the relationship between VAT and increased fracture risk.

In Korean men diagnosed with prediabetes using an oral glucose tolerance test, no significant difference in BMD T-score was found as compared with subjects having normal glucose metabolism^[122]. Despite no difference in total body BMD between prepubertal overweight children with prediabetes *vs* non-prediabetic controls (as assessed by OGTT)^[123], total body bone mineral content (BMC) was found to be significantly lower in prediabetic children. Inverse associations were found between BMC and markers of insulin resistance and inflammation (C-reactive protein).

Bone turnover

Bone turnover may be assessed by measuring bone turnover markers (BTMs), which reflect the bone resorption and formation processes.

Bone turnover in T1D: In general, both T1D and T2D are considered as states of low bone turnover. Different studies have shown that worse glycemic control is associated with lower bone turnover markers in T1D^[124-126], suggesting a negative effect of hyperglycemia on bone turnover. More specifically, patients with T1D exhibit higher sclerostin levels and lower C-terminal telopeptide of type I collagen (CTX) and osteocalcin levels as compared with non-diabetic controls^[127].

Bone turnover in T2D and prediabetes: Bone turnover markers are generally reduced in patients with T2D^[126,128,129], to a greater extent than patients with T1D^[130]. However, not all studies yielded consistent findings. Osteocalcin and CTX are the BTMs most consistently found to be lower in T2D and patients with as compared with subjects without diabetes, whereas sclerostin and osteoprotegerin are generally elevated (Table 1). Conflicting findings have been reported for other markers but, overall, the evidence seems to point towards a suppression of bone formation and bone resorption, both in prediabetes and T2D. Histomorphometric evaluation of bone tissue biopsies from T2D patients confirmed reduced bone turnover^[131,132]. The suppression of bone turnover reported in T2D patients is associated with higher risk of vertebral fractures^[133,134], independent of BMD. This is consistent with the concept that the impairment in bone strength in T2D is due to impaired material properties, which may be caused by low bone turnover, as well as by elevated concentrations of advanced glycation endproducts (AGEs)^[135].

Fewer studies have assessed bone turnover in prediabetes. Impaired fasting glucose (IFG) was associated with lower osteocalcin^[128], CTX and N-amino terminal propeptide of T1D procollagen (P1NP)^[136,137] in women, and lower CTX and P1NP in men^[136], suggesting that, similar to T2D, prediabetes is associated with reduced bone turnover.

Increased bone marrow adiposity

Bone marrow adipose tissue (MAT) has gained increasing attention in recent years as a single anatomic entity, together with its relations with various clinical conditions, including diabetes. MAT consists of MSC-derived adipocytes located within the bone marrow niche. The distribution of MAT around the skeleton is not homogenous, and regulation of marrow adipose depots varies at different skeletal sites. While peripheral depots of MAT (also termed constitutive MAT) rarely change, MAT depots at more central sites (*e.g.*, spine, pelvis and sternum, proximal regions of the long bones) are more diffuse within the red marrow and may increase or decrease in response to environmental or pathological factors (regulated MAT)^[138]. Interestingly, hyperglycemia increases the expression of PPAR genes, which stimulates differentiation of MSC into bone marrow adipocytes^[139]. Similarly, the antidiabetic PPAR γ agonists thiazolidinediones (TZDs) are thought to increase fracture risk through promotion of marrow adipogenesis at the expense of osteogenesis^[140] (Figure 1). Until recently, MAT was thought to be just a reserve of adipose tissue, negatively associated with hematopoiesis, but its complete function has just begun to be revealed. *In vivo* studies using magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) or computed tomography (CT) to assess MAT quantity and composition have helped understand the mechanisms of increased skeletal fragility and metabolic risk associated with several clinical conditions, including diabetes^[141].

MAT in T1D: In animal models of T1D, hyperglycemia is associated with increased marrow adiposity and bone loss^[37,38,142], whereas no differences in MAT were identified between male patients with T1D and healthy controls^[143,144], and neither duration of disease nor glycemic control were related to bone marrow adiposity. This lack of association between MAT and T1D was confirmed in young women with T1D compared with healthy controls^[145]. Irrespective of the presence of diabetes, in young women MAT was inversely associated with BMD^[145]. Carvalho and colleagues showed that MAT quantity and lipid composition (saturated and unsaturated lipids) were similar between male T1D subjects and controls^[144]. There was, however, a significant inverse correlation between MAT saturated lipids and BMD.

MAT in T2D: In T2D men participating in the Osteoporotic Fractures in Men (MrOS) Study, a large epidemiological study of nearly 6,000 men, vertebral MAT was increased as compared with nondiabetic controls, and inversely associate with BMD^[146]. Although no differences were detected in total MAT content in postmenopausal women, those with T2D and previous fractures had the lowest MAT

Table 1 Bone turnover markers in prediabetes/insulin resistance and type 2 diabetes

BTM	Meaning	Pre-DM / IR	Ref.	T2D	Ref.
CTX	Bone resorption	↓ or ↔	[136,276,278-280]	↓	[129,132,134,137,281-286]
TRAP	Bone resorption	↑?	[287]	↓ or ↔	[132,281]
uNTX	Bone resorption			↓	[285]
Sclerostin	Inhibition of bone formation	↑	[65]	↑	[284,285,288,289]
OC	Bone formation	↓ or ↔	[128,276-278,280,290]	↓ or ↔	[129,132,134,281,283,285,286,291-294]
P1NP	Bone formation	↓ or ↔	[136,277,280]	↓ or ↔	[88,132,134,137,282,283,285,286]
BAP	Bone formation	Direct association with IR	[295]	↔ or ↓ or ↑	[132,281,284,286,292,294]
ALP	Bone formation	?	?	↔ or ↑	[292-294]
OPG	Inhibition of bone resorption	↑	[296]	↑	[293,296]

BTM: Bone turnover marker; pre-DM: Prediabetes; IR: Insulin resistance; T2D: Type 2 diabetes; CTX: Carboxy-terminal cross-linking telopeptide of type I collagen; OC: Osteocalcin; P1NP: Procollagen type 1 amino-terminal propeptide; TRAP: Tartrate-resistant acid phosphatase; uNTX: Urinary N-telopeptide of type I collagen; BAP: Bone-specific alkaline phosphatase; ALP: Alkaline phosphatase; OPG: Osteoprotegerin; ↑: Increased; ↓: Decreased; ↔: Similar to healthy controls; ?: Unknown.

lipid unsaturation and highest MAT saturation levels independent of age, race, and BMD, highlighting the importance of MAT composition in addition to the degree of marrow adiposity^[147]. Furthermore, gender-related differences have been reported in the association between MAT and visceral adipose tissue (VAT)/subcutaneous adipose tissue (SAT) volumes or BMI. While in obese or diabetic women MAT is associated with VAT and SAT^[148,149], no such association was found in older men^[150]. In men, a negative association between MAT and DXA-derived BMD of femoral neck and total hip was reported. Data on MAT in pre-diabetes is scanty, but a potential relation between hyperglycemia and MAT has been suggested^[151].

ADVANCED GLYCATION END PRODUCTS-BONE MATRIX IN DIABETES

AGEs are protein or lipid complexes formed through non-enzymatic reactions in the presence of high sugar levels. Their accumulation is thought to play a role in aging and some degenerative diseases^[152]. In *in vitro* studies, AGEs deposits have been demonstrated in bone matrix, where they may exert a direct toxic effect on OBs^[153]. AGEs inhibit bone remodeling and indirectly up-regulate the production of interleukin 6 (IL-6)^[154], a catabolic factor that attenuates OBs activity^[53] and vascular endothelial growth factor A (VEGF-A) by osteocytes, inducing also their apoptosis^[155].

AGEs in T1D: In murine models of T1D, the AGE pentosidine (PEN) in bone is significantly increased, this increase being paralleled by an impairment in bone mechanical properties^[156]. Similarly, PEN levels in bone biopsies from fractured T1D patients were higher than in controls^[80], and circulating PEN levels are associated with prevalent fractures in T1D^[157]. Carboxymethyllysine (CML), another type of AGE that correlates with fracture risk^[158], is increased in mouse models of T1D and inversely associated with bone strength^[159].

AGEs in T2D and prediabetes: Bone strength in T2D postmenopausal women is reduced as compared with non-diabetic controls, and this reduction appears to be associated with increased AGE accumulation, as indirectly estimated by skin autofluorescence (SAF)^[160]. Consistently, increased urinary or serum PEN levels have been associated with greater fracture risk in T2D^[161,162]. To the best of our knowledge, no data are available on AGEs and bone health in prediabetes.

Bone geometry and microarchitecture

Bone geometry and microarchitecture contribute to bone strength. Tools such as high-resolution peripheral quantitative computed tomography (HR-pQCT), micro-magnetic resonance (μ-MRI) and TBS acquired through dual-energy X-ray absorptiometry (DXA) are available to study bone structure in diabetes^[163,164], offering enough resolution to assess microarchitecture and providing indirect indexes of bone quality.

Bone geometry and microarchitecture in T1D: In rodent models of T1D, deletion of

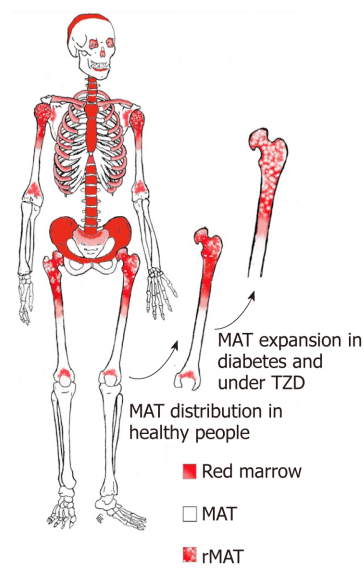


Figure 1 Schematic representation of the anatomical distribution of bone marrow adipose tissue depots.

Both hyperglycemia and the antidiabetic drugs thiazolidinediones may induce marrow adipose tissue (MAT) expansion by increasing the expression of peroxisome proliferator-activated receptor genes, which in turn stimulates adipogenesis. rMAT: Regulated MAT (MAT depots that increase or decrease in response to different stimuli).

the insulin receptor from OBs at different stages of maturation leads to anomalous trabecular architecture and higher bone fragility^[3,4]. In adults with T1D, trabecular bone quality is lower as compared with non-diabetic age-, BMI-, and sex-matched controls and is negatively associated with insulin resistance, as assessed by the hyperinsulinemic euglycemic clamp^[165]. Studies using HR-pQCT demonstrated higher cortical porosity, thicker trabeculae and larger spacing between trabeculae in T1D patients with microvascular complications, compared to those without, and in T1D patients compared with matched non-diabetic controls^[166]. Similar findings were reported using μ -MRI^[167]. Moreover, using μ -CT in T1D subjects without vascular complications, worse bone quality was found in those who did experience fractures as compared with those who did not^[166]. An insufficient peak bone mass at the end of skeletal maturation may result in smaller and shorter bones, a geometry that could favor bone fragility^[130]. However, the contribute of altered geometry and defective trabecular and cortical bone to the increased risk of fracture in T1D is yet to be clarified.

Bone geometry and microarchitecture in T2D and prediabetes: The increased fracture risk in T2D may be related to distorted bone microarchitecture, especially in cortical bone^[168-170].

Bone micro-indentation allows measuring the bone material strength index (BMSi), which estimates the resistance to crack propagation in bone^[171]. BMSi is reduced in patients with T2D as compared to healthy controls^[88,93], suggesting a lower resistance to fractures. Increased cortical porosity has been identified as a possible causative factor. Patients with T2D have higher porosity in trabecular bones, as assessed by MRI^[170]. Studies using HR-pQCT confirmed a similar trend in porosity. Deficits in cortical bone of T2D patients were more marked in patients with previous fractures compared to those without^[169], or present only in T2D patients with microvascular complications compared with patients without complications^[169]. In a cross-sectional analysis of nondiabetic postmenopausal women, higher levels of insulin resistance were associated with lower cortical bone volume, independent of age and weight^[172]. Consistently, female obese late-adolescents had worse trabecular bone microarchitecture at the radius and tibia as compared with non-obese controls, as well as lower bone volume and estimated bone strength^[173]. T2D diabetes and insulin resistance are almost invariably associated with obesity and increased central adiposity, which reflects increased VAT. Studies that explored the relationship between VAT and bone microarchitecture suggest a possible detrimental effect of VAT on bone microarchitecture. Studies have reported a negative impact of VAT on bone microarchitecture, as suggested by a negative association between central adiposity measures and TBS^[174,175]. Furthermore, a negative effect of VAT on femoral

cross-sectional area, cortical bone area and bone strength indexes has been reported^[176]. On the other hand, higher VAT was associated with improved microarchitecture with the exception of higher cortical porosity at the distal radius in the Framingham osteoporosis study^[177]. However, this association lost significance after adjustment for BMI or weight, suggesting that the effects of VAT may not have a substantial effect on the skeleton independent of BMI or weight. In non-diabetic men at the age of peak bone mass, insulin resistance (as assessed by HOMA-IR) was found to be inversely associated with trabecular and cortical bone size, independent of body composition^[178]. Overall, these data suggest a detrimental role of hyperinsulinemia on bone microarchitecture and geometry. Central adiposity might have a negative effect on bone microarchitecture, but this possibility needs to be further explored.

Vascular disease: microangiopathy

Diabetic microvascular complications such as retinopathy and neuropathy may indirectly potentiate the fall risk, impairing vision or physical perception. Diabetic microangiopathy may involve all organs, including bone, possibly contributing to bone fragility. Histomorphometric assessments found microangiopathy in 82% of bone biopsy specimens from diabetic patients, and a concomitant reduction of bone marrow capillaries^[179]. To date, there is no other direct evidence of bone vascular alteration in humans. In mouse models of T1D, administration of an angiogenic factor to ovariectomized mice led to improvements in bone quality^[180]. As mentioned, reduced trabecular BMD, cortical BMD, thinner trabeculae and cortex were reported in T1D patients with known vascular complications, as opposite to T1D patients without complications and non-diabetic controls^[166]. Similarly, in a cross-sectional study that assessed peripheral bone microarchitecture, bone strength and bone remodeling in T2D patients with or without diabetic microvascular disease only T2D patients with established microvascular disease displayed lower cortical volumetric BMD and cortical thickness and higher cortical porosity at the radius compared to controls without microvascular disease^[181]. Impaired microvascular circulation might lead to hypoxia, which in turn may lead to enhanced adipogenesis within the bone marrow and downregulation of OB differentiation^[182].

Pharmacological treatments for diabetes

Metformin. Metformin is widely prescribed for the management of T2D, being recommended as the first-line treatment by international guidelines^[8,183]. It reduces hepatic glucose production and improves peripheral insulin sensitivity, thereby enhancing peripheral glucose disposal^[184]. Metformin has been shown to promote the osteogenic differentiation of adipose-derived MSC, and in general to exert pro-osteogenic effects in preclinical studies^[185-188]. Clinical observations indicate that metformin has a neutral^[28,189] or even a favorable effect on fracture risk^[12,190,191].

Glucagon-like peptide-1 (GLP-1) receptor agonists (RA): GLP-1 RAs (liraglutide, exenatide, lixisenatide, dulaglutide, semaglutide) are recommended as the best choice for a second agent when combination treatment is needed to achieve glycemic control in patients with T2D in whom atherosclerotic cardiovascular disease, heart failure, or chronic kidney disease predominates^[8,183]. By activating the GLP-1 receptor, GLP-1 RAs slow gastric emptying, suppress glucagon secretion while also stimulating glucose-induced insulin secretion^[192]. These effects result in the suppression of hepatic gluconeogenesis and increased peripheral glucose disposal. *In vitro*, activation of GLP-1 receptors promotes differentiation of MSC into osteoblasts^[193] and inhibits osteoblast apoptosis^[194], suggesting an anabolic effect on bone. Studies in rats support these findings^[195]. Of note, in animal models of T1D administration of liraglutide significantly improved bone strength and reduced collagen degradation in the bone matrix, although no changes in trabecular or cortical microarchitecture were observed^[196]. Case-control studies and meta-analyses of population-based studies and randomized clinical trials including patients with T2D treated with GLP-1 RAs indicate no effect on fracture risk^[197-199]. However, evidence exist that different GLP-1 RAs may exert opposite effects on fracture risk, which appears to increase or decrease in patients treated with exenatide or liraglutide, respectively^[200]. Furthermore, liraglutide was reported to prevent a reduction of BMC after weight loss in obese nondiabetic women, although BMD was not affected^[201,202].

Dipeptidylpeptidase 4 (DPP4)-inhibitors: DPP4-inhibitors (sitagliptin, linagliptin, saxagliptin, vildagliptin, alogliptin, *etc.*) exert their action by inhibiting the enzyme DPP-4, which is responsible for the rapid degradation of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and GLP-1, thereby enhancing glucose-induced insulin secretion^[203]. Preclinical studies indicate a possible anti-osteoclastogenic and anti-resorptive effect of DPP4-inhibitors^[204,205]. Clinical data

support a neutral^[189,206,207] or even favorable^[208,209] effect of DPP4-inhibitors on fracture risk. In particular, alogliptin may be associated with a lower risk of bone fracture compared with placebo and other drugs in the same class^[210].

Sodium-glucose cotransporter 2 (SGLT2) inhibitors: By inhibiting the renal SGLT2, these drugs (empagliflozin, dapagliflozin, canagliflozin) reduce glucose reabsorption in the kidney, thus increasing urinary glucose excretion and decreasing blood glucose^[211]. Associated increases in serum phosphate may lead to changes in PTH and fibroblast growth factor 23 (FGF23) that could affect bone metabolism^[212]. Along with GLP-1 RAs, SGLT2 inhibitors are recommended as the best choice for a second agent when combination treatment is needed to achieve glycemic control in patients with T2D in whom atherosclerotic cardiovascular disease, heart failure, or chronic kidney disease predominates^[8,183]. Initial reports of increased frequency of bone fractures associated with SGLT2 inhibitors treatment, particularly with canagliflozin, raised concerns about the skeletal safety of these compounds^[213]. Furthermore, increased bone turnover and reduced total hip BMD have been reported in patients with T2D treated with canagliflozin^[214]. Nevertheless, recent population studies and meta-analyses including several thousands of patients consistently failed to demonstrate an association between SGLT2 inhibitor treatment and increased fracture risk in patients with T2D^[215-219].

Sulfonylureas and glinides: Sulfonylureas (*e.g.*, glimepiride, gliclazide, glybenclamide) and glinides (*e.g.*, repaglinide) stimulate glucose-independent insulin secretion by binding to specific sites at the β -cell membrane^[220,221]. It has been postulated that sulfonylureas do not affect bone directly, but may increase fracture risk by inducing higher rates of hypoglycemic events^[222]. Studies that assessed the effect of sulfonylureas and glinides on fracture risk yielded conflicting results, with most studies indicating increased risk^[28,189,223-225], but also no effect^[191] even decreased risk^[12].

Thiazolidinediones (TZDs): TZDs (rosiglitazone, pioglitazone) are insulin-sensitizing agents that exert their action by activating the peroxisome proliferator-activated receptor γ (PPAR γ)^[226]. Besides enhancing peripheral insulin sensitivity and suppressing hepatic glucose production, activation of PPAR γ stimulates adipogenesis and suppresses osteoblastogenesis, thereby reducing the osteoblast pool in the bone marrow^[227]. A detrimental effect of TZDs on bone health has been consistently shown. In a cohort study including more than 5000 patients with T2D, current use of TZDs was associated with increased hip fracture risk^[190]. Treatment with pioglitazone significantly increased fracture risk compared with placebo in a randomized double-blind, placebo-controlled study^[228]. The increase in risk has been confirmed in population-based studies^[189] and meta-analyses^[229], although the impact on bone seems to be more pronounced in women than in men^[190,229].

Insulin in T1D: Insulin is the pillar of T1D treatment. As previously discussed, insulin exerts anabolic effects on bone. Intensive insulin treatment has been associated with increased BMD in patients with T1D^[82]. Consistently, no association between insulin treatment and single nor multiple fractures was found in a recent study that assessed risk factors for fragility fractures in T1D^[230].

Insulin in T2D: Insulin treatment in patients with T2D is initiated when disease progression overcomes the effect of non-insulin agents^[8,183]. Thus, patients with T2D started on insulin generally have longstanding diabetes, and may have developed serious complications such as retinopathy-related impaired vision, peripheral artery disease and neuropathy, which in turn are risk factors for falls^[20,21]. Insulin use is associated with a 1.4- to 2-fold increase in fracture risk as compared with no insulin use^[189,231], and with a 1.6-fold increase in risk as compared with metformin monotherapy^[232]. However, not all studies point towards a negative effect of insulin on fracture risk^[12,191]. The association between insulin and increased fracture risk despite the anabolic effects of insulin on bone is likely due to the increased risk of falls and hypoglycemic episodes associated with insulin treatment^[222].

Surgical treatments for diabetes

Pancreas and islet transplantation in T1D: Beta cell replacement through pancreas or pancreatic islet transplantation is the only currently available cure for T1D in humans, with pancreas transplantation being more often associated with insulin independence and longer graft function. Successful pancreas transplantation provides physiological insulin repletion, without the risk of hypoglycemia associated with exogenous insulin administration. Evidence exists that combined pancreas-kidney transplantation leads to improvements in BMD^[233], and that fracture rates in patients with T1D are lower

after transplantation with a simultaneous pancreas–kidney compared with kidney transplantation alone^[234], suggesting that T1D remission by pancreas transplantation favorably impacts fracture risk. However, individuals with T1D undergoing pancreas–kidney transplantation also have end-stage renal disease, which strongly affects bone health. A study assessing the effect of diabetes remission following pancreas transplantation alone on bone health in individuals with T1D and preserved kidney function is currently ongoing (NCT03869281).

Metabolic surgery for T2D diabetes: Metabolic surgery is now included as a treatment option for appropriate candidates with T2D^[8,235]. Patients undergoing metabolic surgery experience rapid and massive weight loss, which translates into several metabolic benefits, but may be detrimental to bone health. Most available data relate to the Roux-en-Y gastric bypass (RYGB), a restrictive procedure that also involves a malabsorptive component. Sleeve gastrectomy (SG), which has now overcome RYGB and has become the most common bariatric procedure worldwide^[236], is a restrictive procedure. Other bariatric procedures, such as the malabsorptive biliopancreatic diversion and the restrictive laparoscopic adjustable gastric banding (LAGB), are being gradually abandoned. Available data indicate that fracture risk after bariatric surgery varies depending on the bariatric procedure, being lowest in patients undergoing LAGB^[237] and greatest in those undergoing malabsorptive procedures^[238–241], and increases with time after surgery^[237,239–242]. However, weight loss-related reductions in BMD have even been reported 6–12 months after minimally invasive bariatric procedures not involving resection of the stomach and/or intestine, such as use of the intragastric balloon or an intraluminal liner implanted into the small intestine^[243,244]. Mechanisms underlying the negative effects of bariatric surgery on bone health may involve nutritional factors, mechanical unloading, hormonal factors, and changes in body composition and bone marrow fat^[245]. To the best of our knowledge, no studies have specifically addressed the issue of diabetic bone disease in patients with T2D undergoing bariatric surgery.

PERSPECTIVES: POSSIBLE PREVENTIVE AND THERAPEUTIC APPROACHES

Modifiable risk factors for fracture, including factors that affect fall risk and glycemic control should be tackled to reduce fracture risk, although no prospective studies are available to show the antifracture efficacy of preventive lifestyle and/or treatment strategies. Drugs shown to be associated with increased fracture risk in T2D, such as insulin and TZDs^[231,232,246] should be avoided, when possible. Strict monitoring should be implemented for T2D patients undergoing bariatric surgery in order to prevent nutritional deficiencies that could worsen weight loss-associated bone loss.

Several alterations in calcium homeostasis have been described in diabetic patients, including reduced intestinal calcium absorption and renal tubular calcium reabsorption, and impaired vitamin D synthesis^[247]. It is also recognized that individuals with diabetes, both T1D and T2D, have lower vitamin D levels as compared with non-diabetic controls^[248,249]. Overall, these alterations may negatively impact calcium homeostasis and bone mineralization. International guidelines recommend vitamin D supplementation for the prevention and/or treatment of osteoporosis and osteoporotic fractures in men and postmenopausal women^[250–252], although recent findings bring into question the efficacy of vitamin D supplementation in preventing fractures or falls, or improving BMD^[253]. Vitamin D supplementation was shown to increase bone formation markers^[254] and reduce bone resorption markers^[255] in postmenopausal women with T2D, not to affect bone turnover markers in patients with T2D and chronic kidney disease^[256], and to preserve femoral neck BMD in men with prediabetes^[257]. Few data are available about the effect of the use of osteoporosis medications in patients with diabetes.

Stemming from some positive preclinical results^[258], few recent human studies have focused the attention on nutrients containing antioxidants such as resveratrol, providing encouraging results in terms of on bone density and on bone loss prevention in obese patients^[259] and patients with T2D^[260,261] have been reported.

Recently, hyperbaric therapy^[262,263] has been shown to promote bone regeneration in animal models of diabetes, but further studies are needed to clarify whether this could be an effective approach in humans.

Raloxifene, a second generation selective estrogen receptor modulator (SERM) indicated for the prevention and treatment of postmenopausal osteoporosis^[264], was shown to improve bone material properties (femoral toughness) in diabetes-prone rats^[265]. In postmenopausal women, raloxifene may decrease the bone resorption

marker NTX and it has been speculated that it might improve bone quality by reducing AGEs, although no information is available on the effect on reliable bone quality indicators or relevant clinical outcomes such as fracture risk^[265]. In a pilot study that assessed the skeletal effects of a third generation SERM, bazedoxifene, in postmenopausal women with T2D, all bone resorption markers decreased significantly after 12 weeks of treatment. Homocysteine and pentosidine, which were used as bone quality markers in this study, were not affected^[266].

Little is known about osteoporosis therapies in T1D young patients. As T1D usually manifests in young individuals, it is important to remember that caution must be taken in women during reproductive age, as bisphosphonates are stored and released from bones for long time and may affect fetal skeletal ossification. In elderly, postmenopausal, osteoporotic obese women with T2D treated with long-term bisphosphonates, no difference in spine BMD but a significantly greater decline in BMD in regions of the hip, femoral neck, and forearm were observed as compared with non-diabetic controls^[267]. However, the efficacy of these medications must be assessed based on clinically relevant outcomes. Despite being a condition of reduced bone turnover, epidemiological data indicate that diabetes (either T1D or T2D) was shown not to reduce the antifracture efficacy of antiresorptive drugs, which also reduce bone turnover^[268].

In a large study on the efficacy of recombinant PTH (rhPTH 1-34, teriparatide), similar reduction in nonvertebral fracture incidence and increase in BMD were observed in postmenopausal osteoporotic women with or without T2D^[269].

Denosumab is a RANKL-specific antibody indicated as osteoporosis treatment known to increase particularly cortical BMD. This property might be of particular value, as cortical compartment is the most involved in the diabetic bone. A phase 2 clinical trial to assess the skeletal effects of denosumab in T2D is ongoing (NCT03457818). Interestingly, denosumab was shown to improve hepatic insulin sensitivity in humans^[270,271] and, consistently, to reduce fasting plasma glucose in women with diabetes not on antidiabetic medications^[272]. Preclinical studies also indicate that denosumab may stimulate human β -cell proliferation^[273].

Sclerostin seems to have a central role in the pathogenesis of diabetic bone disease. In mouse models of T1D^[273] and T2D^[274], administration of anti-sclerostin antibodies seems to reverse the deficits in bone density and micro-fracture healing. No data are currently available on romosozumab, an anti-sclerostin antibody shown to reduce the risk of clinical and vertebral fractures in postmenopausal women with osteoporosis^[275].

CONCLUSION

Diabetes has a strong impact on bone health, and skeletal fragility is now recognized as a complication of both T1D and T2D. Fracture risk is greater in patients with T1D, and increases with increasing disease duration. Individuals with T1D have decreased BMD, possibly due to absolute insulin deficiency and the inability of exogenous insulin to mirror endogenous insulin secretion. However, the relatively small reduction in BMD does not appear to completely explain the increase in bone fragility observed in T1D^[276-296]. On the other hand, individuals with T2D have either normal or increased BMD, which is in contrast with the increased fracture risk observed in this population. Therefore, it is likely that factors that affect bone quality, rather than bone mass, impact the resistance of T2D bones to fracture (Table 2). Increased non-enzymatic glycation of bone matrix proteins, impaired microcirculation and glucotoxicity itself, *i.e.*, the direct detrimental effect of high glucose on bone cells, may all play a role. Reduced bone turnover and increased bone marrow adipogenesis at the expenses of osteogenesis may also contribute. Despite a clear association between T2D and increased fracture risk, evidence supporting an association between prediabetes and fracture risk is inconsistent, and further studies are needed to clarify whether insulin excess has either a beneficial or rather detrimental effect on bone health. The incomplete understanding of the mechanisms underlying diabetic bone disease makes it difficult to develop reliable tools for fracture risk prediction. To date, no single method is deemed optimal for predicting all fracture outcomes in patients with diabetes^[32]. Fracture history and risk factors should be assessed in older patients with DM, and measurement of BMD is recommended, if appropriate for the patient's age and gender^[8]. Caution should be used with antidiabetic drugs known to negatively affect bone health, such as TZDs and insulin in patients with T2D. Healthcare professionals involved in the management of T2D patients undergoing bariatric surgery should be aware of the possible detrimental effects on bone health, and implement appropriate nutritional strategies. Due to the lack of randomized

clinical trials to evaluate the efficacy of antifracture drugs in diabetes, and observational data indicating similar efficacy in those with or without diabetes, such drugs should be used according to existing indications.

Future studies should focus on the mechanisms underlying diabetic bone disease, and on preventative and treatment strategies to implement in order to reduce the morbidity associated with fractures in this frail population.

Table 2 Effects of diabetes and prediabetes on bone health

	T1D	T2D	Prediabetes
Fracture risk	↑↑	↑	?
Bone mineral density	↓	↔ or ↑	↔ or ↑
Bone turnover	↓	↓↓	↓?
Bone marrow adiposity	↔	↑	↑?
Bone matrix - AGEs	↑	↑	?
Microarchitecture/geometry	↑ cortical porosity	↑ cortical porosity	↓ trabecular and cortical bone size

AGEs: Advanced glycation endproducts; T1D: Type 1 diabetes; T2D: Type 2 diabetes; ↑: Increased; ↓: Decreased; ↔: Similar to healthy controls; ?: Unknown.

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Development of therapeutic options on type 2 diabetes in years: Glucagon-like peptide-1 receptor agonist's role in treatment; from the past to future

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. Type 2 diabetes (T2DM) accounting for 90% of cases globally. The worldwide prevalence of DM is rising dramatically over the last decades, from 30 million cases in 1985 to 382 million cases in 2013. It's estimated that 451 million people had diabetes in 2017. As the pathophysiology was understood over the years, treatment options for diabetes increased. Incretin-based therapy is one of them. Glucagon-like peptide-1 receptor agonist (GLP-1 RA) not only significantly lower glucose level with minimal risk of hypoglycemia but also, they have an important advantage in the management of cardiovascular risk and obesity. Thus, we will review here GLP-1 RA's role in the treatment of diabetes.

Key words: Incretin-based therapy; Incretin mimetics; Glucagon-like peptide-1 receptor agonist; Dipeptidyl peptidase-4 inhibitor

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Core tip: The prevalence of type 2 diabetes and its complications rising dramatically over the last years. It is well known that diabetes and its complications; especially cardiovascular complications lead to increased morbidity and mortality. Treatment options for diabetes have increased as the pathophysiology was understood. We discuss the incretin-based therapy, especially Glucagon-like peptide-1 receptor agonists and the beneficial effects on comorbidities besides glucose lowering effect.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. Depending on etiology; decreased insulin secretion, decreased glucose utilization and increased glucose production contribute to hyperglycemia^[1]. There are several distinct types of DM. Type 2 DM (T2DM) accounting for 90% of cases globally^[2]. T2DM demonstrate insulin resistance in peripheral tissues, defective insulin secretion particularly in response to glucose stimuli and increased glucose production by the liver as three cardinal abnormalities^[2]. Increased lipolysis in fat tissue, increased production of glucagon, incretin hormone deficiency and resistance, increased renal tubular glucose reabsorption and central nervous system role in metabolic regulation also contribute to the pathophysiology of T2DM^[3]. The worldwide prevalence of DM is rising dramatically over the last decades, from 30 million cases in 1985 to 382 million cases in 2013^[4]. It's estimated that 451 million people had diabetes in 2017^[4]. As the pathophysiology was understood over the years, treatment options for diabetes increased. Thus, we will review here Glucagon-like peptide-1 receptor agonist (GLP-1 RAs) role in the treatment of diabetes. We aimed to summarize not only their glucose lowering effect but also their efficacy on the comorbidities come along with diabetes, such as obesity and cardiovascular disease (CVD).

We selected the articles by searching an electronic database (PubMed) with the following terms; glucagon-like peptide 1 agonists, glucagon-like peptide 1 agonists and CVD, glucagon-like peptide 1 agonists and obesity, dipeptidyl peptidase-4 (DPP-4) inhibitors. The articles not related to diabetes, the case reports, abstract only, comments and conference papers were excluded. Only studies in English language were included. Cardiovascular safety trial of each molecule (GLP-1 RA and DPP-4 inhibitor) were also included. All the included articles reviewed for full text.

ROLE OF INCRETINS IN GLUCOSE HOMEOSTASIS

Glucose is the most important physiologic substance involved in the regulation of insulin secretion from the pancreas^[5-7]. Glucose has a dose-dependent effect on the beta cells. It's well known that oral glucose administration has a greater effect on insulin release than intravenous glucose administration^[8-10]. Known as the incretin effect. In a study, insulin secretion was detected 26% lower in response to IV administration than oral administration^[10]. This increased response to oral glucose shows that glucose absorption from the gastrointestinal tract may cause secretion of some hormones which have an effect on B-cell sensitivity^[5-10]. GLP-1 and glucose-dependent insulintropic polypeptide (GIP) are the major incretin hormones in humans^[11]. GIP is produced in the K-cells and these cells are located predominantly in the proximal parts of the intestine, especially in the duodenum. GLP-1 is produced by the L-cells which distally situated especially in the ileum. L-cells also found in the colon in high density^[12]. Both K-cells and L-cells can be situated throughout all parts of the intestine. It's also detected that there is a population of cells which contain both GLP-1 and GIP^[13]. Secretion of incretin hormones is correlated with food intake and the driving factor is the presence of nutrients in the lumen, not distension since loading of water does not cause a significant increase in GLP-1 and GIP concentrations^[14-16]. The incretins are cleaved by the enzyme DPP-4 and lose their biologic activity^[1,2].

INCRETIN EFFECT IN DIABETES MELLITUS

The incretin effect found substantially reduced or even absent in patients who have T2DM and hyperglycemia^[17-19]. As the fasting plasma glucose level increases above the level defining diabetic state (126 mg/dL), incretin effect seems to start to reduce^[20].

This reduced effect is universal with the possible exception of East Asians^[21].

T2DM patients almost completely lost response to GIP^[22]. Because much of the incretin effect in healthy individuals is mediated by GIP, lack of activity may explain the reduced incretin effect in T2DM patients^[20]. Besides this; the substantial insulinotropic activity of GLP-1 retains in these patients and GLP-1 activity related to dose and concentration, linearly^[23-25]. However, GLP-1 insulinotropic effect is reduced compared with healthy individuals; a result of reduced B-cell mass, most likely^[25,26]. The effects of GLP-1 on appetite, gastrointestinal motility, food intake, and suppression glucagon secretion are retained^[23,27]. Parenterally given GLP-1 significantly increase insulin secretion, suppress glucagon secretion and normalize glucose concentration^[22].

INCRETIN-BASED THERAPY IN T2DM

As the research in the field of diabetes progressed and the pathophysiologic processes were understood, new therapeutic options were invented. Incretin-based treatment is one of them. Practically, DPP-4 inhibitors or GLP-1 RAs can be used for this therapeutic approach. Besides that, GLP-1 gene transferring has studied in animal models and it was showed that GLP-1 gene transfer may be an alternative to GLP-1 infusion or multiple daily or weekly injections, in the future^[28,29].

There are several GLP-1 agonists used in daily clinical practice. Some of them are listed below in Table 1^[30]. All of the GLP-1 agonists administered by subcutaneous injection but semaglutide also has an oral form^[31]. On the other site, all of the DPP-4 inhibitors are given orally. Alogliptin (25 mg, once daily), linagliptin (5 mg, once daily), saxagliptin (5 mg once daily), sitagliptin (100 mg, once daily) and vildagliptin (50 mg, twice daily) are the DPP-4 inhibitors used in daily clinical practice^[32].

GLP-1 RA and DPP-4 inhibitors are important therapeutic options for patients with T2DM^[33]. European Association for the Study of Diabetes and the American Diabetes Association recommend these agents as the second line for the treatment of T2DM^[34]. The glucose-lowering effect of these agents with minimal risk of hypoglycemia is well studied. They also have a favorable effect on body weight and blood pressure^[35-43]. The efficacy of GLP-1 RAs is greater than DPP-4 inhibitors, in general^[44]. While patients who receive GLP-1 RA experience significant weight loss, the effect of DPP-4 inhibitors on body weight is neutral^[44,45]. In a systematic review of comparative effectiveness of GLP-1 RAs, it was concluded that GLP-1 RAs are similar or more effective than oral glucose-lowering agents in improving glycemic parameters. In the same review, GLP-1 RAs found to provide similar or less decrease in HbA1c level compared with insulin therapy, with less hypoglycemia^[46].

CARDIOVASCULAR OUTCOMES OF INCRETIN-BASED THERAPY IN T2DM

After the meta-analysis, published by Nissen and colleagues in 2007, suggesting that rosiglitazone (an anti-diabetic agent) was associated with increased risk of myocardial infarction (MI) among T2DM patients, United States Food and Drug Administration (FDA) mandated the conduct of large, randomized, placebo-controlled cardiovascular safety trials for all new anti-diabetic agents^[47,48]. FDA defined the standards of these studies^[48]. Several large randomized controlled trials (RCT) have been completed since that time. The RCT examined saxagliptin for cardiovascular safety established an unexpected increased risk of hospitalization for heart failure among patients randomized to saxagliptin^[49,50]. The RCT's examined other DPP-4 inhibitors didn't establish such results^[51-59]. Vildagliptin haven't been studied in RCT for examining cardiovascular safety.

Because the GLP-1 RAs promote weight loss, reduce blood pressure, decrease myocardial and vascular inflammation and decrease platelet aggregation behind their effect on blood glucose level, they thought to reduce cardiovascular risk^[60,61]. Cardiovascular safety was established for the whole class in the RCTs of cardiovascular outcomes with GLP-1 RAs (liraglutide, semaglutide, lixisenatide, and extended-release exenatide). Besides that, the results for cardiovascular efficacy was mixed^[62-65]. Among these RCTs in two studies (SUSTAIN 6 and LEADER) a significant reduction in three-point major adverse cardiovascular events (non-fatal stroke, non-fatal MI and cardiovascular mortality) was shown^[63,64]. Questions emerged after these varying findings about the generalizability of the trials to the drug class. The data available from the RCTs of cardiovascular outcomes with GLP-1 RAs was synthesized in a meta-analysis to examine the overall effect on cardiovascular efficacy and

Table 1 Glucagon-like peptide-1 receptor agonist

Drug	Administration	Phase 3 clinical trial
Exenatide	Twice daily (5 µg or 10 µg)	Amigo
Liraglutide	Daily (0.6 mg or 0.8 mg or 1.2 mg)	Leader
Exenatide ER	Weekly (2 mg)	Duration
Lixisenatide	Daily (10 µg or 20 µg)	Getgoal
Dulaglutide	Weekly (0.75 mg or 1.5 mg)	Award
Semaglutide	Weekly (0.5 mg or 1.5 mg)	Sustain
Albiglutide	Weekly (30 mg or 50 mg)	Harmony

safety^[66]. According to this meta-analysis; cardiovascular safety appointed for all GLP-1 RAs, use of GLP-1 RAs was associated with a significant 10% relative risk reduction for the three-point major adverse cardiovascular events, also associated with risk reduction in cardiovascular mortality of 13% and all-cause mortality of 12% compared with placebo^[66]. Likewise, it was determined in a retrospective epidemiological study that patients who treated with exenatide were less likely to have CVD, CVD related and all-cause hospitalizations^[67]. The trial of cardiovascular outcomes in patients with T2DM on albiglutide was completed in 2018 and it was shown that albiglutide was both as safe as placebo in terms of cardiovascular outcomes and superior to placebo in efficacy even in short period of time (1.6 years)^[68].

The effect of incretin-based therapy on atherosclerosis was examined in a meta-analysis of RCTs. Incretin-based therapy showed significant improvement of carotid intima media thickness in the long term (2 years) but it has failed to show this effect in 1 year follow up^[69].

Certain experimental studies examined incretin receptors on vascular smooth muscle cells and showed their role in causing atherosclerosis^[70,71]. Also, the efficacy of DPP-4 inhibitors on improvement of endothelial function was shown^[72].

It was generally shown in observational studies that there is a relationship between hyperglycemia and CVD but reduced CVD by reducing hyperglycemia haven't confirmed in clinical trials^[73-78]. Moreover, one trial terminated early because in the intensive glycemic treatment arm, all-cause mortality was increased and, in each subgroup, it was associated with hypoglycemia^[74,79]. It's an important point that GLP-1 RAs and DPP-4 inhibitors have a glucose lowering effect with less hypoglycemia (GLP-1 RAs are more potent than DPP-4 inhibitors)^[35-44].

According to the recent meta-analysis, GLP-1 RAs are seemed to be cardio-protective as a whole class^[80]. They have pleiotropic actions on cardiovascular risk factors with a direct effect on the cardiovascular system (Table 2)^[69,80,81].

A recently published review in which several preclinical studies were examined, it was concluded that using GLP-1 agonists improve functional outcome after ischemic stroke. It's unknown whether these results are valid for humans in clinical practice^[82].

THE EFFECT OF INCRETIN-BASED THERAPY ON BODY WEIGHT

Obesity is an important risk factor and comorbidity of T2DM, and it also elevates cardiovascular risk. Obesity must also be managed for effective treatment of T2DM. GLP-1 RAs were studied in several trials and it was established that GLP-1 RAs cause significant weight loss in T2DM patients with obesity^[46,83,84]. The effect of DPP-4 inhibitors on weight in neutral^[44,45,83]. Although GLP-1 RA's cost and administration route may be limitations for generalized acceptance, they may also offer a reasonable alternative choice for obese patients (liraglutide 3 mgr.) without diabetes who don't achieve weight-loss goals with lifestyle modification alone^[84].

CONCLUSION

T2DM is a chronic disorder which comes along with several comorbidities like obesity, CVD, kidney disease, hypertension, *etc.* As long as the pathophysiologic process of DM was understood over the years, several new therapeutic options emerged. Individualizing care gained importance in the last years for the management of DM. It's important to manage obesity, hypertension, hyperlipidemia

Table 2 Cardiovascular effect of glucagon like peptide-1 receptor agonists

Anti-atherosclerotic effect	Decrease matrix metalloproteinase 2; decrease vascular smooth muscle cell proliferation
Improves endothelial function	Increase nitric oxide-induced vasodilation; decrease oxidative stress
Anti-inflammatory effect	Suppress human macrophages by inhibition of protein kinase C
Decrease infarct/injury size	Decrease glucose-induced apoptosis; decrease intracellular calcium overload
Modifies risk factors	Improve glycemic control; decrease body weight; decrease blood pressure; decrease low-density lipoprotein

and total cardiovascular risk together with lowering glucose level with minimal risk of hypoglycemia. GLP-1 RAs not only significantly lower glucose level with minimal risk of hypoglycemia but also, they have an important advantage in the management of cardiovascular risk and obesity.

All GLP-1 RAs are administered parenterally but semaglutide also can be given orally by now. Besides that, it was showed that GLP-1 gene transfer may be an alternative to GLP-1 injections, in the future.

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Competences for self-care and self-control in diabetes mellitus type 2 in primary health care

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Abstract

The purpose of the guidelines of self-care and self-control of type 2 diabetes mellitus proposed by the Brazilian Ministry of Health is to strengthen and qualify users and health care professionals through the integrality and longitudinality of care with this disease. This article aims to present the self-care and self-control of people with type 2 diabetes mellitus in objective terms, taking into account the current recommendations based on scientific evidence and also from the subjective point of view, that is, emphasizing the aspects related to experience and subjectivity of these people. Next, we present the essential skills for self-care and self-control of users and professionals working in primary health care.

Key words: Diabetes mellitus type 2; Self-care; Primary health care

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Core tip: This article aims to present the self-care and self-control of people with type 2 diabetes mellitus under the objective point of view, taking into account the current recommendations based on scientific evidence, and also from the subjective point of view, emphasizing the aspects related to experience and the subjectivity of these people. Next, we present the essential skills for self-care and self-control of users and professionals working in primary health care.

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INTRODUCTION

Type 2 diabetes mellitus (DM2) is currently a global epidemic. Incidence and prevalence are increasing in developing and newly industrialized countries. Its impact on public health worldwide consists of social problems, such as reduced quality of life and reduced survival of people with DM2, and economic problems, such as reduced productivity and high treatment costs^[1].

Among the several types of diabetes, DM2 accounts for 90%-95% of cases. It is characterized by an imbalance of the metabolism of carbohydrates, lipids, and proteins and is associated with a deficiency in the secretion and/or action of the hormone insulin secreted by the pancreas. As a consequence, there is a decrease in tissue sensitivity or insulin responsiveness and an increase in blood glucose levels. As a way to combat the complications of hyperglycemia, the goal of treatment is to achieve normal blood glucose levels^[2].

An individual with DM2, if not properly treated and controlled, may develop acute complications, such as hypoglycemia, hyperglycemia, and chronic progressive changes in the retina, kidneys, and peripheral nerves, and may trigger atherosclerotic lesions of the heart, brain, and peripheral members^[2].

Due to the requirement of constant glycemic control, chronicity, and lack of cure, the person with DM2 remains linked to the health system for decades and needs continuous attention focused on the integral care provided by family health and family support nucleus in actions to promote, monitor, and prevent complications of DM2. The complexity of care for people with DM2 requires an interdisciplinary approach with health professionals open to dialogue and willing to plan appropriate consultations and interventions to the specific needs of people with DM2 that are centered on the actions of self-care and glycemic control^[3].

The purpose of the guidelines of self-care and self-control of DM2 proposed by the Brazilian Ministry of Health is to strengthen and qualify care to users and to health professionals through the integrality and longitudinality of care with this disease. Thus, users with DM2 and health professionals who work in primary care should have competencies for self-care and self-control in this pathology. According to Cyrino^[4], competence is a person's ability to mobilize different knowledge to master specific problematic situations faced in daily life and to develop attitudes and practices.

To achieve the goals detailed by the Strategy for the Care of People with Diabetes Mellitus published in the Basic Care Book number 36 of the Ministry of Health^[5], it is proposed that primary care professionals adopt the approach of person-centered health with DM2^[6,7]. This approach allows primary care professionals to use objective methods such as anamnesis, physical examination, and laboratory tests as well as subjective methods for analyzing and understanding feelings and ideas, the effects of DM2 on one's life, and expectations of treatment^[8]. Thus, health professionals, in addition to the epidemiological and pathophysiological knowledge of DM2, must understand the psychosocial aspects of people; have pedagogical skills, communication skills, listening, understanding, and negotiating with the interdisciplinary health team^[9]. On the other hand, people with DM2 must have the skills and autonomy to assume self-care and self-control.

In this way, this article aims to present the self-care and self-control of people with DM2 under the objective point of view, taking into account the current recommendations based on scientific evidence, and also from the subjective point of view, emphasizing the aspects related to experience and the subjectivity of these people. Next, we present the essential skills for self-care and self-control of users and professionals working in primary health care.

SELF-CARE AND SELF-CONTROL OF PEOPLE WITH DM2 UNDER THE OBJECTIVE POINT OF VIEW

Self-care and self-control are shown as possibilities for the person with DM2 to reduce the repercussions caused by the disease. Self-care is understood as the set of activities that involve dietary, corporal, drug, and glucose monitoring practices performed by

the patient to promote his health, minimizing hypoglycemia and excessive weight gain. Self-control is the monitoring of the conditions of health and disease by the subject himself, according to objective parameters obtained by biochemical tests of blood glucose and glycohemoglobin^[4].

The main goal of self-management and self-control of people with DM2 is metabolic control and includes tests for fasting blood glucose and glycated hemoglobin^[10]. Glycated hemoglobin is the gold standard that provides an index of glycemic control for 6 to 12 wk^[11], dosed quarterly until reaching control and then every 6 mo^[3]. In order not to increase the risk of hypoglycemia or other complications of treatment, the patient aims to reach values lower than or equal to 6.5 a 7.0^[1,10].

The monitoring of the annual lipid profile (triglycerides, total cholesterol and its fractions) is of fundamental importance for the control of DM2, since this indicator is associated with cardiovascular diseases, obesity, and arterial hypertension, which may favor the development of insulin resistance and metabolic syndrome^[10].

Blood pressure should be measured query, with ideal targets for systolic pressure < 130 mmHg and diastolic blood pressure of < 80 mmHg. In addition, ophthalmologic evaluations, urinary albumin excretion, and comprehensive examination of feet should be made after the diagnosis in order to avoid retinopathies, nephropathies, ulcers, and amputations, respectively^[10].

For self-care and self-management of DM2 in order to maintain glycemic control, to avoid acute complications, and to reduce the risk of long-term complications, it is recommended that people with D2M regularly participate in medical appointments and care health monitoring of biochemical and blood pressure tests, weight and abdominal circumference measurements, as well as evaluation of drug treatment, diet, and physical activity^[10]. But not enough people attend consultations regularly, making necessary adherence to self-care and self-control, which begins with the incorporation of dietary practices and physical activity prescribed by professionals in primary health care.

Behavioral modification related to dietary practices is a requirement imposed by the disease, and the selection of foods and fractionation of meals, energy consumption for the purpose of reducing or avoiding weight gain, and decreased consumption of trans and saturated fats, cholesterol, and sodium should be reviewed. These modifications improve insulin resistance and decrease plasma glucose, abdominal circumference, and visceral fat levels by improving the metabolic profile with reduced levels of low-density lipoprotein, triglycerides and increased high-density lipoprotein^[12,13].

As for the body practices, 150 min per week of aerobic physical activity of moderate intensity is recommended. These activities include walking, cycling, running, swimming, and dancing, preferably three times a week, provided that there is no medical contraindication. Exercise improves glycemic control, reduces glycated hemoglobin and cardiovascular risk, contributes to weight reduction, and improves self-esteem^[1]. When associated with changes in eating habits, important components of maintenance of glycemic control and weight loss programs are important^[10].

When the desired glycemic levels have not been reached after the use of dietary measures and exercise, antidiabetic medicinal products should be used. Some people with DM2 will require insulin therapy soon after the diagnosis and many throughout the treatment^[1].

SELF-CARE AND SELF-CONTROL OF PEOPLE WITH DM2 FROM THE POINT OF VIEW OF THEIR EXPERIENCE AND SUBJECTIVITY

The subjects should be prepared and motivated from diagnosis to take the treatment. Although people are adaptable to the realization of self-care and self-control, compliance with these practices is not so easy for most people with DM2. At the moment the disease is discovered, the structure of daily life and the forms that sustain it are interrupted. First, ruptures occur with the new limits of normal daily life, as behaviors performed before being sick must be changed, potentially leading to deep breaks in one's biography and self-concept. Finally, in the various segments of daily life, due to the care they need, people with DM2 must mobilize resources to face the changed situation^[14].

At this stage, the person may be faced with the obstacle of food (one of the most difficult to overcome), the non-acceptance of DM2, fear of insulin, a lack of knowledge about the disease and self-care, the need for commitment and discipline, unfavorable financial situation, and the emotional component involved with feeding^[4,15].

Thus, living with the limits imposed by a diagnosis of DM2 is full of conflicts,

ruptures, questioning, and nonconformity. Knowing the experience and the subjectivity of these people, of the meanings attributed to them by the disease, favor the identification of limiting aspects and the way in which they articulate different aspects that interact in the production of self-care and consequently in self-control.

Some studies seek to approach the subject and his experience with the disease, taking into account the vision and participation in the management of care^[16] treatment adherence^[17], the involvement of friends and family in the treatment^[18], as well as support or self-help groups and social networks^[19].

The experiences of individuals with the disease are socially shared, and their analysis is possible when expressed as subjective narration, that is, the conscious or unconscious mind of people. Thus, one approach to the subjective questions is the social representations, understood as complex subjective productions, because they have an impersonal aspect, in the sense of belonging to all; they are the representation of others, belonging to other people or to another group and are also a personal representation, perceived effectively as belonging to the ego^[20].

Social representations play a fundamental role in the dynamics of social relations by understanding and explaining reality, guiding behaviors and practices, explaining and justifying behaviors in a situation or with partners, and defining identity^[21]. The author emphasizes a clear relationship between social representation, identity, and the behavior of people.

It is necessary, then, to understand the social representations in which people with DM2 are anchored and the social identities that underlie them. With this intention, Amorim *et al.*^[22,23] investigated the identity representations of users with DM2 of a basic health unit, located in Belo Horizonte, Brazil. From the guiding question: "what comes to mind when I speak, I am diabetic", the speeches were categorized and interpreted by the technique of content analysis and theories of social representation and social identity. As a result of this research, some people with DM2 studied are considered normal, others accept the disease, there are those who are dissatisfied, and others lead a life with difficulty. The "normal" participants coexist with illness in a positive way and minimize the impact of DM2 on their identity when they experience the process of normalization of illness and care, in which the changes and adaptations required to the treatment become routine and are incorporated into daily life. Participants who "accept the disease" do not ideally accept their chronic illness. The ideal acceptance of a disease consists of a psychological state in which the illness is part of the perception of reality and is not perceived as a factor that limits the person. The unfavorable attitudes of the "non-conforming" participants, the information about the risks of the disease and the image of danger that they elaborate on the illness, help to understand the sense that the participants attribute to the "diabetic being". Participants who think that they "have a life fraught with difficulties" face obstacles in taking care of themselves, culminating in negative feelings and attitudes about the disease. It is possible that people with "distressed" DM2, not feeling confident about the future and facing adversity, do not make sustained efforts to achieve their goals, neglecting self-control and self-care. Thus, the obstacles faced by participants who think they are "accepting the disease, think they are "discontented" and "have difficulties" when they put into practice self-care, especially in relation to food, should be understood by the team that works in primary health care, biomedical logic^[22,23].

The social representations about the feeding of these people with DM2 were investigated. Some respondents indicated that the person with DM2 should eat healthy. Others relied on the quality of food, representing it as "eating vegetables and fruits" and "avoiding sweets." There are still those whose speech was based on eating little, worrying about the quantities of food eaten. There are those who represented eating as not eating too much, focusing on the frequency of feeding, as they consider that breaking down the food in many meals is not appropriate. Others focused their speech on selective food intake, specifically those that do not harm the body. Finally, others considered that food does not imply following a specific diet^[24,25].

In analyzing the social representations of the diet of people with DM2 as they represent their identity and its implications for glycemic control, it was found that adequate HbA1c values of the participants considered to be "normal" are adequate and are related to the actions of self-care, allowing to infer about the effectiveness of feeding. Proper nutrition improves insulin resistance, decreases the levels of plasma glucose and waist circumference, and improves metabolic visceral fat profile of triglycerides and cholesterol. People who think they have a normal life represent eating in the categories eating healthy, eating reduced, eating vegetables and fruits, and divert from sweets^[26].

The particular way in which the participants who judge "accepting the disease", "having difficulties", and "nonconformists" perform the self-care related to the alimentary practice is derived from the different processes of subjectivation in which each one of them relies on to construct its social representations on the identity and

feeding and consequently have mean values of HbA1c above normal values. Participants who "accept the disease" are based on "no" to represent their diet: do not eat too much and do not eat at all. Participants who "have a life with difficulties" represent their eating in the negative categories: do not eat too much, do not eat at all, and do not follow the diet. A participant who represents eating in eating vegetables and fruits, unlike normal people, has difficulty putting their thinking into practice. "Nonconformists" represent their food in the negative categories: not eating much and not eating at all. Two participants represented their diet in eating vegetables, but in practice they eat the forbidden foods^[26].

USER ABILITIES REQUIRED FOR SELF-CARE AND SELF-CONTROL

The adherence of people with DM2 to self-care and self-control therapies is still low in developed countries, with around 50%, and it is estimated that in developing countries this percentage is lower, compromising the effectiveness of the treatment^[27].

Due to the complexity of self-care and self-control in DM2, Cyrino^[4], based on the literature and the joint evaluation with specialists in the area, defined a list of competencies required by people with DM2 to conduct the treatment. A total of 47 skills classified in the fields of knowledge - technical dimension of illness and know-how-practical dimension were elaborated, contemplating the general notions about DM2 and its complications, glycemic self-control, self-care in acute complications, and self-care in drug treatment.

In addition to the knowledge and skills portrayed in the competency roll, it is necessary to consider the attitudes and the necessary awareness that influence the user's behavior and consequently the health improvement^[28]. For Sousa *et al*^[29] the increase in knowledge when correlated significantly with attitude is associated with the predisposition to assume self-care.

In order to verify the knowledge and attitudes of people with DM2 who participated in a self-care education program, Rodrigues *et al*^[30] used the instruments validated for use in Brazil, the Diabetes Knowledge Questionnaire and the Diabetes Attitude Questionnaire. The Diabetes Knowledge Questionnaire covers issues related to knowledge about basic physiology, hypoglycemia, food groups and their substitutions, management of DM2 in the course of another disease, and general principles of care. The Diabetes Attitude Questionnaire presents issues that include stress associated with DM2, treatment receptivity, treatment confidence, personal efficacy, health perception, and social acceptance^[30]. After applying these two instruments, Rodrigues *et al*^[30] concluded that although participants had a good level of knowledge, they still did not change their attitude towards coping with the disease.

As knowledge does not always lead to a change in attitude towards the daily demands that treatment imposes on daily life, it is necessary to listen to the feelings, the hidden complaints of the person with DM2. In this line of reasoning, according to which the subjective perspective of the patient is considered and valued, Cyrino^[4] developed a study with the objective of knowing the skills developed by users with DM2 of a health service for self-care and self-control in DM2, from their testimonials. A set consisting of 98 competences derived from the knowledge of the experience of those who live the disease was raised, distributed in the fields of knowledge, know-how and know how to be and know how to communicate. The competences related to psychological and social difficulties to self-care were expressed by people with DM2, showing differences in conceptions about the disease and care among health professionals and their patients^[4].

To know the skills of people with DM2 for self-care, a scale containing 27 items was developed and validated, assessing physical abilities (vision, touch, dexterity, and manual ability), mental abilities (reading, attention, memory, discrimination and classification of knowledge within certain situations, judgment of certain situations, and conceptualization of a system of actions to act in certain situations), and motivational and emotional capacities^[31,32]. This scale of identification of the competence of the person with DM2 for self-care (ECDAC) allows a qualitative and quantitative evaluation of the capacities of people with DM2 for the exercise of the self-care actions necessary for the maintenance of health^[33]. The deficiencies in the physical, mental, and motivational capacities pointed out by the ladder provide subsidies for the planning and implementation of intervention methods based on the person-centered approach, favoring a global and individualized assistance practice.

ABILITIES OF HEALTH PROFESSIONALS NECESSARY FOR SELF-CARE AND SELF-CONTROL

The complexity involved in self-care and self-management of people with DM2 requires an approach of interdisciplinary care with family health strategy professionals and family health support nucleus open to dialogue, with the ability to communicate, employing person-centered care and valuing the objective and subjective aspects. In this sense, it is recommended that these professionals overcome the biomedical paradigm of being the experts responsible for curing diseases and help people achieve health and normality^[34], through a systemic and comprehensive view of the individual, family, and community in the promotion, specific protection, rehabilitation, and care, working with creativity and critical thinking^[35].

Within this context, Torres *et al*^[36] developed a training program for primary health care professionals for DM2 education. The competency role required by people with DM2 to conduct the treatment developed by Cyrino^[4] was adapted and applied, including questions related to pathophysiology, nutrition, physical exercise, and insulin therapy, to assess the knowledge of the professionals of basic health units concerning self-care. The difficulties identified by the professionals pointed out the need for continuing education and supported the planning and development of the educational program in DM2. For the professionals' training, the work workshops modality was used to motivate the exchange of experiences and knowledge and reflection on the obstacles they experienced in their daily lives when caring for people with DM2.

In order to overcome these obstacles, primary health care professionals should value the individual's own experience, his subjectivity, his conceptions of illness^[17,37], as well as his beliefs^[38,39]. By living the disease in their everyday experience, the subject mobilizes knowledge and attributes meanings to master specific problematic situations, developing the skills (*i.e.* attitudes and practices related to self-care and self-control in DM2)^[4].

Thus, in practicing the person-centered approach, health professionals should have the ability to distinguish the disease from the experience of the disease, so that they find methods of health promotion and preventive care more appropriate to the world of the person, varying according to the person, the moment, and the question of health care. The use of qualitative methodologies provides an in-depth understanding of the broad context of understanding the subjective issues of the person being treated^[40].

After this stage, health professionals and the person in care should work together on a joint problem management plan to define goals, care priorities, and care roles^[40]. To apply problem solving requires the ability to recognize the problem, ability to generate alternative solutions, and insight to select an appropriate option^[41].

This pathology requires a holistic view of the health-disease process by the health professional, with the apprehension of the subject in its biopsychosocial dimension, integrating preventive, promotional, and coordinated assistance actions, for a more comprehensive understanding of the disease and to favor more effective interventions and accession. A structured intervention in multidisciplinary teams for the effective development of programs of education and health promotion of these patients and relatives is fundamental^[42].

The abilities for self-care and self-control in DM2 of users and health professionals are illustrated in Table 1.

CONCLUSION

People with diabetes face obstacles, often filled with social representations. The rules to be followed by these individuals should be adapted to deal with the restrictions, prohibitions, and difficulties that act as contingency to put into practices the desired behavior.

So, to ensure the effectiveness in meeting the people with DM2, the experience of listening, the ability to communicate and understand the subjective aspects of people and the context in which they operate, is a key skill to be developed by health professionals in primary health care. A change in the behavior of health professionals is possible to be motivated by the institution in which it is linked as well as by an internal involvement mediated by the self-conscience of its professional activity.

On the other hand, people with DM2 should have knowledge about the disease, motivation and positive attitude from diagnosis to self-control and self-care, and support from the social network and family. Participation in the educational process should be active, this essential condition to ensure effective results for better

Table 1 Abilities for self-care and self-control in diabetes mellitus type 2

Abilities for self-care and self-control in diabetes mellitus type 2	User	Health professionals
	Physical abilities	Interdisciplinary approach
	Mental abilities	
	Motivational abilities	Person-centered approach
	Emotional abilities	

acceptance of the disease, treatment adherence, metabolic control, and quality of life.

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Retrospective Study

Comparison of awareness of diabetes mellitus type II with treatment's outcome in term of direct cost in a hospital in Saudi Arabia

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Abstract

BACKGROUND

Saudi Arabia is among the top 10 countries with the highest prevalence of diabetes. Cost of prevention and the indirect cost must be calculated to increase the awareness of society and to emphasize disease prevention and limit further complications.

AIM

To understand the importance of awareness and the impact on the expenditure of diabetes mellitus and treatments outcomes.

METHODS

A prospective descriptive and comparative survey was carried out among patients with diabetes mellitus in Saudi Arabia.

RESULTS

One hundred and one participants were included in the study of which 40% were female and one third were above the age of 50. The mean of the first HbA1c reading was 6.95, and the median was 7. The mean of the second reading of HbA1c was 7.26, and the median was 7. The mean body mass index was 32.1, and the median was 30.9. The average yearly cost of the medication was 995.14 SR. Comparing participants who think that a healthy low-sugar diet can affect blood sugar with those who do not, showed a statistically significant difference when cost was considered (P value = 0.03). Also, when comparing the group of participants who know when to take their oral hyperglycemic medicine and their yearly direct cost and those who do not know when to take it, by using independent sample T test, showed significant statistical difference (P value = 0.046).

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CONCLUSION

It is essential for the governments to invest in ways to prevent and help in the early detection of such an expensive disease by performing national screening and education programs. Many pharmaco-economic studies can be done to help the decision-maker in our hospitals think about strategies to help the patient to be physically fit by offering gymnasium or places to walk or contract.

Key words: Middle East; Diabetes; Lifestyle; Hypoglycemic

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Core tip: This study evaluated diabetic patients' compliance to hypoglycemic medications, dietary control, and their impact on cost effectiveness. It shows that lack of compliance has negative impact on patients' therapeutic outcomes, which in turn affects cost of medications and management of diabetic complications. Further educational campaigns are important among diabetic patients in order to reduce negative health consequences and economic outcomes.

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INTRODUCTION

Diabetes mellitus (DM) is a non-communicable metabolic degenerative disorder associated with a high risk of chronic complications and comorbidities^[1]. Obesity and many other inabilities could lead to diabetes if they happen in pre diabetic patients^[2].

Around 422 million people are diagnosed with DM, and 80% of diabetes deaths occur in low- and middle-income countries. Approximately 1.5 million deaths in 2012 were directly caused by diabetes worldwide, while 2.2 million deaths were caused by higher blood glucose level due to the increases of risk of cardiovascular disease in the same year. The prevalence of the disease increased dramatically many fold during the last 3 decades, aligning with the increase of prevalence of obesity, overweight, and physical inactivity^[3]. If no drastic actions are taken, the number of people living with diabetes is expected to reach 552 million by 2030^[4-6]. Cost of prevention and indirect cost must be calculated to increase the awareness of society and to emphasize the importance of disease prevention and limiting further complications^[7]. Saudi Arabia is among the top 10 countries with the highest prevalence of diabetes^[8-10]. Early prevention can limit the complications and their impact on the person's quality of life, reducing the cost with positive impact on the Health system^[11]. Most countries spend between 5% and 20% of their total health expenditure on diabetes^[12,13]. Fourteen percent of the population in the Eastern Mediterranean Region has diabetes^[14], approximately 35 million people. The expected prevalence of diabetes in Middle East and North America (MENA) will be 60 million in 2030^[15,16].

The sixth edition of the International Diabetes Federation Diabetes Atlas reports that only 2.5% of global health expenditure on diabetes is spent in the MENA Region^[17]. The anticipated prevalence for diabetes 2010-2030 in the Gulf countries are: United Arab Emirates 18.7%-21.4%, Kingdom of Saudi Arabia 16.8%-18.9%, Bahrain 15.4%-17.3%, Kuwait 14.6%-16.9%, and Oman 13.4%-14.9%^[18,19]. The recent and rapid socio-economic development of the Gulf Cooperation Council countries has been associated with this rising prevalence.

"The prevalence of obesity in adults of 30-60 years in Saudi Arabia increased by 1.5% for women and 4.1% for men annually between 1992 and 2005. In Qatar and Kuwait, 35% and 36% of male; and 45% and 48% of female adults were found to be obese"^[20]. Equally alarming are the numbers for younger age cohorts: In Kuwait, 21% of males and 18% of females aged 10-19 years were obese^[21-23].

The statistics of World Health Organization in Saudi Arabia in 2016 showed that the rate of diabetes in males was higher than that in females. Also, the level of overweight females was higher than that in males, and the rate of physical inactivity

was higher among women 67.7%, while in men it was 52.1%^[24].

The purpose of this study was to describe the relationship between direct medical costs and individual demographic characteristics, different regimen of treatment, and glycemic control. Here, we include the monthly cost of medications and the pharmacy average consumption of each oral hypoglycemic medication listed in the formulary. In addition, awareness of these patients of the disease and the role of lifestyle modifications in addition to oral hypoglycemic medication are explored. Lack of sufficient awareness will lead to high treatment cost with low therapeutic outcomes.

MATERIALS AND METHODS

A prospective descriptive and comparative face-to-face survey was carried out among patients with DM in Saudi Arabia. The study included both genders of patients visiting the primary care medical center. Patients aged between 35 to 75 years who were on oral hypoglycemic were selected within the inclusion criteria. Pregnant women were excluded from the study. The prices and quantities of average monthly ordering costs of the medicine were collected from the institution.

A random convenience sample of patients following up with the chronic disease clinic (CDC) were selected for this study to help ensure a representative sample. The participants were males and females from different backgrounds and educational and socio-economic levels. The total number of patients registered to follow up in December 2016 was 371, among which 196 patients were not able to come to the appointment and therefore considered as no shows. Among the remaining, 112 patients were involved in this study. The sample size for the study was calculated using raosoft online calculator (<http://www.raosoft.com/samplesize.html>), with a margin of error of 9%, confidence interval of 96%, and response distribution of 50%, and the population number of patients is 371 was used.

A structured questionnaire was used to collect data. The questionnaire was translated into Arabic, the national language of Saudi Arabia, to ensure proper understanding of the questions. The questionnaire was collected by the researcher. The questionnaire was divided into two parts. The first section included questions about the respondents socio-demographic data including, gender, age range, onset of the disease, medical history, and the and the regimen of the hyperglycemic medication. The second part was used to determine the level of knowledge about DM type 2 by checking the awareness of disease, their knowledge about its complications, and how far they are trying to control it by healthy diet and exercise. After finishing the data collection process, data were extracted as an Excel file, and then data were copied on SPSS (version 24, Armonk, NY, United States). Responses were coded and entered into SPSS for analysis using basic frequencies, descriptive, independent samples *t*-test.

Ethical standards for conducting the study were maintained as follows: (1) Confidentiality of all patients guaranteed; (2) Patients' information obtained from the survey was confidential; and (3) Patients can withdraw from the study at any time.

RESULTS

A total of 112 questionnaires were collected, of which 11 responses were incomplete and hence excluded from the study. At the end, a total of 101 responses out of 112 received responses were adopted for the study. Socio-demographic characteristics are listed in [Table 1](#).

Health status of respondent

One third of the participants had only DM (30.7%) as past medical history. More than half (63.4%) suffered from DM with other cardiovascular comorbidities, and 5.9% had diabetes with other diseases.

During 2015 to 2016, the last subsequent two reading of HbA1c of intervals from 3-9 mo were recorded from patients' files, the mean of the first reading was 6.95, and the median was 7. The mean of the second reading of HbA1c was 7.26, and the median was 7. The mean body mass index (BMI) was 32.1, and the median was 30.9.

Lifestyle behavior

Among all participants, 36.6% were not doing any exercise, the remaining (*n* = 65) were classified according to the type of exercise they do, which was mostly walking 55.4%. About one forth (25.7%) of 952 of the people doing exercise said they do it daily, and 13% said they exercised once a week. The mean was 2.7, and the median of

Table 1 Socio-demographic characteristics

Characteristic	Frequency	Percentage
Gender		
Male	61	60.4
Female	40	39.6
Age		
30-39	12	11.9
40-49	22	21.8
50-59	34	33.7
60-69	27	26.7
70-79	6	5.9
Onset of the disease		
< 1	7	6.9
1-5	37	36.6
6-10	28	27.7
> 10	29	28.7
Regimen of treatment		
No medicine, only healthy lifestyle	2	2
Single therapy	51	50.5
Double therapy	35	34.7
Triple therapy	13	12.9

the time to exercise per week was 2. Around one third (33.7%) of the participants exercised between 30 to 59 min every time they exercised, while 36.6% did not do any exercise at all.

In their daily diet, more than half of the participants ate three meals/d (60.4%), 25.7% ate two meals/d, 9.9% ate four meals/d, 3% ate one meal/d, and 1% ate five meals/d. Concerning preferred food, 53.5% prefer mixed refined carbohydrates and complex carbohydrates, 31.7% said they prefer refined carbohydrates, 12.9% prefer protein-based diet, and 2% prefer complex carbohydrates only. About their daily consumption of dates, their answers varied between 5.9% did not eat any dates, to 1% eating 22 dates/d. The mean of their consumption was 6.12 dates/d and the median was 5.

General awareness of participants

Approximately 75% of participants believe that healthy diet can help control blood sugar level, 11.9% did not know, while 12.9% did not believe that it has an effect on blood sugar and suggested that diabetes is a result of if emotional and genetic factors. More than half of the participants (51.5%) were not following any healthy low sugar diet. As regard to exercise, 67.3% believe that it can lower blood sugar level, and 32.7% did not believe that it has any direct effect on blood sugar but did think it is good for general health. Most of the participants (93%) know when to take their oral hyperglycemic medication, while 8% did not know exactly the correct time to take their medicine either before or after food. Around half of them (45.5%) will skip their tablet if ever missed, 35.6% will take the tablet once they remember, 10.9% will double the next dose, and 7.9% said they did not have an idea what to do if ever they missed their oral hyperglycemic medications.

Regarding hypoglycemic symptoms, one third of them (28%) did not know how to deal with them, and 73% knew how to deal with them. More than half of them (63.4%) never visited a diabetic educator. Sixty-five percent said they have full awareness of the disease, while around one third of participants (34.7%) think they are not aware enough. The average yearly direct cost of the hyperglycemic medication of the participants (without any medicine used to treat its complications) was 995.14 SR. The median was 614.4SR with results of being widely distributed. Only two of the participants were not on any medicine because they do not adhere to the regimen (yearly cost is zero), and they were instead following a strict healthy diet and exercise only. The maximum yearly direct cost was 3417 SR, and this patient was taking 6 mg of Glimeperide once a day and 50 mg of Vildagliptine twice a day.

When comparing participants who think a healthy low-sugar diet can affect blood sugar level with their yearly direct cost (mean of yearly direct cost is 952.8 SR) and

those who think low-sugar diet has no effect on their blood sugar level (mean of yearly direct cost is 1334.6 SR) the difference is statistically significant. This is when using independent sample *t*-test, with *P* value = 0.03. Comparing participants who know when to take their oral hyperglycemic medicine and their yearly direct cost (the mean of direct cost = 976.7 SR) and those who did not know (the mean of direct cost = 1209.1 SR) by using independent sample *t*-test, showed significant statistical difference with *P* value = 0.046.

On the other hand, when comparing the yearly cost between the group of participants who are following low sugar diet and those who are not following such a diet, it showed no significant statistical difference by independent sample *t*-test with *P* value = 0.656. Also, there was no statistically significant in the yearly direct cost between the group of participants who think exercise can lower blood sugar level and those who think it has no effect on blood sugar with *P* value = 0.141.

Comparing male and female genders regarding lifestyle showed a statistically significant difference between the number of dates consumption with a *P* value = 0.003 by Levene's test for Equality of Variance by Independent Samples Test. Also, when comparing the type of food preferred as refined carbohydrates and the awareness of participants about the importance of a healthy diet on blood sugar level *versus* gender the *P* value = 0.004 and 0.009, respectively, by using Linear-by-Linear association Chi square test. Using the same type of test to compare gender *versus* physical activity, the *P* value = 0.002. Using Chi square test to compare gender *versus* full awareness of disease, the *P* value = 0.078. When comparing gender *versus* how to deal with hypoglycemic attack with *P* value = 0.026 by using Linear-by-Linear Association. On the other hand, there was no significant statistical difference for gender *versus* following healthy diet and visiting diabetic educator.

Awareness of a healthy lifestyle

The mean HbA1c for the second reading of the participants who said a low-sugar diet can help to decrease blood sugar level *versus* participants who said there is no effect of a low-sugar diet on blood sugar-level was 7.04 *versus* 7.98, respectively, which was statistically significance different (*P* value = 0.007) by independent sample test. On the other hand, there was no relationship between awareness of the significance of healthy diet and BMI levels. The mean BMI of the participants who said the healthy low-sugar diet can lower blood sugar level was 31.6 and the mean of those who said it has no effect on the blood sugar level was 31.8 (independent sample *t* test, *P* value = 0.951).

Thirty-eight percent of the participants were not following a low-sugar diet, although they had the awareness of the impact of a healthy low-sugar diet on blood sugar results. Eight-point nine percent of participants were not following such a diet because they did not have an idea if low-sugar diets had an effect or not. The significant statistical difference according to Pearson chi-square asymptotic significance had a *P* value of 0.001.

Regarding the awareness of the importance of exercise, the mean BMI of the participants who think exercise can lower blood sugar level was 32.05, and the mean of those who said it had no effect on the blood sugar level was 32.20 (independent sample *t*-test, *P* value = 0.695). When comparing the second HbA1c reading between people who think exercise would improve blood sugar level (the mean is 7.11) and those who think it would not (mean is 7.57), it was statistically significant (*P* value = 0.049, Levene's test for equality of variance descriptive data). Of those patients who think exercise could decrease blood sugar, 41.1% of them did not exercise, 10 of 68 exercised once/wk, and only 16 of 68 exercised daily. On other hand, 10 out of 33 who did not think exercise has an effect on blood glucose level do exercise daily for general health only, not because of its importance on blood sugar level. While 28.7% (19 out of 66) think they have full awareness of the disease, they do not think exercise can lower blood sugar level.

Visiting diabetic educator

Among participants who have visited a diabetic educator, 48.6% will skip the missed dose (18 out of 37), 32.4% will take it once remember, and 18.9% of them will double the next dose to compensate for the missed one. There was no statistically significant difference between the people who ever visit diabetic educator and their daily preferred type of food (*P* value = 0.832). Data taken from the pharmacy and supply department in the hospital where the study was conducted showed that the direct cost of diabetes is 133258620 SR.

Participants who are aware of the importance of a low sugar diet have better HbA1c (7.04) in comparison to those who do not have this awareness (HbA1c = 7.98) (*P* value = 0.007). There is, however, no significant difference in BMI between participants who have an awareness of healthy diet (31.6) and not (31.8). Both

categories are obese. On the other hand, participants who are aware of the importance of exercise have better a HbA1c result (mean of HbA1c is 7.11) in comparison to those who did not have this awareness (mean of HbA1c is 7.57) (P value = 0.049, Levene's test for equality of variance descriptive data). These data will encourage us to increase their awareness in order to give better HbA1c results.

DISCUSSION

This study explored participant awareness of DM and the importance of a healthy lifestyle (diet and physical activity) and its impact on their health from a financial and therapeutic point of view. The main past medical history among participants is diabetes with other cardiovascular diseases. Since diabetes is associated with many comorbidities, it is recommended that individuals maintain a healthy lifestyle and HbA1c levels below 7.0%^[25]. The International Expert Committee recommended that persons with HbA1c level between 6.0 and 6.5% were at particularly high risk and might be considered for diabetes prevention interventions^[26,27]. As mentioned in results, HbA1c score worsened instead of improving during the treatment course, which reflected some defect in the chain of treatment. United Kingdom Prospective Diabetes Study and Diabetes Control and Complications Trial demonstrated that improving HbA1c by 1% for diabetic patient cuts micro-vascular complications risk by 25%^[28]. In addition to other research that has also shown that people with type 2 diabetes who reduce their HbA1c level by 1% are 19% less likely to suffer cataract, 16% are less likely to suffer heart failure and 43% are less likely to suffer amputation or death due to peripheral vascular disease^[29,30]. Diabetic patients must be encouraged to lose weight, be more physically fit, and follow a healthy diet and active lifestyle to minimize their risk of complications and increase their quality of life. A high BMI score is associated with substantially shorter healthy and chronic disease-free life expectancy. Physical inactivity has been identified globally as the fourth leading risk factor for mortality. It becomes increasingly important to identify high-risk populations and to implement strategies to delay or prevent diabetes onset^[31]. It is recommended to all individuals with diabetes to have physical activity as part of the therapy plan^[32]. The recommendation is to exercise at least 150 min/wk. It is recommended to do at least 30 min of moderate or vigorous physical activity 5 d of the week. To lose weight or maintain weight loss, they might need to do 60 min or more of physical activity 5 d/week^[33].

In this study, the results are far away from the international recommendations; participants were not following the correct duration and frequency of exercise. Studies have shown that weight loss of 5%-7% improves blood glucose control in type 2 diabetes, reduces cardiovascular risk factors, reduces insulin resistance, contributes to weight loss, and improves well-being^[34,35]. Another way of lowering BMI and controlling blood sugar is to follow a healthy diabetic diet, it is one of the most important services that should be offered to diabetic patients. The recommendation is to limit refined carbohydrates and processed meals. They should focus on high fiber diet and complex carbohydrates like vegetables. Complex carbohydrates are digested slowly, thus preventing the body from producing too much insulin. Carbohydrate counting is a way to plan meals. It has a bigger impact on blood sugar levels than fats and proteins. Some studies have shown that eating too much protein, especially animal protein, may actually cause insulin resistance. A key factor in diabetes is a healthy diet that includes protein, carbohydrates, and fats^[36]. According to this study's results, when compared with the recommended diabetic diet, most of the participants preferred to eat carbohydrates with a smaller number of meals. This result when compared with another study conducted in Iran in 2015, showed that consumption of 24.2 g of one type of dates (approximately two dates) at the snack time did not cause significant alterations in blood glucose level^[37]. However, as sugar caused the same effect on blood glucose, these snacks may not be considered very healthy for patients with type 2 diabetes, even though they have good content of minerals, vitamins, fiber, and antioxidants^[38,39].

Lack of knowledge among participants regarding hyperglycemic medicine affects the incidence of hypoglycemic reactions, which is considered as indirect cost. Unfortunately, many studies from both developed and developing countries have reported that diabetes knowledge is generally poor among diabetic patients^[40-43]. Health care clinic programs to increase patients' awareness about DM and to keep them educated and motivated are essential in order to improve their understanding, compliance, and management and, thereby, their ability to cope with the disease. According to Canadian guidelines for diabetes care, they recommend that all people with diabetes who are able should be taught how to self-manage their diabetes and

offered timely diabetes education that is tailored to enhance self-care practices with comprehensive programs. Incorporate behavioral/ psychosocial interventions, as well as knowledge and skills training with shared decision making, and problem-solving skills are more likely to improve a diabetic's glycemic control^[44-46]. Education of diabetics is one of their rights to be offered in the healthcare system to enhance their treatment outcomes and to minimize the side effect and complications. In this study, the yearly direct cost was higher with the group of participants who had less awareness about the impact of a healthy low-sugar diet on blood sugar level and the group of participants who did not know how to take their oral hyperglycemic medications. But the difference was not statistically significant between the yearly direct cost and those of participants who said they were on diabetic diet (maybe due to their misunderstanding of the best type of carbohydrate and portion recommended), number of meals, dates consumption, and other techniques of a diabetic diet. The same result was found for yearly direct cost and the group of participants who think exercise can lower blood sugar level, which was maybe due to the improper and insufficient time of exercise that does not follow the guideline recommendations mentioned above.

Although around 60% of both genders never visited a diabetic educator, they are almost the same in the awareness of healthy diet and its impact on blood sugar level. Men prefer refined carbohydrates + complex carbohydrates (which are healthier) compared to half of women who mainly prefer refined carbohydrates. The male participants consumed more dates than women, and this difference was highly statistically significant (P value = 0.003). With all of this similarities and differences, there was no significant difference in the yearly cost of both genders.

The rise in diabetes in the gulf region has been linked to many different factors, including diet, exercise, and lifestyle changes due to rapid economic change, increased fast food, and sedentary lifestyle^[47]. In Saudi Arabia, for example, the consumption of meat for each person had increased by 2.2% per year between 1993 and 2003, while fiber rich food has decreased^[48]. The dietary regime in the Gulf Cooperation Council region has moved away from "predominantly consuming dates, milk, fresh vegetables and fruit, whole wheat bread, and fish to mostly foods rich in high saturated fats and refined carbohydrate diets coupled with a low dietary fiber intake"^[49].

In conclusion, poor awareness and limited diabetic education service were considered barriers to get better treatment outcomes. Male patients were more likely to be aware about the disease and adhere more to physical activity than females. There is a greater need for primary care providers to offer continuous diabetes awareness to the public whenever possible to provide the knowledge of preventing disease progression as it is global endemic disease with rapidly increasing prevalence. According to the World Health Organization, it can be prevented and managed through diet and physical activity. The burden of diabetes is huge worldwide; this study showed that the standards of diabetes care in the region can be improved. It may be useful to consider some of the interventions applied worldwide. These could potentially be as effective, and there is a degree of overlap. For example, the use of patient education by small group or a one-on-one setting education programs, diabetes specialist nurses, and self-glucose monitoring appear to be potentially useful and are relatively well-developed components of systems elsewhere.

It is essential for the governments to invest in ways to prevent and help in the early detection of such an expensive disease by performing national screening and education programs. Many pharmaco-economic studies can be done to help the decision maker to think about strategies to help the patient to be physically fit by offering gymnasiums or places to walk or to have a contract with a specialized gym to refer them there. Even if this seems costly, it has a good economic impact.

ARTICLE HIGHLIGHTS

Research background

Saudi Arabia is among the top 10 countries with the highest prevalence of diabetes. Cost of prevention and indirect cost must be calculated to increase the awareness of the society and to emphasize the importance of disease and limiting further complications.

Research motivation

Diabetes complications are the most expensive medical consequences encountered during diabetes management. Lack of patient education regarding lifestyle changes and medication use leads to treatment failure, which adds burden to both patients and the government.

Research objectives

The purpose of this study was to describe the relationship between direct medical costs and individual demographic characteristics, different regimen of treatment, and well glycemic control. Here, we include the monthly cost of medications and the pharmacy average consumption of each oral hypoglycemic medication listed in the formulary. In addition, awareness of these patients of the disease and the role of lifestyle modifications in addition to oral hypoglycemic medication are explored. Lack of sufficient awareness will lead to high treatment cost with low therapeutic outcomes.

Research methods

A prospective descriptive and comparative face-to-face survey was carried out among patients with diabetes mellitus in Saudi Arabia. The study included both genders of patients visiting the primary care medical center. Patients aged between 35 to 75 years who were on oral hypoglycemic were selected within the inclusion criteria. Pregnant women were excluded from the study. The prices and quantities of average monthly ordering costs of the medicine were collected from the institution.

Research results

Results of this study show a lack of proper counseling about lifestyle changes and medication use among patients with diabetes. This study urges other researchers to focus on patient counselling techniques and the barriers diabetic patients encounter during therapy.

Research conclusions

This study shows that there is a lack in patient education about the proper way to manage diabetes, which affects money expenditure on diabetic management. This study proposes the use of well-structured techniques by diabetic educators that include organized follow up plan and utilization of modern technology to reduce diabetic complications and improve quality of life.

Research perspectives

Future research should focus on the utilization of social media in promoting diabetes education in both diabetic and pre diabetic patients.

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Future technology-enabled care for diabetes and hyperglycemia in the hospital setting

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Abstract

Patients with diabetes are increasingly common in hospital settings where optimal glycemic control remains challenging. Inpatient technology-enabled support systems are being designed, adapted and evaluated to meet this challenge. Insulin pump use, increasingly common in outpatients, has been shown to be safe among select inpatients. Dedicated pump protocols and provider training are needed to optimize pump use in the hospital. Continuous glucose monitoring (CGM) has been shown to be comparable to usual care for blood glucose surveillance in intensive care unit (ICU) settings but data on cost effectiveness is lacking. CGM use in non-ICU settings remains investigational and patient use of home CGM in inpatient settings is not recommended due to safety concerns. Compared to unstructured insulin prescription, a continuum of effective electronic medical record-based support for insulin prescription exists from passive order sets to clinical decision support to fully automated electronic Glycemic Management Systems. Relative efficacy and cost among these systems remains unanswered. An array of novel platforms are being evaluated to engage patients in technology-enabled diabetes education in the hospital. These hold tremendous promise in affording universal access to hospitalized patients with diabetes to effective self-management education and its attendant short/long term clinical benefits.

Key words: Diabetes; Inpatients; Continuous subcutaneous insulin infusion; Continuous glucose monitoring; Clinical decision support; Patient education; Self-management

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Core tip: Achieving optimal glycemic control in inpatients with diabetes and hyperglycemia remains a challenge for hospital providers. An array of technology-supported systems are evolving to assist providers and patients in meeting this challenge. Next generation, robust clinical decision support systems embedded in the electronic medical record are well positioned to replace structured order sets in the near term. If demonstrated to be cost effective, fully automated electronic glycemic management systems may become commonplace, in particular in intensive care unit settings. Novel media platforms hold tremendous potential for expanding access to crucial, effective self-management education for all patients with diabetes in hospital settings.

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INTRODUCTION

Adults with diabetes mellitus in the United States account for 7.2 million hospital discharges and 40.3 million hospital days annually^[1,2]. Inpatient glycemic control remains suboptimal both in the United States^[3] and abroad^[4]. Numerous variables impact inpatient glycemic control, including: the pre-admission level of glycemic control^[5]; medications prescribed for acute conditions (*e.g.*, steroids)^[6]; comorbidities such as acute or worsened renal failure; and nutritional status^[7]. Throughout the stay, providers need to identify glycemic trends in the context of multiple dynamic factors to safely and effectively optimize the insulin regimen.

In response to these challenges, technology-enabled systems are being evaluated and adapted for inpatient use. There is significant outpatient experience with diabetes technologies such as continuous subcutaneous insulin infusion (CSII) and continuous glucose monitoring (CGM) systems. Experience with emerging technology systems such as electronic medical record (EMR) based clinical decision support (CDS) for insulin prescription remains limited. Finally, inpatient engagement technology for diabetes education holds the potential to allow access to survival skills education for all inpatients with diabetes.

This editorial will focus on future directions evolving as technology-enabled supports for inpatient diabetes care delivery. For purposes of this discussion, we have grouped these endeavors into three broad categories shown on [Table 1](#).

OUTPATIENT TECHNOLOGIES ADAPTED FOR INPATIENT USE CSII

In 2016 five million persons with diabetes were utilizing CSII pumps^[8-10]. Inpatient CSII use is not well characterized, but is likely to grow. CSII for hospital diabetes self-management is considered by the American Diabetes Association (ADA) to be appropriate for select patients^[11].

CSII pumps deliver basal insulin (units per hour) to meet insulin requirements in the fasting state and between meals. The pump delivers bolus insulin doses (units) to match nutritional intake and as correction doses when blood glucose (BG) levels are high. Hospital providers need to be cognizant of these basics to safely supervise glycemic management when these patients are under their care. Patient ability to continue pump use in the hospital can be assessed by asking patients to describe essential pump skills such as how to adjust the basal rate, administer a bolus dose, and problem solve correction of an out of target BG^[12]. A dedicated insulin pump protocol should address hospital use of CSII, including its use during procedures and in the operating room^[13]. Training in pump basics should be provided to nurses and non-endocrinologist inpatient providers, including hospitalists and anesthesiologists, who may be called upon to write CSII orders and oversee glycemic management^[14].

Potential CSII safety issues in the hospital include insertion site infections; mechanical pump failure; the need for frequent pump interruptions (*e.g.*, radiology

Table 1 Technology-enabled strategies for inpatient glycemic management and diabetes care

Technology category	Purpose	Technologies
Outpatient technologies adapted for inpatient use	Support insulin management	Personal continuous subcutaneous insulin infusion pumps Continuous glucose monitoring sensor systems
Technologies developed for inpatient use	Diabetes and glycemic care management, including care transitions	Electronic medical record based clinical decision support Electronic glycemic management systems
Technology-enabled diabetes education	Engagement in diabetes survival skills education	Electronic medical record-generated, printed education content “SMART” TVs Web-based education platform

tests involving ionizing radiation); and handoffs for procedures and diagnostic testing. Expert consensus recommends that appropriate patient selection is essential to safe hospital CSII use. Limited retrospective case series suggest a good safety record. The largest series ($n = 164$ admissions) found no surgical site infections, mechanical failures, or hospital-acquired diabetic ketoacidosis^[15,16]. Both retrospective studies and a single, small randomized trial suggest that when compared to usual care, inpatient CSII use is equivalent for hyperglycemia events and possibly superior in hypoglycemia prevention^[17,18].

CGM

CGM systems measure and report BG every 5-15 min. CGM technology is estimated to be used by 4%-26% of Americans with type I diabetes^[19]. CGM systems use subcutaneously placed sensors that measure BG in interstitial fluids and typically require changing every 10-14 d. Intensive care unit (ICU) CGM use has been studied for over ten years in both observational and prospective randomized studies of varying size. CGM systems accuracy compared to venous/arterial BG performed in the hospital laboratory and efficacy compared to usual care glycemic outcomes have been examined. The accuracy studies have found data generated by CGM systems to be acceptable. With regards to efficacy, a recent systematic review identified five randomized clinical trials. Most reported no significant difference in glycemic control (*i.e.*, mean glucose or time in range) while two found significant reduction in severe hypoglycemia favoring CGM^[20,21]. Concerns regarding appropriateness of CGM use when factors which may impact subcutaneous circulation such as hypotension have been raised^[22]. Larger randomized studies are needed to confirm benefits in hypoglycemia prevention for CGM in ICU settings and its cost effectiveness when compared to usual care.

Studies assessing routine CGM use in non-ICU settings are limited to small, uncontrolled prospective studies^[23-26]. These studies report no difference in mean daily glucose, and CGM identified more hypoglycemic events compared to traditional point of care testing. However, for patients wishing to use their home CGM devices in the hospital, expert consensus has articulated several important potential safety concerns including the accuracy of CGM data when acute physiologic disturbances are present (*i.e.*, hypoxemia, vasoconstriction, and rapidly changing glucose levels in diabetic ketoacidosis) as well as concerns over correct CGM data interpretation by non- Endocrine inpatient care providers^[18], and as a result, routine use of patient-generated CGM readings to guide inpatient insulin prescribing is not currently recommended.

Several insulin pumps now utilize CGM data to auto-modify insulin dosing via computerized algorithms. While there have been studies looking at use of “closed loop” insulin delivery systems for inpatients^[27-29], to our knowledge, none have used the commercially available pump devices to date.

INPATIENT SPECIFIC TECHNOLOGIES CDS SYSTEMS

Structured insulin order sets are now widely used in hospitals for subcutaneous insulin ordering and have been shown to improve daily average glucose, reduce glycemic extremes, and reduce prevalence of sliding scale only regimens^[30-32]. Based on this evidence, current guidelines recommend the use of structured, electronic order

sets that include advice for optimal insulin prescription^[8].

CDS refers to electronic systems which assist in clinical decision making via provision of recommendations based on processing and presenting patient specific data at an appropriate time. This contrasts with passive order sets that provide advice that is not patient specific. The ubiquity of inpatient EMRs combined with guidelines for the use of insulin to manage most cases with hyperglycemia make inpatient insulin prescribing ideal for incorporation of CDS into workflow. Controlled evidence of the impact of CDS for inpatient insulin prescribing are lacking. However, the safety and acceptability of the Gluco Tab[®] mobile insulin prescription CDS system^[33] has been reported and recently, the creation and implementation of an inpatient insulin prescription CDS module for the Epic EMR system has been described. This utilizes interactive computerized physician order entry elements which prompt the provider to input relevant factors (*e.g.*, indication for insulin - acute hyperglycemia without prior DM *vs* established DM not on insulin *vs* established DM on insulin) while also extracting other relevant factors (*e.g.*, insulin received in last 24 h) in order to process each element into formulating insulin prescription recommendations; the provider then selects one of the provided options^[34]. Studies on the efficacy and safety of this CDS module are in progress.

ELECTRONIC GLYCEMIC MANAGEMENT SYSTEMS

While CDS systems rely on user input and chart extraction of key information, more automated CDS systems require minimal provider input and are termed electronic glycemic management systems (eGMS). Several proprietary eGMS systems have been developed for intravenous insulin infusion and subcutaneous administration. Examples include Glytec's GlucomanderTM system^[35], GlucoStabilizer[®]^[36] by Medical Decision Networks, and Monarch's EndoTool[®]^[37]. These software systems use multivariate algorithms to continuously recalculate the appropriate insulin dose, adjusting to patient specific variables. Generally, the initial insulin dose is set by the provider based on a weight-based calculation or custom order and the algorithm makes subsequent insulin dosing adjustments. There are several potential advantages to such a system, including reduction in hypoglycemia and hyperglycemia, reduction in the cost of care, improvements in patient safety and provider satisfaction.

Reduction in hypoglycemia rates has been shown in several eGMS studies. Rabinovich *et al*^[38] used the Glucomander eGMS to show reduction in BG < 3.9 mmol/L from 21.5% to 1.3% ($P < 0.0001$) and severe hypoglycemia reduction from 5.4% to 0.01% ($P < 0.0001$) in a retrospective review of critically ill patients on insulin infusions. A comparison between the eGMS and a computerized basal-bolus order set for non-critically ill patients on subcutaneous insulin also found a difference in glucose < 3.9 mmol/L (1.9% *vs* 2.8%, $P = 0.001$)^[39]. These results may be magnified when an eGMS is implemented where basal-bolus insulin therapy is not prevalent. Newsom *et al*^[40] found the rates of use of sliding scale insulin go from 95% to 4% after eGMS implementation, moderate and severe hypoglycemia rates drop by 21% and 50% respectively, reduced length of stay and fewer point of care tests per patient. Although there is limited data demonstrating potential cost savings^[41], convincing hospital leadership to invest in them may present a challenge. It remains to be determined where they will fit in the big picture of technology supported inpatient glycemic management as CDS tools evolve and data to support each model accumulates.

TECHNOLOGY-ENABLED DIABETES EDUCATION IN THE HOSPITAL

Deficits in diabetes knowledge and self-care management skills contribute to hospitalizations among persons with diabetes. Hospitalization presents an opportunity to provide education to this population, many of whom may not otherwise have access to this service. An accumulating body of evidence suggests that inpatient diabetes education, improving communication of discharge instructions and involving patients in medication reconciliation may reduce risk for early readmissions^[42] and improve outcomes, including hemoglobin A1C and risk of readmission to the Emergency Department^[43-46].

The ADA recommends that education be provided during an admission when a need is identified^[8]. Content focused on "survival skills" to enable safe self-management until further outpatient instruction, as needed, is recommended. Inpatient diabetes education should also include a discharge plan for continuity of diabetes care

as the transition from hospital to home is especially challenging and is associated with a high risk of negative outcomes, including readmissions^[8]. Inpatient diabetes education delivery may be supported by both “low-tech” and increasingly by “high-tech” patient engagement strategies as shown on [Table 2](#).

While patient engagement technologies offer the potential to expand the reach of education, in the hospital setting research in this field is emergent and outcomes data is lacking. It is crucial to patient engagement that technology tools are user friendly from a human factors perspective and that support is available to assure patient access and movement through the education content. Finally, if education is to be individualized, data security and privacy need to be assured^[47].

Patient education systems are evolving from basic methods to high-tech-enabled systems. Low-tech methods include generic diabetes education sourced from providers such as KRAMES and Healthwise® and delivered *via* “SMART” TVs. These systems offer the advantage availability at every bed in the hospital and delivery through a familiar platform. Reports assessing the impact this type of education are lacking. In addition, whether hospitalized patients would choose to watch health information videos in large numbers remains in question. The Diabetes To Go study explored the effectiveness of video-based inpatient diabetes education in a large urban teaching hospital. Adults with diabetes participated in survival skills education delivered at the bedside *via* DVD player. Significant improvements in diabetes knowledge and medication adherence, as well as a trend towards reduction in hospital admissions in the 3 mo post- intervention were observed^[37].

High-tech support for individualized diabetes education can potentially be delivered from the internet *via* tablet computer or smartphone using a web-interface from an education platform or embedded directly onto a tablet computer. Such platforms have ability to administer surveys and subsequently auto-direct the user to content tailored to responses. Staff must deliver the devices to the bedside, if they are not included with each bed, and staff time is often required to familiarize the patient with the platform.

Education delivery *via* personal-use devices also requires attention to infection control, physical device management and ergonomics. While web-based patient education technologies are being studied in the outpatient setting, inpatient studies are needed.

Finally, there are over 5000 technology applications and a wide variety of telehealth coaching programs available for diabetes education support. Among these technologies, very few have reported data or conducted clinical trials to assess impact on outcomes and none to-date has targeted education for inpatients with diabetes^[48].

CONCLUSION

Despite the current challenges in achieving optimal glycemic control in the hospitalized patient, there are an array of technology-based systems that have the potential to impact the future of inpatient glycemic management. Of the systems reviewed to-date, EMR-based CDS systems which facilitate insulin management and technology-enabled education would appear to hold the greatest potential for widespread dissemination and impact in a cost-effective fashion. Inpatient use of personal CSII pumps and CGM systems will likely continue to grow making it necessary for hospitals to develop policies and familiarize providers with their use. Electronic Glucose Management systems, whether EMR-based or provided by third parties, will also likely play a role in inpatient glycemic management, particularly in intensive care units. Long after an admission, it is reasonable to believe that technology-enabled diabetes education delivered in the hospital could afford the patient clinical benefit, such as has been documented with traditional outpatient diabetes education approaches. Ongoing research to compare and contrast the potential for impact of each of these technologies in hospital diabetes care management and to develop the business case for their use is needed to help enlighten future use strategies.

Table 2 Inpatient diabetes education delivery - current and future states

Modality	Current state	Future state
1:1 at the bedside	Unit nurse/Physician/educator provides basic education- often skills based, <i>e.g.</i> , insulin instruction, and/or printed generic content	Supplemented by printed individualized electronic medical record clinical decision support generated content based on diagnoses, procedures, medications
Low-tech	Generic education content delivered <i>via</i> SMART TV or video	Video-based survival skills education content individualized for diabetes medications prescribed at discharge Provider and/or electronic medical record clinical decision support prescribes targeted generic education content
High-Tech	Generic education content prescribed for delivery at bedside on TV or tablet computer from web-based platform	Individualized education delivered via an interactive patient engagement platform Content auto-directed to learner based on embedded survey responses “App” for telehealth coaching prescribed, <i>e.g.</i> , BlueStar ^[49]

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Another simple regimen for perioperative management of diabetes mellitus

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Abstract

Persons with diabetes who require surgical procedures are increasing day by day. Many of the regimens available to manage patients with diabetes perioperatively are complex. Hence, the junior doctors and the paramedics (Primary care providers on a 24/7 basis) find it difficult to execute them. We need a simple regimen that can be executed in a primary care setting/general floor as it is becoming difficult to accommodate the patients in a sophisticated setting because of the increasing burden of the disease. We suggest a simple regimen in this article (Ram's regimen) which we believe safer, economical and more effective than few simple regimens available to date. Moreover, this regimen does not require any additional equipment such as syringe pumps, measured-volume set, etc. Hence, this regimen can be implemented in a primary care setting/general floor easily and we hope that it will be useful for doctors of various specialties and their patients.

Key words: Diabetes mellitus; Insulin therapy; Perioperative management; Simple regimen

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Core tip: Peri-operative management of diabetes is like walking a tightrope. Complexity of the regimens adds fuel to the fire. We propose a simple regimen, which we believe safer, economical and more effective. "User-friendly" for the primary care providers on 24/7. Executable in a primary care set-up/general floor too, which is becoming inevitable because of the increasing burden of the disease.

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INTRODUCTION

Patients with diabetes who require surgical procedures are increasing day by day. The frequency of surgical procedures as well as the duration of stay in the hospital is more in them when compared to those who do not have diabetes^[1]. The two major types of regimens^[2] available for managing patients with diabetes peri-operatively are (1) subcutaneous insulin; and (2) variable-rate intravenous insulin infusion (VRIII) administered continuously. In our opinion, the latter one, which is commonly followed currently in many parts of the world, is cumbersome for the patients as well as the junior doctors/paramedics (Primary care providers on 24/7) as they require hourly checking of glucose. Also, there is a potential possibility of equipment failure resulting in unintended dose or total stopping of insulin being delivered to the patient in this method, leading to extremes of blood glucose levels. Concerning the subcutaneous regimens, there is a possibility of “peaks and valleys” in the blood glucose levels because of mismatching between the duration of action of insulin and intravenous dextrose/uncertainty of oral intake (as the case may be). Also, the absorption of subcutaneous insulin is unpredictable particularly in the perioperative period^[3].

“No Glucose-No Insulin” method adopted by some anesthesiologists (probably due to the complexity of the regimens and/or fear of hypoglycemia) is not acceptable on many occasions or dangerous sometimes as there is a potential chance of starvation ketosis and electrolyte imbalances^[4]. Although the peri-operative team (anesthesiologists, surgeons, and paramedics) is overburdened with many tasks, the management of diabetes cannot be put on the backburner. Nevertheless, it is unfortunate that it (ignoring the management of diabetes) happens commonly. One of the important causes for this could be the complexity of the regimens, which cannot be brushed aside as a “lame excuse”.

WHAT SHOULD BE THE OBJECTIVES OF THE REGIMEN?

It is disheartening to note that the level of confidence in managing patients with diabetes among junior doctors in the United Kingdom was poor, according to an article published in 2011^[5]. We are afraid that the scenario would be “no different” anywhere, even now. As we are aware of the fact that the peri-operative period consists of inherent problems such as starvation, anxiety, pain, unstable hemodynamics, *etc.* which would have a major impact on patients with diabetes, the complexity of the regimens of perioperative management would add only fuel to the fire. Furthermore, a recent review article about the update of peri-operative hyperglycemia has stated that many studies have established the fact that hyperglycemia is an important cause for increased mortality and morbidity in general surgery patients^[2]. On the other hand, it is mentioned in the same review article that there is a potential chance of hypoglycemia causing death in intensive insulin regimen compared to the moderate one^[2]. Hence, a simple regimen having features such as a moderate target, which can be followed by the trainee doctors and the paramedics round the clock easily thereby improving their confidence, which would also provide a stable blood glucose level, is the need of the hour. Besides, it should be executable in any variety of the places of a hospital (operating room, recovery area and general floors)^[3].

Mode of the regimen

Ram’s regimen (Table 1) suggested in this article is based on an old concept concerning the dose of insulin only (Incidentally, we found that it was originally suggested for continuous insulin infusion)^[6] and modified in all other aspects by the first author who has been adopting this regimen for over two decades. Indeed, the dose of insulin is also modified slightly to remember it easily in the increments of numerical five (5, 10, 15, 20 Units at the rate of 25 drops per minute). After preparing the solution with calculated insulin (Table 1), it can be administered through a separate small-bore intravenous line (Metabolic line) in addition to a large-bore intravenous line (Hemodynamic line) or as a piggyback through a three-way connector to only one line according to individual preference at the rate of 100 mL per hour (*i.e.*, 25 drops per minute or by drop-infusion pump if available). In emergencies where we might encounter a case with very high levels of glucose too, it can be initially stabilized with short-acting insulin (one unit of insulin for every 30 mg/dL rise above 180 mg/dL) administered in 100 mL of isotonic saline over 30 min to one hour. Once the target glucose level (140-180 mg/dL) is achieved, we can switch over to the regimen. Similarly, if a slightly more strict control (120-150 mg/dL) is needed (for instance, joint replacement surgeries) 2.5 U of insulin (0.5 U/h) can be added in

the 500 mL solution in addition to the calculated insulin.

WHY ANOTHER SIMPLE REGIMEN?

To our knowledge, there are only a few simple regimens available to date. The Alberti and Thomas regimen^[7], a simple algorithm for the VRIII^[3], and the Vellore regimen^[8] to name a few. We believe that our regimen is simpler, safer and economical than those few simple regimens, on the following grounds:

(1) Despite its great features such as safety, simplicity and classical concepts, there is a chance of hyponatremia in Alberti regimen^[4] (we believe that it is quite possible in Vellore regimen too), which is unlikely in our regimen as we recommend dextrose in isotonic saline instead of plain dextrose. Moreover, a majority of the VRIII regimens do not recommend routine administration of the required dextrose on an hourly basis (which is mandatory), yet Marks JB recommends 5 g of dextrose per hour as a separate infusion which would prevent protein breakdown, ketosis or hypoglycemia^[3]. Nonetheless, hyponatremia is more likely to happen in any regimen that advocates plain dextrose for prolonged duration^[9]. We recommend 10% dextrose for patients who are susceptible to water load. The dose of insulin needs to be doubled and the rate of administration has to be halved in that case. Despite this, if any patient develops hyponatremia, it should be corrected judiciously by administering hypertonic saline through a central line and/or diuretics according to the case.

(2) Vellore regimen suggests potassium supplementation only when the level reaches 3.5 mEq/L or below, whereas Alberti regimen recommends 10 mmol for every bottle. We suggest it for selected patients (Table 1).

(3) This regimen doesn't require even the 100 mL measured-volume-set as well as hourly checking of glucose, unlike the Vellore regimen. Hence, it is simpler and economical.

(4) We suggest the target glucose of 140-180 mg/dL, which is moderate, aimed to prevent both extremes of glucose levels.

(5) Technically analyzing, Vellore regimen is similar to VRIII regimens with only a change of administering the calculated insulin in 100 mL of 5 % dextrose together instead of insulin as a separate infusion. All the VRIII regimens, as well as the Vellore regimen, require hourly checking of glucose level. In addition to being cumbersome to patients as well as care providers, we believe that there is a possibility of fluctuations in the blood glucose values in these regimens which could be due to the fact that the controlling of diabetes happens retrospectively *i.e.*, the dose of insulin is calculated on the glucose level which probably reflects the metabolic trend of the previous hour, but administered for the subsequent hour. In this context, it is worth to note that it is a usual practice to adjust the night dose of insulin for any deviations of fasting blood glucose and the morning dose of insulin concerning post-lunch values, hence considered a prospective approach. Although the scenario is different (longer-acting subcutaneous versus short-acting intravenous insulin, oral feeds versus intravenous glucose *etc.*) concerning the perioperative period, we believe that the retrospective element would probably play a lesser role in our regimen when compared to VRIII or Vellore regimen. This is because we recommend administration of required dextrose (5 g/h) and the calculated insulin (based on clinical conditions and other factors) together from the beginning to achieve a moderate glucose level (140-180 mg/dL). Hence, our regimen requires only two-hourly checking of glucose until four hours and fourth hourly once stabilized, as it is expected to provide a reasonably stable glucose level. Furthermore, it is easier for the junior doctors/paramedics to follow-up, as the crucial period of control would be usually over within the first few hours under the supervision of a senior physician. Once stabilized, the patient can be managed on the general floor also.

(6) Marks JB mentions that glucose-insulin-potassium (GIK) regimen has easier maintenance following initial stabilization despite its drawback of inability to adjust the dose of insulin and the dextrose administration independently, warranting preparation of new solution^[3]. Nonetheless, our regimen (having a similar concept) requires preparation of a new solution only for a rare occasion (glucose value of less than 100 mg/dL). We can add the extra units of insulin in the remaining solution taking sterile precautions, for any value of above 180 mg/dL.

And (7) Alberti regimen is safe because of its salient feature of administering the combination of insulin with dextrose^[4]. Although our regimen is based on a similar concept, the following variations are worth noting: (1) 5% dextrose with isotonic saline versus plain 10 % dextrose; (2) The dose of insulin is based on clinical conditions and other factors thus tailoring to individual needs. Alberti *et al* had stated this point in 1979 itself, that the starting therapy of their GIK regimen should not be

Table 1 Ram's regimen (dose of insulin)* Target glucose level 140-180 mg/dL.

Condition of the patient	Dose of insulin, i.e., units of insulin per gram of dextrose per hour (U/g per hour) ^[6]	Total insulin in 500 mL of 5% dextrose isotonic saline solution
General guideline	0.2-0.4	5 to 10 U
Obese/hepatic dysfunction	0.6	15 U
Severe infections/sepsis/steroid therapy	0.6-0.8	15 to 20 U

* The dose of insulin can be chosen based on the clinical condition mentioned above as well as other factors such as preoperative requirement of insulin/other anti-diabetic drugs, the preoperative blood glucose level. The solution can be administered at 100 mL/hour. Check capillary glucose two hourly for the first four hours. Add 1 U of insulin per hour for every 50 mg of rising of glucose level above 180 mg (e.g., If it is 280 mg after two hours, it would become 2 U/h, i.e., 6 U for the remaining 300 mL of solution). If the glucose level is between 100 and 140 mg, start 10% dextrose at 50 mL per hour simultaneously, and reduce the insulin dose by 0.5 U per hour in the subsequent preparations. If <100mg, give only 10% dextrose until it reaches 140mg, and reduce the insulin dose by 1 U per hour (minus five of total dose calculated previously) in the subsequent preparations. Once stabilized, the capillary glucose can be checked every four hours. Potassium can be added if required as in cases of (1) hypokalemia; (2) gastrointestinal procedures; and (3) requirement of infusion for more than five hours.

adhered blindly to each and everybody ("Patients will always vary") rather it must be flexible with an application of common sense too^[7]; and (3) Addition of potassium in selected patients.

CONCLUSION

As the perioperative management of diabetes is inherently complex, simpler and safer the regimen better for all persons involved in the care. We hope that the regimen suggested here will be useful for all care providers, educators as well as the patients regardless of the care setting. We certainly agree that our regimen needs to be studied in the future to prove the advantages we have claimed here.

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Oral manifestations in patients with diabetes mellitus

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Abstract

The purpose of this article was to increase the knowledge about oral manifestations and complications associated with diabetes mellitus. An overview was performed on Google, especially in recent reliable papers in relation to diabetes mellitus and its oral manifestations (keywords were “diabetes mellitus”, “oral manifestations”, and “oral complications”). Data were collected and the results were declared. Diabetes mellitus is one of the most common chronic disorders characterized by hyperglycemia. This disease can have many complications in various regions of the body, including the oral cavity. The important oral manifestations and complications related to diabetes include xerostomia, dental caries, gingivitis, periodontal disease, increased tendency to oral infections, burning mouth, taste disturbance, and poor wound healing. Oral complications in diabetic patients are considered major complications and can affect patients' quality of life. There is evidence that chronic oral complications in these patients have negative effects on blood glucose control, so prevention and management of the oral complications are important.

Key words: Diabetes mellitus; Oral complications; Oral manifestations; Periodontal disease; Xerostomia

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Core tip: Since diabetes mellitus is a common disease and can have some annoying manifestations in the patient's mouth, it is important for physicians to be aware of these manifestations and to treat them properly.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia due to either a deficiency of insulin secretion or resistance to the action of insulin or both^[1-3]. Chronic hyperglycemia leads to different complications in various regions of the body including the oral cavity, so blood glucose control is very critical^[4]. Possible mechanisms that may be related to oral complications of diabetes include impaired neutrophil function, increased collagenase activity, and a reduction in collagen synthesis, microangiopathy, and neuropathy^[4].

The oral manifestations and complications related to DM include dry mouth (xerostomia), tooth decay (including root caries), periapical lesions, gingivitis, periodontal disease, oral candidiasis, burning mouth (especially glossodynia), altered taste, geographic tongue, coated and fissured tongue, oral lichen planus (OLP), recurrent aphthous stomatitis, increased tendency to infections, and defective wound healing^[1-8]. The intensity of diabetic complications is usually proportional to the degree and duration of hyperglycemia^[5]. In this study, we briefly reviewed DM and its oral manifestations and complications in recent reliable scientific papers.

XEROSTOMIA

People with diabetes experience salivary dysfunction, which can lead to decreased salivary flow and change in saliva composition. The estimated universal prevalence of xerostomia among diabetic patients ranges between 34% and 51%^[1,2]. Xerostomia can lead to numerous problems such as difficulty in eating, swallowing, and speaking. It can actually have a negative effect on patients' quality of life. Many studies have detected impaired salivary function in adults with diabetes. The etiology is unknown, but may be related to polyuria, autonomic neuropathies, and microvascular changes and alterations in the basement membranes of salivary glands^[2,4,5,7,8]. There is a significant relationship between the degree of xerostomia and glucose levels in saliva. Notably, the highest level of salivary dysfunction is observed in diabetics with poor glycemic control^[4,5].

DENTAL CARIES

Diabetic patients are susceptible to the development of new and recurrent dental caries. Reduced cleansing and buffering capacity of the saliva, increase of carbohydrate in the saliva, and increased level of oral yeasts, mutans streptococci and lactobacilli can lead to an increase in the incidence of tooth decay. In addition, chronic hyperglycemia may cause irreversible pulpitis leading to pulp necrosis^[1,2,5,7,8]. Some studies have shown that apical periodontitis and radiolucent periapical lesions are more common in diabetic compared to nondiabetic individuals^[1,5,9].

PERIODONTAL DISEASE

Poor glycemic control can be associated with the outbreak and progression of gingivitis, periodontitis, and alveolar bone loss. Periodontal disease has been reported with increased incidence and prevalence in patients with type 1 and 2 diabetes. Prevalence of severe periodontitis in diabetic patients compared to nondiabetics has been found to be 59.6%:39%^[3,7,8,10].

Possible mechanisms for explanation of increased susceptibility to periodontal diseases include alterations in host defense response (such as neutrophil dysfunction), subgingival microflora, structure and metabolism of collagen, vascularity, and gingival crevicular fluid and also, inheritance patterns. Furthermore, several risk factors have been reported, which make these patients more susceptible to the development of periodontal disease including poor oral hygiene, poor metabolic control, longer duration of diabetes, and smoking^[3,6-8].

It is noteworthy that numerous studies have shown that periodontal disease has a negative impact on diabetes, and the treatment of periodontal disease has a desirable effect on blood glucose control. The elimination of pathogens by treatment leads to a decrease of inflammation, which in turn reduces insulin resistance; this in turn decreases glucose levels. Therefore, there is a two-way relationship between periodontal disease and diabetes^[1,3,5,10]. In adults, periodontal disease is the main reason for tooth mobility and consequently, loss of it. Therefore, treatment of periodontitis, in addition to lowering blood glucose levels, can prevent tooth loss^[11].

ORAL INFECTIONS

Patients with diabetes are more susceptible to the development of various oral infections including fungal and bacterial infections. Decreased salivary flow rate and the absence of its antimicrobial effects can cause these infections. In addition, an impaired defense mechanism and poor metabolic control may play an important role in developing infection^[2,7,8].

Oral candidiasis is an opportunistic fungal infection. The prevalence of that is increasing, as it is one of the most common fungal infections. Higher candida colonization rates were reported in patients with diabetes type 1 when compared to type 2 (84% *vs* 68%, respectively), while the percentage in nondiabetic subjects was about 27%^[2,12].

Oral candidiasis can be developed by numerous predisposing factors including xerostomia. Salivary dysfunction in these patients can contribute to higher carriage of fungi. Candida-related lesions include denture stomatitis, angular cheilitis, and median rhomboid glossitis^[2] (Figure 1). Candida infection is more prevalent in diabetic patients who smoke, wear dentures, have poor glycemic control, and use steroids and broad spectrum antibiotics^[2,7,8].

BURNING MOUTH

Burning sensation or dysesthesia in the oral cavity of diabetic patients is attributed to poor glycemic control, metabolic alterations in oral mucosa, angiopathy, candida infection, and neuropathy^[1]. Neuropathic pain in these patients can be manifested as burning, tingling, or even as electric shock or stabbing sensation that these symptoms may be very debilitating. These pain sensations have a considerable effect on the physical and psychological functions, and are associated with the level of sleep disturbance, anxiety, and depression^[1,4].

TASTE DYSFUNCTION

Taste dysfunction can occur in patients with poorly controlled diabetes. In a cross-sectional study, among diabetic or prediabetic patients, 5.7% had a sweet taste disorder and 8.6% had a salt taste disorder^[8,13]. Salivary dysfunction can cause altered taste sensation or raise of detection thresholds. Neuropathy also increases the threshold of taste. This sensory dysfunction can inhibit the ability to maintain a good diet and can lead to poor glucose regulation^[1,2,4,7,8].

ORAL MUCOSA ALTERATIONS

Some oral mucosa alterations such as coated and fissured tongue, geographic tongue, recurrent aphthous stomatitis, and some premalignant lesions including lichen planus can be associated with diabetes^[1,2,5,7,8] (Figure 2). Susceptibility of these patients to oral cavity changes is still controversial, but insufficient control of diabetes, immunological alteration, microcirculatory changes with decline of blood supply, xerostomia and alteration in salivary flow and composition, and smoking have been mentioned^[1]. OLP occurs more frequently in patients with type 1 diabetes compared to type 2, because type 1 diabetes is considered an autoimmune disease, and OLP has an underlying autoimmune mechanism^[2,8]. Acute hyperglycemia causes changes in the immune responsiveness in diabetic patients^[2].

POOR ORAL WOUND HEALING

Delayed healing of soft and hard tissues in diabetic patients is a well-known complication during oral surgeries^[2,8]. Based on some studies, effective factors in the prolonged wound healing of these patients include delayed vascularization, diminished blood flow and hypoxia, a reduction in innate immunity, decreased growth factor production, and psychological stress^[2,14].

CONCLUSION



Figure 1 Candida-related lesions. A: Denture stomatitis; B: Angular cheilitis; C: Median rhomboid glossitis.

Oral complications in patients with DM are considered major complications of the disease and can impress the patients' quality of life. There is evidence that chronic and persistent oral complications in these patients adversely affect blood glucose control. Thus, prevention and management of oral complications due to diabetes are considerable.

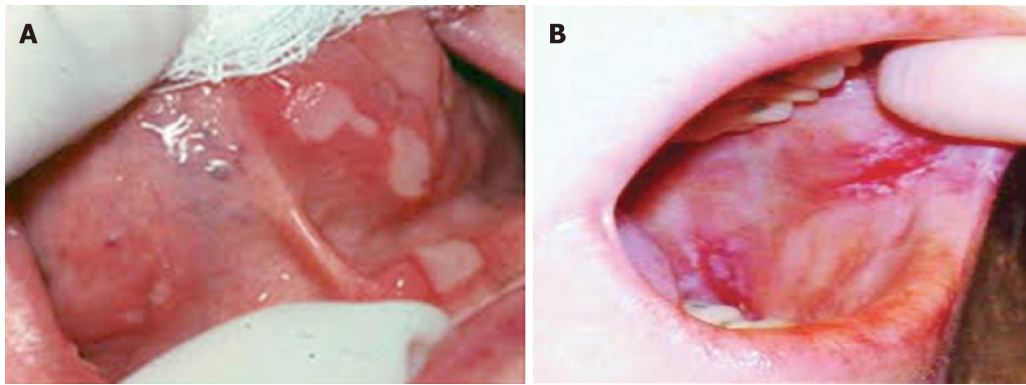


Figure 2 Oral mucosa alterations. A: Recurrent aphthous stomatitis; B: Oral lichen planus.

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**REVIEW**

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Diabetic cardiomyopathy: Pathophysiology, theories and evidence to date

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Abstract

The prevalence of type 2 diabetes (T2D) has increased worldwide and doubled over the last two decades. It features among the top 10 causes of mortality and morbidity in the world. Cardiovascular disease is the leading cause of complications in diabetes and within this, heart failure has been shown to be the leading cause of emergency admissions in the United Kingdom. There are many hypotheses and well-evidenced mechanisms by which diabetic cardiomyopathy as an entity develops. This review aims to give an overview of these mechanisms, with particular emphasis on metabolic inflexibility. T2D is associated with inefficient substrate utilisation, an inability to increase glucose metabolism and dependence on fatty acid oxidation within the diabetic heart resulting in mitochondrial uncoupling, glucotoxicity, lipotoxicity and initially subclinical cardiac dysfunction and finally in overt heart failure. The review also gives a concise update on developments within clinical imaging, specifically cardiac magnetic resonance studies to characterise and phenotype early cardiac dysfunction in T2D. A better understanding of the pathophysiology involved provides a platform for targeted therapy in diabetes to prevent the development of early heart failure with preserved ejection fraction.

Key words: Diabetic cardiomyopathy; Cardiac metabolism; Myocardial steatosis; Myocardial strain

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Core tip: Altered myocardial metabolism and impaired metabolic flexibility are key

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mechanisms implicated in diabetic cardiomyopathy. Glucotoxicity, lipotoxicity, coronary microvascular dysfunction and suboptimal substrate utilisation are examined in detail. The mechanisms implicated and the impact on myocardial structure and function have been scrutinised within this review.

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INTRODUCTION

Type 2 diabetes (T2D) is now a global pandemic. The disease is characterised by insulin resistance, relative impairment of insulin secretion and increased hepatic glucose output resulting in high blood glucose levels. It is now among the top 10 causes of death and represents a major cause of mortality and morbidity in the world^[1]. Globally there were 422 million patients with diabetes in 2014, with growing prevalence from 4.7% in 1980 to 8.5% in 2014^[2]. Heart failure (HF) has emerged as the most common initial cardiovascular complication of diabetes^[2,3]. T2D is likely to contribute to the development of HF through a variety of mechanisms, including disease specific myocardial structural, functional and metabolic changes. In the 2015-16 England and Wales National Diabetes Audit, there were 115695 emergency admissions for patients with diabetes and HF compared to 21399 with myocardial infarction and 29392 with stroke^[4]. Once HF diagnosis is established in T2D patients over the age of 65, mortality risk increases tenfold, and five-year survival reduces to 12.5%^[5].

The diabetic population has been shown to pose a marked preponderance to developing HF following a myocardial infarction^[6-8]. In addition to this, systolic and diastolic left ventricular dysfunction has also been described unrelated to the presence of macrovascular coronary disease^[9-11]. The prevalence of HF in the general population has been estimated to be 11.8%^[12], whereas in clinical trials of cardiovascular outcomes in T2D patients, the prevalence of HF at baseline has varied between approximately 10% and 30% encompassing both HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF) with a doubling of the risk of developing HF in those aged 75-84^[13-23].

Diabetic cardiomyopathy is defined as cardiac dysfunction encompassing structural, functional and metabolic changes in the absence of coronary artery disease (CAD)^[24-26]. This distinct clinical entity was first proposed by Lundbaek^[27] in 1954 as diabetic heart disease independent of hypertension and CAD that commonly co-exist with T2D. Rubler *et al*^[9] in 1972 went on to confirm these observations, describing diabetic-related post-mortem findings in four patients with T2D, glomerulosclerosis and HFrEF with normal epicardial coronary arteries the absence of hypertension, CAD, valvular or congenital heart disease. A large United States nationwide case-control study by Bertoni *et al*^[28] in 1995 further confirmed an association between non-ischaemic idiopathic cardiomyopathy and diabetes. Another recent large population study showed that despite good control of all cardiovascular risk factors, the risk of HF hospitalisation in T2D was still consistently higher although there was little or no increase in risk of mortality, myocardial infarction, or stroke in comparison to the general population^[29].

Experimental studies have shown that functional and structural alterations within the diabetic heart may be caused by a significant difference in underlying metabolic mechanisms and substrate utilisation. These have been supported by multiple hypotheses on how T2D disease processes affect the structure and function of the myocardium resulting in a HF-like phenotype^[30]. The aetiology of diabetic cardiomyopathy is multifactorial. Despite decades of research aiming to determine the causes of this distinct pathology, more research is needed. Current hypotheses include insulin resistance, endothelial dysfunction, fibrosis, cardiac lipotoxicity and energetic impairment. In particular, cardiac metabolism may play a central role in diabetic cardiomyopathy.

Type 1 diabetes (T1D) is a condition of absolute insulin deficiency due to T-cell-mediated autoimmune destruction of pancreatic β -cells. Cardiovascular disease is again a major long-term sequelae of the disease with an impact on healthcare

resources. This encompasses coronary artery disease, cerebrovascular disease, peripheral artery disease, heart failure and cardiomyopathy. The pathophysiology of these processes vary and most of the data from population studies and large databases are focused on T2D. Studies looking at atherectomy samples have previously shown that the pathology of atherosclerosis did not differ between diabetes type^[31]. Angiographic evidence showed that T1D caused more multivessel, distal and severe stenosis^[32]. T1D appear to be affected more by hypoglycaemia and inflammation. Inflammatory markers such as C reactive protein, interleukin receptors and CD4 ligands are higher in T1D^[33,34]. Excess adiposity and altered fat distribution have been shown to contribute to diabetic cardiomyopathy in T1D similar to T2D. For the purposes of this review, we will be focusing on the evidence base behind diabetic cardiomyopathy in T2D.

MYOCARDIAL ENERGY METABOLISM

Normal vs the diabetic heart

The heart converts chemical energy present in the form of substrates and oxygen to mechanical energy and heat^[35,36]. Maintenance of adequate levels of cardiac high-energy phosphate metabolites, adenosine triphosphate (ATP), the energy source for contraction, and phosphocreatine (PCr), the major energy storage compound, are of vital importance for normal heart function^[37]. A healthy heart is capable of metabolising a range of substrates, including fatty acid (FA), glucose, amino acids, ketones and lactate, to produce ATP^[38,39]. In the normal heart, the main substrates for acetyl-CoA and therefore ATP formation are long chain FAs (60%-90%) and glucose and pyruvate oxidation (10%-40%, formed from glycolysis). The normal cardiac metabolic process involves energy transfer initially from substrate to the high energy phosphate metabolite ATP. This occurs through the generation of acetyl-CoA, that then enters the tricarboxylic acid cycle (TCA), also known as Krebs or citric acid cycle, followed by oxidative phosphorylation^[40] resulting in the production of ATP (Figure 1). This is then transferred through facilitated diffusion to the areas requiring the high energy released through ATP hydrolysis, *i.e.*, myosin and sarcoplasmic reticulum^[37,41-43]. Figure 2 shows the role of carnitine in the mitochondrial oxidation of fatty acids contained within Figure 1. Figure 3 details the Randle cycle, also referred to as the glucose-fatty acid cycle which helps regulate uptake and utilisation of glucose within the muscle dependent on the rate of fatty acid oxidation.

Altered myocardial substrate metabolism may play a central role in cardiac dysfunction in T2D patients^[40,44,45], by affecting myocardial oxygen demand which in turn results in reduced metabolic flexibility^[39,46,47]. Metabolic flexibility describes the ability of an organism to respond to changes in metabolic or energy demand as well as the prevailing conditions or activity^[38,39]. There are no myocardial ATP reserves^[35,48,49]. Energy in the heart is stored in three forms. The first form of stored energy is phosphocreatine, which can rapidly donate its high energy phosphates to produce ATP from ADP. The second endogenous form of energy is glycogen. The third form of stored energy is represented by triglycerides.

Stress metabolism studies and metabolic flexibility

Myocardial substrate metabolism can be measured directly by cardiac catheterization and transmyocardial blood sampling. Cardiac metabolic flexibility can be evaluated by coronary sinus (CS) studies invasively and positron emission tomography (PET) studies. Substrate concentrations in arterial and CS blood, in combination with the measured CS flow, yield information about the myocardial use of substrates and oxygen^[50]. Simultaneous CS and coronary artery blood sampling allow detailed analyses of myocardial substrate metabolism obtained by measuring the arteriovenous extraction of carbohydrates, fat, ketones, and amino acids. The flow in the CS correlates with cardiac output, mean arterial pressure, and the flow through the coronary arteries and varies with myocardial oxygen demand^[51]. The technique has been used in healthy individuals^[52], in patients with dilated cardiomyopathy^[53], and syndrome X^[54] during incremental pacing. These studies looked into FA, glucose and lactate metabolism in the heart as key sources of energy production as well as myocardial oxygen consumption at rest and during periods of increased cardiac work. They confirmed that in healthy individuals, cardiac metabolism relies predominantly on oxidation of FAs, but during increased cardiac workload with maximal pacing stress, glucose uptake increases by a factor of two, while FA metabolism does not change. In summary these studies confirmed that, although FAs are the predominant fuel for energy provision in the human heart in the resting state, carbohydrates are the fuel for the heart in a state of exercise or stress. In T2D,

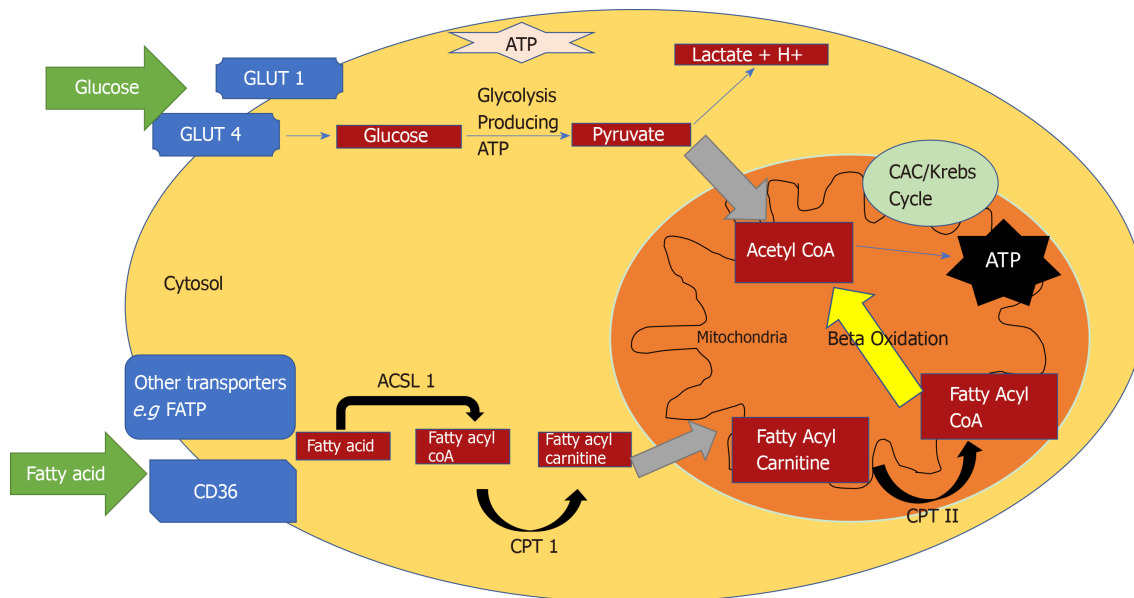


Figure 1 An overview of myocardial energy substrate utilization. Fatty acids and glucose are the major substrates used for ATP generation. The pyruvate generated from glycolysis is metabolised within the mitochondria to produce the majority of carbohydrate-derived ATP while fatty acids undergo β -oxidation. The majority of myocardial ATP originates from the mitochondria via the Krebs Cycle. A preferential increase of activity in one pathway over the other can result in imbalances in substrate uptake and utilization by the mitochondria^[151]. GLUT 1: Glucose Transporter 1; GLUT 4: Glucose Transporter 4; FATP: Fatty Acid Transport Protein; CAC: Citric Acid Cycle; ATP: Adenosine Triphosphate; CPT-1: Carnitine palmitoyltransferase-1; CPT-2: Carnitine palmitoyltransferase-2.

cardiomyocytes have been shown to exhibit insulin resistance, decreased glucose utilisation and increased fatty acid oxidation in response to stress, insulin or variability in FA availability confirming that cardiac metabolic reserve is impaired in diabetes^[55].

Stress metabolism can also be assessed non-invasively by PET. There have been only a few studies to date using PET to assess cardiac metabolism in patients with T2D. However, there has been conflicting outcomes from these studies^[56] regarding the changes in myocardial glucose uptake and insulin resistance in T2D patients. While one study showed reduced myocardial glucose uptake and myocardial insulin resistance, another study showed preserved myocardial insulin responsiveness in patients with T2D^[46,57]. One small study using PET imaging to characterize myocardial glucose metabolism in patients with T2D evaluated the influence of chronic hypertriglyceridemia on myocardial glucose uptake. They compared five T2D patients with high triglyceride (TG) levels to 11 T2D patients with normal TG. Patients were matched by age, gender, blood pressure and glycaemic control. They showed 40 and 65% lower myocardial glucose uptake in the high TG group compared normal TG group, respectively, and evidence of myocardial insulin resistance in T2D. Another small PET study assessing skeletal muscle and myocardial glucose uptake in 10 patients with T2D compared to nine age and weight-matched healthy male controls under normoglycaemic hyperinsulinaemic conditions, showed preserved myocardial glucose uptake^[57]. A third PET study compared glucose utilization in diabetic ($n = 19$) and nondiabetic CAD patients ($n = 35$) undergoing coronary artery bypass graft surgery grafting surgery (CABG) also showed lower myocardial glucose uptake in diabetic cohort, but this study did not assess myocardial insulin resistance^[56].

Myocardial energetics

The phosphocreatine to ATP (PCr/ATP) ratio is a sensitive measure of myocardial energetic status. ³¹-Phosphorus magnetic resonance spectroscopy (³¹P-MRS) provides a non-invasive quantification of myocardial PCr/ATP. Normally, the body is able to increase phosphotransferase reactions, glycolysis and glycogenolysis to function under higher energy demands^[43,58,59]. Scheuermann-Freestone *et al*^[60] pioneered the study of myocardial energetics in 2003 by studying cardiac and skeletal muscle energetics in T2D. Twenty one diabetics and 15 controls were studied to determine normal energy metabolism and the effect of T2D on circulating glucose and free fatty acid concentrations. They showed that, in the presence of normal cardiac structure, function and morphology, patients with T2D had 35% lower PCr/ATP ratios. There was a significant negative correlation with fasting plasma FA concentration as well as a significant positive correlation with plasma glucose concentration in diabetics

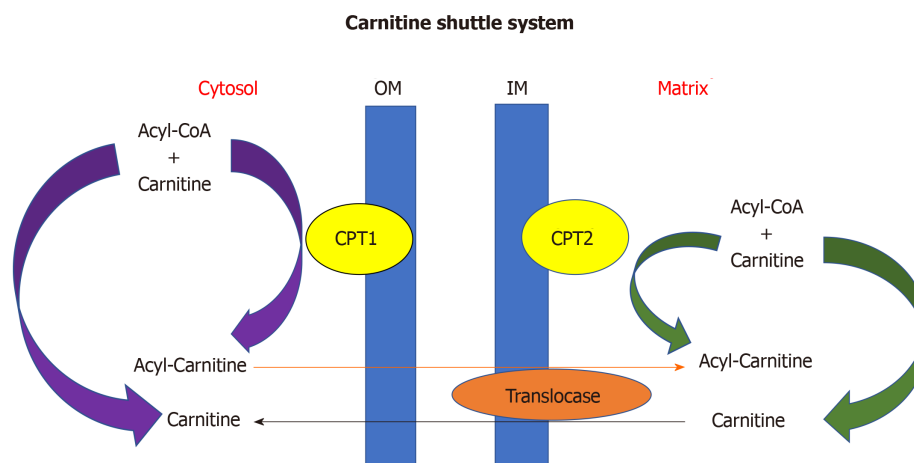


Figure 2 Carnitine shuttle system. This summarises the role of carnitine in the mitochondrial oxidation of fatty acids; contained within **Figure 1**^[152]. CPT-1: Carnitine palmitoyltransferase-1; CPT-2: Carnitine palmitoyltransferase-2; OM: Outer mitochondrial membrane; IM: Inner mitochondrial membrane.

compared to controls. In skeletal muscle, energetics was normal at rest, but the decrease in PCr was faster during exercise in those with diabetes and the recovery was slower after exercise. This lends credibility to the theory that changes in myocardial and skeletal muscle energetics is part of the initial disease process in T2D and this results in changes in substrate use and availability. Cardiac muscle is more affected by the energetic alterations and substrate availability^[60].

Shivu *et al*^[61] looked at 25 asymptomatic type 1 diabetics *vs* 26 healthy controls who underwent ³¹P-MRS and adenosine stress cardiovascular magnetic resonance (CMR) and again this showed significantly reduced PCr/ATP ratio in diabetics, both long-term and newly-diagnosed, compared to healthy volunteers (2.23 ± 0.56 *vs* 1.49 ± 0.44 , respectively, $P < 0.001$). Mean myocardial perfusion reserve index (MPRI) correlated negatively with duration of diabetes, with a significant reduction in long-term diabetes (1.7 ± 0.6) compared to newly diagnosed subjects (2.1 ± 0.2 , $P < 0.05$) and controls (2.3 ± 0.3 , $P = 0.05$). However, there was no significant correlation between PCr/ATP and MPRI implying the myocardial energetic alteration was independent of any coronary microvascular dysfunction primarily results from metabolic dysfunction^[61].

Most recently, Levelt *et al*^[62] have shown significant correlation between myocardial systolic strain and rest and stress PCr/ATP in 31 T2D compared to 16 healthy controls. Subjects underwent ³¹P-MRS performed both at rest and exercise and adenosine stress CMR for assessment of perfusion by MPRI and oxygenation. The PCr/ATP was 17% lower in T2D compared to controls (1.74 ± 0.26 *vs* 2.07 ± 0.35 , respectively, $P = 0.001$) at rest, with a further 12% reduction in PCr/ATP during exercise in T2Ds. However, there was no change in PCr/ATP in the control group during exercise. Similarly, MPRI was lower in diabetes (1.61 ± 0.43 *vs* 2.11 ± 0.68 in controls, $P = 0.002$). At rest, no correlation was observed between PCr/ATP and MPRI, but a significant correlation was noted during exercise. These findings support the notion that under stress microvascular dysfunction may exacerbate energetic derangement^[62].

Cardiac metabolic reserve is impaired in diabetes. Lower metabolic reserve is likely to be the consequence of impaired metabolic flexibility and may be associated with increased mortality, as has been shown in a small study of patients with dilated cardiomyopathy^[63].

DIABETIC CARDIOMYOPATHY AND POTENTIAL MOLECULAR MECHANISMS

Glucotoxicity

Activity of pyruvate dehydrogenase (PDH), which is a key enzyme that regulates the balance between carbohydrate and fat metabolism in the heart, is decreased in diabetes and pyruvate oxidation impaired^[64,65]. Inhibition of PDH limits pyruvate oxidation^[66]. The dissociation of glycolysis and pyruvate oxidation in the diabetic heart results in the accumulation of glycolytic intermediates^[67]. Failure to adequately control intracellular glucose levels has also been implicated in the development of

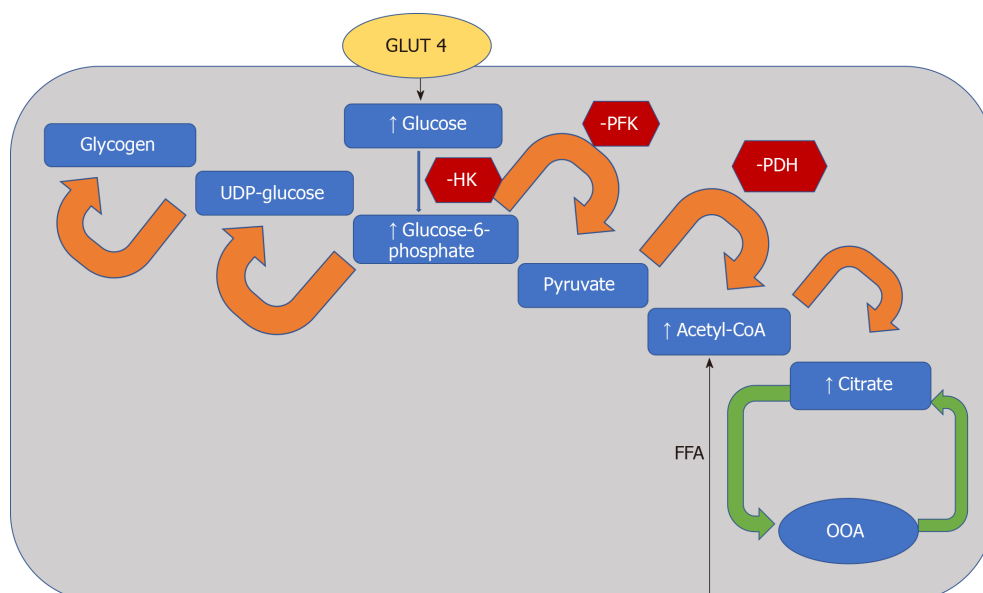


Figure 3 Randle cycle. The glucose-fatty acid (Randle) cycle in muscle. Oxidation of fatty acids inhibits pyruvate dehydrogenase. Citrate inhibits phosphofructokinase. The rise in glucose-6-phosphate inhibits hexokinase^[153]. FFA: Fatty acids; HK: Hexokinase; PDH: Pyruvate dehydrogenase; PFK: Phosphofructokinase; UDP: Uridine diphosphate; GLUT 4: Glucose transporter 4; OOA: Oxaloacetic acid.

insulin resistance and in the generation of reactive oxygen species (ROS). These glycolytic intermediates activate glucose sensing transcription factors^[68,69]. It has been previously postulated that accumulation of glycolytic intermediates decreases the sarcoplasmic reticulum calcium ATPase 2a (SERCA2a) expression^[70], an essential enzyme in calcium homeostasis, which results in diastolic dysfunction^[71]. Confirming this, SERCA2a expression in the heart was shown to be decreased in response to diabetes^[71,72].

Lipotoxicity and myocardial steatosis

In diabetes, a decrease in insulin sensitivity leads to an inability to suppress lipase within adipose tissue and very low-density lipoprotein within the liver. This results in high levels of circulating FAs and as a consequence peroxisome proliferator activated receptor- α (PPAR α) activation, while decreasing glucose-transporter-4 activity^[47,73]. PPAR α is an essential component in cardiac substrate switching. Decreased PPAR α expression (due to pressure overload and/or prolonged exposure to hyperglycemia and/or hyperlipidemia) will limit the FA oxidative capacity of the heart^[74]. The discordance between the rates of FA availability and/or uptake with that of FA oxidation results in increased intracellular long chain fatty acyl-CoA concentrations^[46]. Since cardiomyocytes are not specialised to store lipid, this finding suggests a deleterious effect, and cellular lipid overloading underlies the concept of “lipotoxicity” as a potential mechanism for impaired cardiac function^[75-78]. The excess long chain fatty acyl-CoA is then diverted towards non oxidative processes with the production of lipotoxic intermediates such as ceramide and diacyl-glycerol^[47]. This is postulated to be a potential mechanism for impaired cardiac structure and function^[76-79]. The molecular mechanisms described above that have been implicated in the pathophysiology of diabetic cardiomyopathy are detailed in **Figure 4**.

Imaging myocardial triglyceride

Proton (^1H)-MRS offers a non-invasive method to measure cardiac triglyceride (TG) content. Using this methodology, studies have shown myocardial TG content to be significantly raised in T2D (**Table 1**). The studies are all consistent in reporting an increased hepatic triglyceride content in the diabetic population. Levelt *et al*^[62] has also recently shown that myocardial lipid level is a predictor of concentric LV remodelling independent of BMI, systolic and diastolic blood pressure, and circulating FA, and is associated with subclinical contractile dysfunction in T2D. The study recruited 46 non-hypertensive T2D patients and 20 matched controls. Seventy four percent of the diabetic patients were on statin therapy resulting in lower total cholesterol and low-density lipoprotein cholesterol levels in patients compared to controls. This provides another avenue for potential therapy in T2D as myocardial steatosis has been found to be reversible with cholesterol and triglyceride control in addition to glucose control^[80,81].

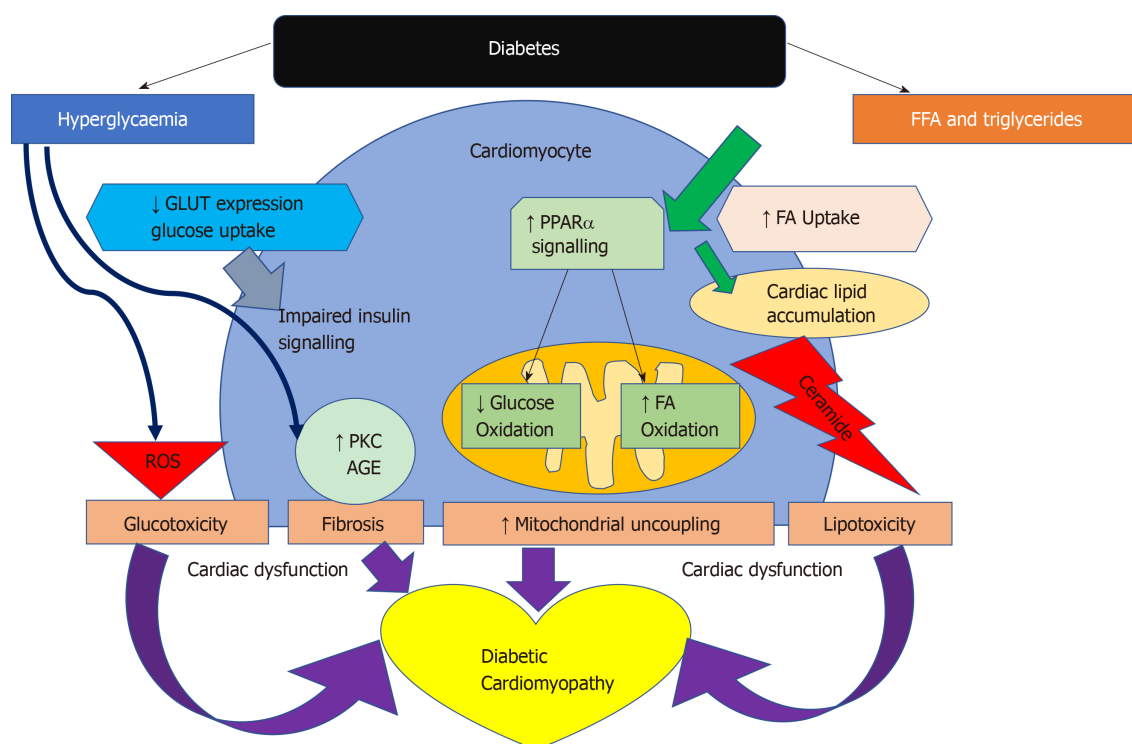


Figure 4 Pathways of cardiac dysfunction leading to diabetic cardiomyopathy. Pathways leading to the development of diabetic cardiomyopathy^[154]. AGE: Advanced glycation end products; FA: Fatty acids; FFA: Free fatty acids; GLUT: Glucose transporters; PKC: Protein kinase C; PPARα: Peroxisome proliferator-activated receptor alpha; ROS: Reactive oxygen species.

Increased myocardial TG content has been shown in T2D. Compared with lean subjects, myocardial TG content was elevated 2.3-fold in impaired glucose tolerance and 2.1-fold in T2D. This was evidenced in a cross sectional study looking at both lean and increased BMI participants with and without T2D^[82]. Myocardial TG has also been shown to be an independent predictor of left ventricular diastolic function in multivariable analysis accounting for BMI, heart rate, visceral fat and diastolic blood pressure. In a study of 38 T2D and 28 matched controls, myocardial TG measured by (¹H)-MRS was significantly higher in T2D. Although there was no significant difference in systolic function, all indices of diastolic function were significantly impaired in T2D^[83]. Interestingly, caloric restriction in obese T2D patients on a very low calorie diet of 450 kcal/d was been shown to decrease myocardial TG content from 0.88 +/- 0.12% to 0.64 +/- 0.14%, respectively ($P = 0.019$) over a 16 wk period, and this was associated with better diastolic function (E/A ratio from 1.02 +/- 0.08 to 1.18 +/- 0.06)^[84]. Myocardial TG level is a predictor of concentric LV remodelling independent of BMI, systolic and diastolic BP, circulating FA and is associated with subclinical contractile dysfunction in T2D^[62].

MYOCARDIAL STRUCTURAL AND FUNCTIONAL CHANGES IN DIABETES – EVIDENCE FROM HISTOLOGY, MRI AND ECHOCARDIOGRAPHY STUDIES

Structural changes

Pathological evidence characterised by myofibrillar hypertrophy with fibrotic strands extending between muscle fibres to cause diffuse myocardial fibrosis first helped distinguish cardiomyocyte damage in diabetes^[9]. The advancement in non-invasive imaging techniques has facilitated further delineation and phenotyping of the diabetic heart. Alterations to left ventricular geometry lead to concentric remodelling, hypertrophy and eventually increased mass. Left ventricular hypertrophy in diabetes includes both concentric and eccentric hypertrophy. Left ventricular concentric remodelling has been shown to have a higher association with cardiovascular mortality than eccentric remodelling on both echocardiographic and CMR studies^[62,85-87].

Increased left ventricular mass had initially been shown to be associated with a rise

Table 1 Myocardial triglyceride content in type 2 diabetes

Study details (Author,Year)	Sample population	Exclusion	Results
Jankovic <i>et al</i> ^[92] , 2012	<p>$n = 18$</p> <p>IT ($n = 10$)</p> <p>Mean age 56 ± 2</p> <p>DM duration 9 ± 2 yr</p> <p>6 males</p> <p>HbA1c 11.1 ± 0.4</p> <p>Oral therapy (OT) ($n = 8$)</p> <p>Mean age 53 ± 2</p> <p>DM duration 3 ± 1 yr</p> <p>4 males</p> <p>HbA1c $9.8\% \pm 0.7\%$</p>	<p>Previous MI, CAD, HF, digitalis use or thiazolidinediones, previous insulin use or T1D</p> <p>Patients on insulin must have had insufficient control on oral, HbA1C > 8% and on oral therapy</p>	<p>Baseline IT myocardial lipid content $0.42\% \pm 0.12\%$ vs $0.80\% \pm 0.11\%$ water signal, ($^aP = 0.034$).</p> <p>Myocardial lipid content decreased by 80% after 10 d IT ($P = 0.008$). No significant change in hepatic lipid content. After 181 ± 49 d, myocardial lipid content returned to baseline (0.37 ± 0.06, $P = 0.692$) Hepatic lipid content decreased by 31% ($^bP < 0.001$)</p>
Korosoglou <i>et al</i> ^[119] , 2012	<p>$n = 58$</p> <p>T2D ($n = 42$)</p> <p>Mean age 62 ± 6 yr</p> <p>26 male</p> <p>Mean BMI 31.6 ± 4.8 kg/m²</p> <p>HV ($n = 16$)</p> <p>Mean age 62 ± 3 yr</p> <p>10 male</p> <p>Mean age 62 ± 3 yr</p> <p>Mean BMI 23.9 ± 2.5</p>	<p>Unstable condition, clinical signs of heart failure or angina</p> <p>contraindications for CMR, insulin use</p>	<p>Significant association between myocardial triglyceride content and mean diastolic strain rate ($r = -0.71$, $^cP < 0.001$) and no association was found between triglyceride content and perfusion reserve ($r = -0.08$, $P = NS$)</p>
Van der Meer <i>et al</i> ^[101,155] , 2009	<p>$n = 72$ T2D</p> <p>All males</p> <p>Pioglitazone ($n = 39$)</p> <p>Metformin ($n = 39$)</p> <p>Baseline age 45-65</p> <p>HbA1C 6.5%-8.5%</p> <p>BMI 25-32</p>	<p>BP > 150/85 mmHg, previous insulin or thiazolidinedione use, previous positive stress echo or arrhythmia, diabetes related complications or significant medical problems</p>	<p>No significant change in myocardial fatty acid uptake at follow up on either arm. Metformin arm showed a significant decrease in fatty acid oxidation and myocardial glucose uptake. No significant change in myocardial triglyceride content in Pioglitazone or Metformin arm after therapy however there was a decrease in hepatic triglyceride content in the Pioglitazone arm</p>
Rijzewijk <i>et al</i> ^[83] , 2008	<p>$n = 66$</p> <p>T2D ($n = 38$)</p> <p>All males</p> <p>mean age 57 ± 1 yr</p> <p>BMI: 28.1 ± 0.6</p> <p>Controls ($n = 28$)</p> <p>All males</p> <p>Mean Age: 54 ± 1</p> <p>BMI: 26.9 ± 0.5</p>	<p>Females, HbA1C > 8.5%, BP > 150/80, hepatic impairment or history of liver disease, substance abuse, known CVD, DM complications, contraindication to MRI, use of lipid lowering therapy.</p>	<p>Myocardial triglyceride content in T2D vs controls ($0.96\% \pm 0.07\%$ vs $0.65\% \pm 0.05\%$). Hepatic triglyceride content in T2D vs controls (8.6% vs 2.2%). Both cases, $^dP < 0.05$ On univariate analysis, myocardial triglyceride content correlated with age, visceral adipose tissue, cholesterol, plasma glucose and insulin and hepatic triglyceride content ($^eP < 0.05$ for all). E/A independently associated with myocardial triglyceride content on multivariate analysis (inverse correlation)</p>
McGavock ^[82] 2007	<p>$n = 134$</p> <p>Lean(L) ($n = 15$)</p> <p>Age 35 ± 3 yr, 47% males</p> <p>BMI 23 ± 2, non T2D</p> <p>Overweight/Obese(O): ($n = 21$) Age 36 ± 12, BMI 32 ± 5, 48% males, non T2D</p> <p>Impaired glucose tolerance(I): ($n = 20$)</p> <p>Age 49 ± 9, 25% males, BMI 31 ± 6,</p> <p>T2D ($n = 78$),</p> <p>Age 47 ± 10, 47% males BMI 34 ± 7</p>	<p>Age > 70 yr, known CAD, Previous MI, contraindications to MRI, thiazolidinedione treatment</p>	<p>↑Subcutaneous, visceral fat and hepatic triglyceride in O,I and T2D vs L, ↑myocardial triglyceride content in I and DM vs L (0.95 ± 0.60 vs 1.06 ± 0.62 vs 0.46 ± 0.30 fat/water content, $^fP < 0.05$), this remained significant after adjusted for serum triglyceride, BMI, age and gender. In multiple regression model, Subcutaneous and visceral fat both independent determinants of myocardial triglyceride content ($^gP < 0.05$) however myocardial triglyceride unrelated to hepatic triglyceride or diastolic function</p>

DM: Diabetes mellitus; MI: Myocardial infarction; T1D: Type 1 diabetic; CAD: Coronary artery disease; BMI: Body mass index; IT: Insulin therapy.

in HbA1c levels in a HF population^[88]. Studies using both echocardiography and MRI have been successful in showing an increase in left ventricular mass associated with diabetes independently of other factors^[10,62,85,87,89-94]. Table 2 details studies that have examined left ventricular mass and concentric remodelling on MRI in T2D. In summary, there is a general trend that shows an increase in left ventricular mass. However, when corrected for body surface area, this does not persist. In two studies, there remained a higher left ventricular mass/volume ratio, but due to a general increase in end diastolic volume this difference is obliterated when corrected for volume. A decrease in stroke volume is as expected in concentric remodelling. Increased left ventricular mass is a known predictor of cardiovascular mortality and morbidity^[95,96].

CMR T1 mapping pre and post-contrast allows calculation of myocardial extracellular volume (ECV) which is a surrogate for diffuse interstitial fibrosis and correlates with pathology samples obtained in patients with heart failure^[97]. Levelt *et al*^[62] used similar techniques and concluded that although there was concentric remodelling and evidence of diastolic dysfunction, there was no evidence of expansion of extracellular matrix in people with well-controlled T2D. However, this is contrary to most existing literature on ECV and T1 mapping to date, which has shown increased ECV and native T1 values in T2D compared to healthy controls^[98,99]. Swoboda *et al*^[100] also showed increased ECV and native T1 relaxation at baseline in T2D and demonstrated that treatment with renin-angiotensin-aldosterone system inhibition led to an improvement in left ventricular ejection fraction and a decrease in myocardial ECV. The differences in literature on extracellular matrix may be related to patient selection for the controls. For example, young and healthy controls rather than age-matched non-diabetics. Sensitivity of different T1 mapping techniques may also play a small role.

Functional changes

Diabetic cardiomyopathy has mainly been linked with features of diastolic dysfunction. This is especially apparent in asymptomatic individuals as the earliest sign of HF. Most of the evidence in imaging of patients with T2D have not shown a significant decrease in ejection fraction/systolic dysfunction^[88,101-103] with the exception of the Strong Heart Study where a direct correlation of ejection fraction was seen associated with HbA1c levels^[87]. Diastolic dysfunction is now regarded as the first functional change occurring in diabetic cardiomyopathy.

Strain is a measure of tissue deformation. As the ventricle contracts, muscle shortens longitudinally and circumferentially and thickens radially. The application of strain to measure deformation is constrained by a number of complexities when the parameter is measured by echocardiography^[104]. Tissue Doppler imaging (TDI) in echocardiography improved the identification of early diastolic dysfunction and recent developments in strain imaging on CMR has very much improved the sensitivity and accuracy of identifying subclinical diastolic dysfunction in the T2D population. Measurement of strain using CMR is now considered the gold standard. Strain rate measures the time course of deformation and this can be derived from speckle tracking echocardiography or measured directly from MRI cine images with dedicated software programmes of which several are available.

Both longitudinal and circumferential strain have been shown to be impaired in T2D. Table 3 identifies the larger and more recent studies that have shown early changes in strain in people with diabetes^[11,88,91,94,105-107]. Different studies have studied and reported different measures of strain making the literature not directly comparable. Global longitudinal strain (GLS) has been shown to be decreased in the diabetic population compared to healthy volunteers in all the studies described across tissue Doppler, speckle tracking echocardiography and CMR. Additionally, some studies have shown a decrease in radial strain. One study has shown that peak early diastolic strain rate (PEDSR) and peak systolic strain rate are both decreased in diabetes even when compared to obese/overweight non-diabetic controls^[11]. Strain and strain rate, especially PEDSR is a good measure contractility and contractile reserve. Its application in clinical practice remains in infancy to be used to predict potential development of heart failure with preserved ejection fraction in the form of a contractility index. It is widely regarded as a precursor to the onset of heart failure in T2D and a predictor of cardiovascular mortality and morbidity even in the absence of symptoms^[108,109].

Coronary microvascular dysfunction

Table 2 Magnetic resonance imaging studies looking at left ventricular mass and concentric remodelling in diabetes

Study details (Author, Year)	Sample population	Exclusion criteria	Main findings
Ng <i>et al</i> ^[91] , 2012	<i>n</i> = 69 DMs (<i>n</i> = 50, 35 T1DM) Mean age 51 ± 10 yr, 54% males. BMI 26.3 ± 3.7 Controls (<i>n</i> = 19), matched for age (45 ± 15), sex (63.2% males) an BMI 26.1 ± 4.4	Age < 18 yr, arrhythmia, CAD, MI, RWMA, segmental LGE, EF < 50%, valve disease	No difference between groups for LVEDVI, LVESVI, LVMI, LVEF.
Wilmot <i>et al</i> ^[93] , 2014	T2D <i>n</i> = 20, mean age 31.8 ± 6.6, BMI 33.9 ± 5.8 kg/m ² Lean Controls: <i>n</i> = 10, Mean age 30.9 ± 5.6, Mean BMI 33.4 ± 2.4, 60% males Obese Controls: <i>n</i> = 10, Mean age 30.0 ± 6.7, Mean BMI 21.9 ± 1.7, 50% males	Weight > 150 kg, contraindications to MRI. In diabetic group BMI > 30 (> 27.5 in South Asians)	↑ LVM (85.2 <i>vs</i> 80.8 g, ⁱ <i>P</i> = 0.002) and LVM/Volume (0.54 <i>vs</i> 0.45 g/m ² , ^k <i>P</i> = 0.029) in participants with diabetes compared to lean controls. No significant difference in LVMI
Larghat <i>et al</i> ^[10] , 2014	T2DM: <i>n</i> = 19 Mean age 59 ± 6, 68% males, BMI 30.81 ± 4.6 Pre-DM: <i>n</i> = 30 Mean age 57 ± 8, 43% males, BMI 30.1 ± 5.0 Non DM: <i>n</i> = 46, Mean age 57 ± 7, 41% male, BMI 29 ± 4.9	Coronary artery Stenosis > 30% luminal narrowing on angiography, previous MI, significant heart disease, contraindications to MRI or adenosine	↑ LVM (112.8 ± 39.7 <i>vs</i> 91.5 ± 21.3 g, ¹ <i>P</i> = 0.01) in participants with diabetes. Participants with diabetes also showed an increase in LVEDV and SV, but non indexed
Levelt <i>et al</i> ^[62] , 2016	T2DM: <i>n</i> = 39, Mean age 55 ± 9, 58% males, BMI 28.7 ± 5.6 Controls: <i>n</i> = 17, Mean age 50 ± 14 yr 53% males, BMI 27.1 ± 5.0	History of CVD, chest pain, smoker, uncontrolled hypertension, contraindications to MRI, ischaemia on ECG, renal dysfunction, insulin use, significant CAD on CTCA	EF, LVM, LVMI, no significant difference between groups. ← LVM/Volume (0.98 ± 0.21 <i>vs</i> 0.70 ± 0.12, ^m <i>P</i> < 0.001), LVDEV (125 ± 30 mL <i>vs</i> 161 ± 39 mL, ⁿ <i>P</i> = 0.001) and lower SV in diabetes

IT: Insulin therapy; OT: Oral therapy; BMI: Body mass index; CAD: Coronary artery disease; MI: Myocardial infarction; RWMA: Regional wall motion abnormality; LVEDVI: Left ventricular end diastolic volume index; LVESVI: Left ventricular end systolic volume index; LVMI: Left ventricular mass index; LVEF: Left ventricular ejection fraction.

Coronary microvascular dysfunction in diabetes is a complex pathophysiological process, which involves structural, functional and metabolic alterations. It is likely to be a multifactorial phenomenon, related to changes in perivascular and interstitial fibrosis^[110], myocardial hypertrophy^[111], reduced capillary density, and autonomic neuropathy^[112]. Coronary microvascular dysfunction has emerged among the potential mechanisms leading to increased incidence of heart failure^[113] and risk of cardiovascular mortality^[3,114] in patients with diabetes. Both hyperglycaemia and dyslipidaemia have been shown to cause abnormal structure and function within the endothelium. High circulating glucose concentration causes downregulation of nitric oxide resulting in increased vasoconstriction^[115]. This in turn results in increased vascular tone, permeability, thinning of vascular endothelium, weakening of intercellular junctions, altered protein synthesis and ultimately causes remodelling^[115].

Using CMR^[10,94,116] and PET^[114,117] myocardial perfusion during vasodilator stress has been shown to be impaired in patients with diabetes^[10,94,116] and in the absence of epicardial coronary artery stenosis, this finding is indicative of coronary microvascular dysfunction. Importantly, coronary microvascular dysfunction is an early precursor of cardiovascular events and was shown to be associated with a 2.5% annual major adverse event rate that includes cardiovascular mortality, nonfatal myocardial infarction, nonfatal stroke, and congestive HF even among patients without epicardial coronary artery stenosis^[118]. Consequently, early identification of coronary microvascular disease may be beneficial in prognosis evaluation and patient stratification for optimal medical therapy

Using adenosine stress perfusion CMR Larghat *et al*^[10] have assessed myocardial perfusion reserve in 65 patients with no significant epicardial coronary artery stenosis on angiography (< 30% coronary luminal stenosis). Left ventricular mass was shown to be higher in diabetics than non-diabetics (112.8 ± 39.7 g *vs* 91.5 ± 21.3 g, *P* = 0.01) and in diabetics than prediabetics (112.8 ± 39.7 g *vs* 90.3 ± 18.7 g, *P* = 0.02). MPR was lower in diabetics than non-diabetics (2.10 ± 0.76 *vs* 2.84 ± 1.25, respectively, *P* = 0.01)^[10]. Khan *et al*^[11] have also studied 40 young patients (mean age 30 years), including 20 diabetics and 10 lean and 10 obese controls who underwent CMR. This study, however, did not show any significant correlation between left ventricular intracardiac measures, including mass, MPR and perfusion and the presence of T2D^[11]. Diastolic dysfunction was found to be associated with TG content but not with

Table 3 Studies on left ventricular function and myocardial strain in diabetes

Publication and imaging modality	Group and baseline characteristics	Exclusion	Main findings
Ernande <i>et al</i> ^[107] , 2010	T2DM: <i>n</i> = 119, 69 males	LVEF < 56%, age < 35 or > 65, signs, symptoms or history of heart disease, no RWMA, valve disease, renal disease, T1DM, poor DM control (HbA1C > 12%)	↓GLS (-19.3% ± 3% <i>vs</i> -22% ± 2%) and GRS (50% ± 16% <i>vs</i> 56% ± 12%, [†] <i>P</i> < 0.003) in participants with diabetes <i>vs</i> participants without diabetes
Echocardiography	Controls: <i>n</i> = 39, 30 males		Multivariate analysis showed DM (<i>t</i> = 3.9, <i>P</i> < 0.001) and gender (<i>t</i> = 3.4, <i>P</i> = 0.001) independent determinants of GLS, DM only independent determinant of GRS.
Ng <i>et al</i> ^[91] , 2012	<i>n</i> = 69	Age < 18 yr, arrhythmia, CAD, MI, RWMA, segmental LGE, EF < 50%, valve disease	↓GLS DM <i>vs</i> controls (-16.1% ± 1.4% <i>vs</i> 20.2% ± 1.0% [†] <i>P</i> < 0.001)
MRI	DMs (<i>n</i> = 50, 35 T1DM) Mean age 51 ± 10 yr, 54% males. BMI 26.3 ± 3.7 Controls (<i>n</i> = 19), matched for age (45 ± 15), sex (63.2% males) an BMI 26.1 ± 4.4		↓GLS DM T2DM <i>vs</i> T1DM (-15.3% ± 1.2% <i>vs</i> 16.4% ± 1.4%, [‡] <i>P</i> = 0.009)
Khan <i>et al</i> ^[11] , 2014	T2D <i>n</i> = 20, Mean age 31.8 ± 6.6, BMI 33.9 ± 5.8 kg/m ²	Weight > 150 kg, contraindications to MRI. In diabetic group BMI > 30 (> 27.5 in South Asians)	↓PEDSR in DMs <i>vs</i> Lean controls <i>vs</i> obese controls (1.51 ± 0.24 <i>vs</i> 1.97 ± 0.34 <i>vs</i> 1.78 ± 0.39 s ⁻¹) and PSSR (-21.20 ± 2.75 <i>vs</i> -23.48 ± 2.36 <i>vs</i> -23.3 ± 2.62 s ⁻¹)
MRI	Lean Controls: <i>n</i> = 10, Mean age 30.9 ± 5.6, Mean BMI 33.4 ± 2.4, 60% males. Obese controls: <i>n</i> = 10, Mean age 30.0 ± 6.7, Mean BMI 21.9 ± 1.7, 50% males		
Levelt <i>et al</i> ^[62] , 2016	T2D: <i>n</i> = 39, Mean age 55 ± 9, 58% males.	History of CVD, chest pain, smoker, uncontrolled hypertension, contraindications to MRI, ischaemia on ECG, renal dysfunction, insulin use, significant CAD on CTCA	↓GLS (-9.6 ± 2.9 <i>vs</i> -11.4 ± 2.8 [†] <i>P</i> = 0.049) and mid ventricular (-14.2 ± 2 <i>vs</i> -19.3, [§] <i>P</i> < 0.001) systolic strain (PCr/ATP ratio at rest and exercise, correlated with reduced systolic strain results; there was also a negative association between the myocardial lipid levels and systolic strain in this cohort)
MRI	Controls: <i>n</i> = 17, Mean age 50 ± 14 yr		

CTCA: Computed topography coronary angiogram; GLS: Global longitudinal strain; GRS: Global radial strain; PEDSR: Peak early diastolic strain rate; PSSR: Peak systolic strain rate; RWMA: Regional wall motion abnormality.

impaired myocardial perfusion reserve in another study with patients with T2D with preserved systolic function^[119].

Another study looking at the interplay between metabolic and ischaemic changes in the hearts of people with well-controlled T2D, even in the absence of arterial hypertension or significant CAD on computed tomography coronary angiography, showed there were significant reductions in MPRI and evidence of blunted oxygenation response compared with controls. These findings support the concept that hypoperfusion as a result of microvascular dysfunction plays a role in the impaired ability to increase and/or maintain myocardial oxygenation during vasodilator stress in diabetes^[94].

In a large study of 2783 consecutive patients (1172 diabetics and 1611 non-diabetics) measuring coronary flow reserve (CFR) by PET^[120], they showed that diabetic patients with preserved CFR have similar rates of cardiac events to nondiabetic patients, whereas impaired CFR was associated with an adjusted 3.2- and 4.9-fold increase in the rate of cardiac death for both diabetics and nondiabetics. This raises the question of whether CFR should be included in a cardiac risk model for patients with diabetes. However, there is selection bias due to patients recruited consecutively, some had regional perfusion defects and the results of angiography were not known therefore the impaired flow reserve cannot be attributed directly to microvascular dysfunction.

In a small (*n* = 64) randomised trial comparing the effects of spironolactone, hydrochlorothiazide or placebo on coronary flow reserve in T2D assessed by cardiac positron emission tomography, spironolactone was associated with modest improvements in coronary flow reserve (from 2.77 ± 0.82 to 3.10 ± 0.74) compared to the other two treatment arms (*P* = 0.01) after six months^[117]. The mechanism for this is thought to be due to expression of the mineralocorticoid receptor in endothelium, vascular smooth muscle cells, cardiomyocytes and circulating leukocytes^[117]. Mineralocorticoid receptor activation causes vascular inflammation with increased

ROS production and increased vascular damage, vascular dysfunction, and perivascular fibrosis. The findings of this study support the potential role of mineralocorticoid use in reversing coronary microvascular dysfunction in T2D. However, there was no reporting of cardiovascular outcomes, relatively small sample size and limited follow up period.

The evidence of the role of coronary microvascular dysfunction in T2D is conflicting. This suggests that the metabolic dysfunction caused by diabetes likely may predispose to some degree of microvascular dysfunction however these may not be the main mechanisms contributing to cardiac dysfunction in diabetes.

Therapeutic developments and cardiovascular outcomes

The evidence for pharmacological therapy for heart failure targeted at the diabetic population thus far has been limited to studies of both patients with and without diabetes. ACE-inhibitors are recommended within the ESC/EASD guidance for diabetes and impaired glucose tolerance. The CHARM, ATLAS and HEAAL trials have all shown the beneficial effects of ACE in HF in terms of morbidity and mortality, however subgroup analyses showed no difference with and without diabetes^[121-124]. This is similarly the case with the use of mineralocorticoid antagonists^[125,126], nitrates and hydralazine^[127], ivabradine^[128] as well as more recently sacubitril/valsartan^[129].

The impact of better glycaemic control on left ventricular systolic and^[130] diastolic function remains debatable due to varying evidence. Previous large studies including United KingdomPDS^[131], ADVANCE^[130], ACCORD^[132] and VADT^[15] have not shown an improvement in cardiovascular outcomes with good glycaemic control. A meta-analysis of 37229 people with T2D data from multiple randomized trials concluded there was no observed benefit of intensive glycaemic control on HF related outcomes^[133]. Patients with T2D who received intensive glucose lowering therapy had a reduced risk of major adverse cardiovascular events, but there was no effect on total mortality, cardiac death, stroke, and heart failure^[133].

There is also a large body of evidence to suggest T2D therapies may increase the risk of developing heart failure. This has been particularly the case with dipeptidyl peptidase-4 (DPP4) inhibitor saxagliptin and thiazolidinediones (TZDs). In SAVOR-TIMI 53 looking at saxagliptin *vs* placebo there was a significantly increased risk of heart failure hospitalisation^[134,135]. These patients then went on to have a high rate of subsequent death. The latter result was also found in the RECORD trial^[136]. The TECOs trial looking at sitagliptin^[17] and EXAMINE for alogliptin^[18], both against placebo there was no increase in heart failure. Further studies are required to consolidate the effects of DPP4 inhibitors on heart failure in T2D. There are current ongoing studies, CARMELINA (Cardiovascular and Renal Microvascular Outcome Study With Linagliptin in Patients with Type 2 Diabetes Mellitus; NCT01897532) and CAROLINA (Cardiovascular Outcome Study of Linagliptin Versus Glimepiride in Patients With Type 2 Diabetes; NCT01243424), looking at Linagliptin in T2D that may provide better insight.

More recent studies have shown favourable cardiovascular outcomes with newer glucose lowering agents. There have recently been numerous non-inferiority cardiovascular outcome trials using newer glucose lowering therapies in T2D, namely GLP1 – Receptor Agonists and sodium–glucose cotransporter 2 (SGLT2) inhibitors.

Glucagon like peptide 1-receptor agonists

Multiple glucagon-like peptide-1 (GLP-1) receptor agonists in the form of once weekly exenatide, dulaglutide, albiglutide and semaglutide have been recently developed. GLP-1 agonists exert their effects by stimulating insulin secretion, suppressing appetite, decreasing circulating glucagon levels and gastric emptying^[137]. They have also been associated with weight loss. The cardiovascular benefits of GLP-1 is multifaceted. The first is glycaemic control. Long acting agonists have been shown to be better at reducing HbA1c than short term^[138]. Despite the failure to show an improvement in cardiovascular outcomes with some large studies in the past^[15,139,140], the United Kingdom PDS study that looked at tight glycaemic control at diagnosis showed a reduction in cardiovascular events after a decade even with similar HbA1c levels^[131]. The second is through blood pressure control. None of the GLP-1 studies conducted thus far have been specifically to look at its' effects on blood pressure however both studies as well as reviews and meta analyses have shown a decrease in BP of both exenatide and liraglutide compared with placebo and other antidiabetic drugs^[141]. It has also been shown to have favourable effects on the vascular endothelium in that Liraglutide has been shown to improve nitric oxide synthase (NOS) activity as well as attenuates plasminogen activator inhibitor type 1 (PAI-1) and vascular adhesion molecule (VAM) within the vascular endothelium. These apply a protective effect against endothelial dysfunction^[142,143]. GLP-1 agonists also appear to

have a cardioprotective effect on atherosclerosis progression and myocardial ischaemia, and this has been shown with the use of exenatide as an adjunct therapy in STEMI and thrombolysis^[144,145]. GLP-1 agonist use has not been associated with a significant effect on heart failure hospitalisation.

Large studies examining the impact of GLP-1 agonists on cardiovascular outcomes in T2D have been summarised in **Figure 5**. In the LEADER trial, patients with T2D on Liraglutide had lower rates of cardiovascular death^[146]. Similarly SUSTAIN-6 also looked at GLP-1 agonists with Semaglutide being an injectable version with longer half-life have also shown improved cardiovascular outcomes. However, in the EXSCEL^[23], Exenatide did not show overall cardiovascular risk benefit compared to placebo. Most recently, the PIONEER 6 trial has studied the non-inferiority of oral daily semaglutide against placebo for cardiovascular outcomes^[147]. It has been shown to have a significant reduction in the composite primary outcome of first occurrence of death from cardiovascular causes, nonfatal myocardial infarction (including silent), or nonfatal stroke, *i.e.*, 8.6% in the semaglutide group *vs* 6.6% in placebo, with a hazard ratio of 0.79, 95%CI: 0.57-1.11, $P < 0.001$ for non-inferiority, $P = 0.02$ for superiority. However, when the components of the primary outcome are looked at, non-fatal MI and death from cardiovascular causes comes up non-significant. However, this study was relatively underpowered compared to the other cardiovascular outcome trials and this may explain why the reduction was not statistically significant. Increasing evidence for oral GLP-1 agonists has marked benefits for those who have reservations regarding all previous GLP-1 agonists that have been in the form of a subcutaneous injection and will allow for better compliance with the new ADA/EASD guidance to use a GLP-1 agonist as second line therapy in diabetes. There is a larger phase three cardiovascular outcome trial underway, the Heart Disease Study of Semaglutide in patients with Type 2 Diabetes (SOUL; NCT03914326), looking at oral semaglutide against placebo and its effects on lowering MACE in over 9000 patients. The results of this is eagerly awaited.

Sodium glucose co-transporter 2 inhibitors

In both the EMPA-REG OUTCOME^[19] and CANVAS^[20] studies (**Figure 6**) there was a relative risk reduction in cardiovascular mortality and hospitalisation for HF in patients with T2D. The hazard ratio for HF hospitalisation in the EMPA-REG OUTCOME trial was 0.65, (0.57, 0.82, $P = 0.002$) for empagliflozin *vs* placebo^[21]. A reduction in hospitalization for HF was also observed with Sodium glucose co-transporter 2 (SGLT2) inhibitor Dapagliflozin in DECLARE-TIMI 58 trial^[148]. An important consideration in interpretation of these results is that the patients in these studies either had established cardiovascular disease or multiple risk factors for atherosclerotic cardiovascular disease risk factors and it is uncertain whether the results can be translated in to lower risk groups with prevention of the development of HF.

The mechanisms by which SGLT2 inhibitors lower HF hospitalisation rates in T2D are still under scrutiny. However, it is hypothesised to be related to the urinary sodium and glucose excretion that leads to increased fluid losses resulting in reduced pre-load and afterload^[149]. Secondary effects include fluid loss and blood pressure lowering. There is also suggestion that SGLT2 inhibitors shift myocardial metabolism towards ketones, resulting in this having a favourable effect on cardiac energetics^[150].

CONCLUSION

Diabetic heart disease is multi-faceted and spans metabolic, structural and functional changes. Recent advancements in imaging has aided significantly in understanding the pathophysiology as well as the remodelling and functional changes within the heart. Further research into the degree of dependence on fatty acid metabolism instead of glucose in the presence of diabetes is required. The relationship between the metabolic changes within the heart and functional measures such as myocardial strain rates as well as triglyceride content will help us better understand how to treat this disease process. This will also add towards increasing the mechanistic accuracy of new therapeutic targets.

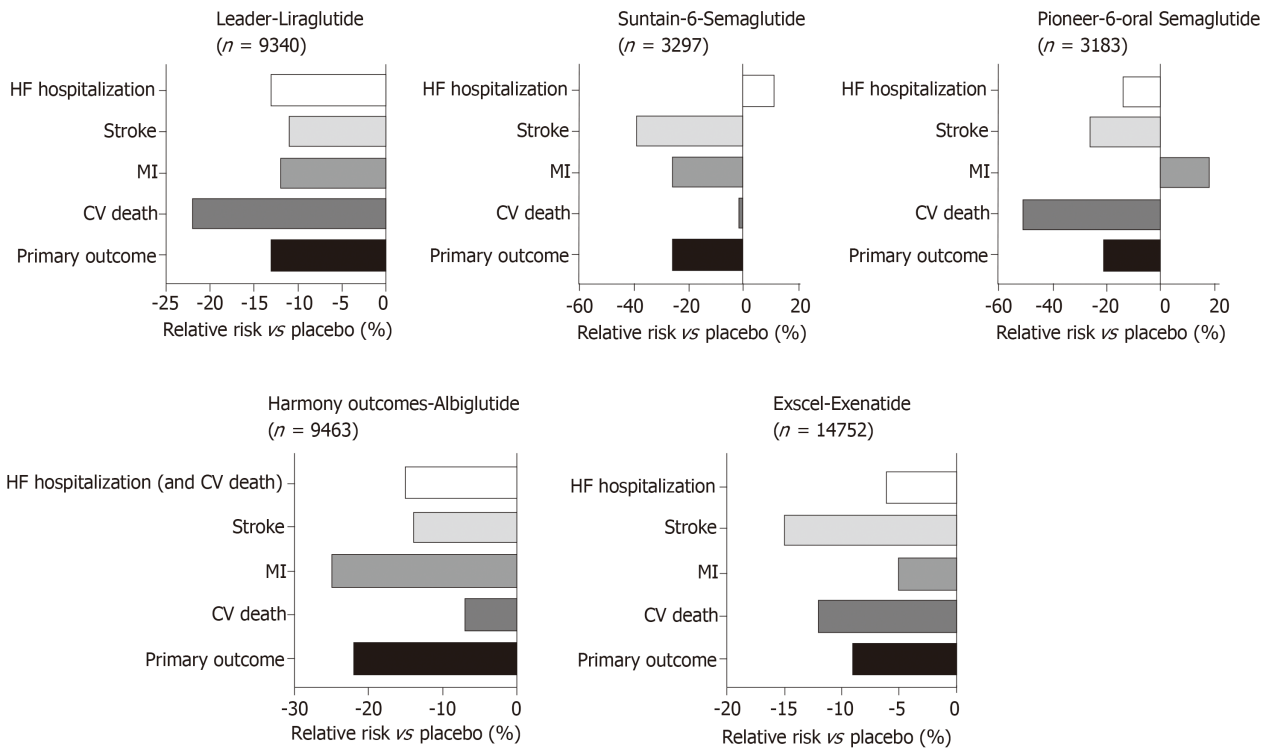


Figure 5 Major cardiovascular outcome trials using GLP1 receptor antagonists.

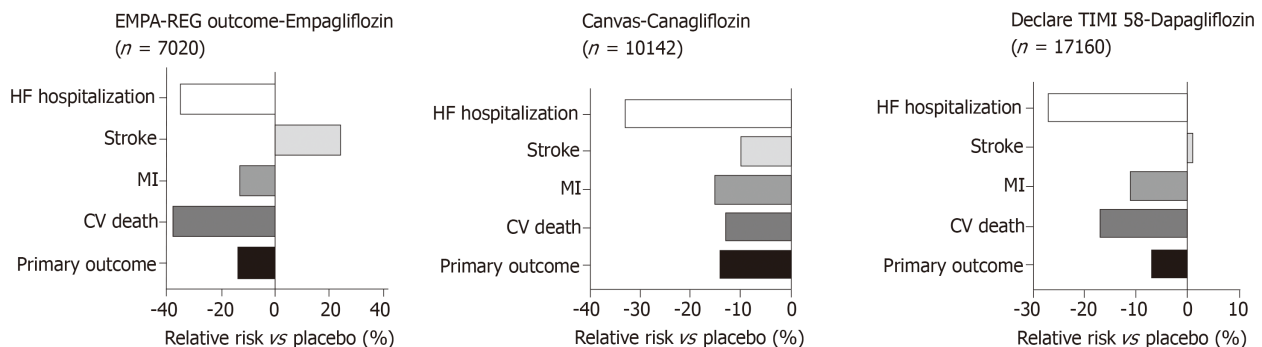


Figure 6 Major cardiovascular outcome trials examining SGLT2 inhibitors.

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Type 1 diabetes in a Nigerian family - occurrence in three out of four siblings: A case report

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Abstract

BACKGROUND

Most occurrences of type 1 diabetes cases in any population are sporadic rather than familial. Hence, type 1 diabetes among siblings is a rare occurrence. Even more rare is for three or more siblings to develop type 1 diabetes. In this report, we describe a case of a Nigerian family in which type 1 diabetes occurred in three siblings among four children with neither parent having diabetes. All three siblings are positive for glutamic acid decarboxylase and anti-islet cell antibodies.

CASE SUMMARY

There were four siblings (three males and one female) born to a couple without a diagnosis of diabetes. The eldest child (male) was diagnosed with diabetes at the age of 15, the second child (female) was diagnosed at the age of 11 and the fourth child (male) was diagnosed at the age of 9. All the siblings presented with similar osmotic symptoms and were diagnosed of diabetic ketoacidosis. All of them had markedly reduced serum C-peptide levels with high levels of glutamic acid decarboxylase and insulinoma-associated protein-2 antibodies. We could not perform genetic analysis of HLA-DR, DQ and CTLA4 in the siblings as well as the parents; hence haplotypes could not be characterized. Both parents of the probands have no prior history of diabetes, and their blood glucose and glycated hemoglobin levels were within normal ranges. The third child (male) has no history suggestive of diabetes, and his blood glucose and glycated hemoglobin have remained within normal ranges.

CONCLUSION

Although the occurrence of type 1 diabetes in proband siblings is uncommon,

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screening for diabetes among siblings especially with islet autoantibodies should be encouraged.

Key words: Type 1 diabetes; Siblings; Case report; Nigerian; Family

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Core tip: Although most occurrences of type 1 diabetes are sporadic, it can also be familial. Type 1 diabetes among siblings is a rare occurrence. Even more rare is for three or more siblings to develop type 1 diabetes. Hence due to the possibility of this familial occurrence, screening for diabetes among siblings should be encouraged. This report describes a case of a Nigerian family in which type 1 diabetes occurred in three siblings among four children with neither parent having diabetes.

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INTRODUCTION

Type 1 diabetes (T1D) is a life-long medical condition that primarily affects young people. It is characterized by immune destruction of insulin-producing beta-cells in the pancreas resulting from the action of environmental factors in genetically predisposed individuals^[1]. It usually begins in childhood or young adulthood but can develop at any age. The presence of any of the following antibodies increases the risk of T1D: Glutamic acid decarboxylase-65, islet cell, insulin autoantibody and insulinoma-associated protein-2^[2]. In general, 70% of people with new-onset T1D have a positive antibody if only one antibody is measured, whereas 90% will have at least one antibody when all forms are measured^[2]. Most occurrences of T1D cases in any population are sporadic; that is, first-degree relatives do not have diabetes at the time patient with diabetes is diagnosed. Nevertheless, siblings of childhood-onset T1D patients are at increased risk of developing the same disease compared with the general population^[3,4].

Genetic susceptibility is important in the development of T1D. In Caucasian populations, the lifetime risk in siblings of type 1 diabetic probands has been reported to be much higher than that in the general population (6% *vs* 0.4%), indicating that T1D clusters in families^[5]. This represents a 15-fold risk tendency in siblings compared to the risk in the general population^[5]. This classic role of genetics in diabetes risk is demonstrated by comparing concordance rates in monozygotic *versus* dizygotic twins. In Finland, with the highest incidence of T1D, concordance rates for T1D were found to be 27% and 3.8% among monozygotic and dizygotic twins, respectively^[6]. Familial clustering (which refers to the occurrence of a disorder at a higher frequency in first-degree relatives of an affected person compared to the general population)^[7] of T1D is a rare occurrence. Even more rare is for three or more siblings to develop T1D.

It is estimated that HLA (a cluster of genes located within the major histocompatibility complex) on chromosome 6p21 accounts for 40%-50% of familial clustering and the strongest genetic association with T1D^[8], in addition to other different genetic loci that contribute susceptibility to the development of T1D^[9]. Although the risk of developing T1D is increased in relatives of individuals with the disease, the risk is relatively low. This risk depends on which HLA haplotypes are shared^[8]. From multiple family pedigrees and HLA typing data, it is estimated that if a sibling shares both HLA-D haplotypes with an index patient, the risk for that individual is 12% to 20%. For a sibling sharing only one haplotype, the risk for T1D is 5% to 7%. For a sibling with no haplotype in common, the risk is only 1% to 2%^[10]. According to Redondo *et al*^[11], approximately 85% of new cases of T1D occur in persons without an affected first-degree relative. Approximately 13% of children and adolescents who develop T1D have a parent or sibling with diabetes. Dahiquist and Gotheffors^[12] reported that among children with newly diagnosed diabetes, 2%-3% had a mother with T1D, 5%-6% had a father with T1D and 4%-5% had a brother or a sister with T1D.

The study of familial clustering is an important concept in genetic epidemiology. The lifetime risk in siblings of type 1 diabetic probands in Nigerians is unknown (it has never been reported). Hence, the purpose of this paper is to report a case of a Nigerian family in which T1D occurred in three siblings among four children with neither of the parents having diabetes. This report will encourage clinicians to assess the possibilities of diabetes in siblings of children with T1D.

CASE PRESENTATION

Chief complaints

The three siblings presented with polyuria, polydipsia and weight loss of about 2 wk duration.

Present illness

Case 1: First child, male, was diagnosed of acute-onset T1D at 15-years-old. He had presented with osmotic symptoms (polyuria and polydipsia) and associated weight loss about 2 weeks before presentation. At presentation, he was drowsy and dehydrated.

Case 2: Second child, female, was diagnosed of acute-onset T1D at 11-years-old. She had presented with polyuria, polydipsia and weight loss.

Case 3: Fourth child, male, was delivered at estimated gestation age of 36 wk and birth weight was 4.65 kg. He presented with features of acute-onset osmotic symptoms at 9-years-old ([Figure 1](#)).

History of past illness

None of the siblings were previously diagnosed with diabetes.

Personal and family history

Both parents of the probands have no prior history of diabetes. Their blood glucose and glycated hemoglobin (HbA1C) levels were within normal ranges. The third child (male) had no history suggestive of diabetes, and his blood glucose and HbA1C were within normal ranges. None of the siblings or parents were smokers or used alcohol. The father was overweight, and the mother was diagnosed with obesity.

Physical examination upon admission

Case 1: At presentation, he was drowsy and dehydrated. His plasma glucose and HbA1C levels were 403 mg/dL (22.4 mmol/L) and 10.6%, respectively. His urinalysis showed glucosuria (4+) and ketonuria (3+). His serum C-peptide level was markedly low, which showed that his insulin secretory capacity was reduced.

Case 2: Her blood glucose at diagnosis was 448 mg/dL (24.9 mmol/L), and HbA1C was 11.0%. Urinalysis showed ketonuria (3+) and glucose (4+) but no proteinuria.

Case 3: His blood glucose at diagnosis was 448 mg/dL (24.9 mmol/L) and HbA1C was 11.0%. Urinalysis showed ketonuria (3+) and glucose (4+) but no proteinuria.

Anti-glutamic acid decarboxylase, anti-insulinoma-associated protein-2 and anti-thyroid peroxidase antibodies were determined by radioimmunoassay. However, the genotype of the HLA could not be done due to lack of facility ([Table 1](#)).

FINAL DIAGNOSIS

All were diagnosed of acute-onset diabetic ketoacidosis and T1D.

TREATMENT

All were treated with intravenous fluid and multiple doses of insulin at admission.

OUTCOME AND FOLLOW-UP

All are currently being treated with multiple injections of insulin with total daily insulin dosage ranges of 90-122 U/d. They all have fair glycemic control with occasional hypoglycemia in the youngest sibling with diabetes.

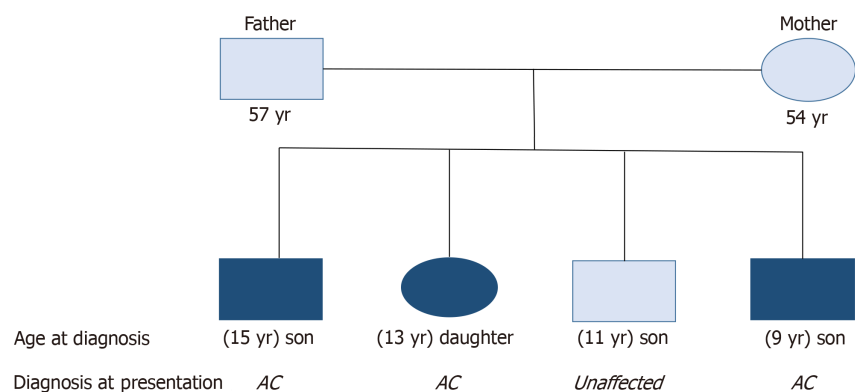


Figure 1 Pedigree of the family with type 1 diabetes. Circle: female; Square: male; Shaded circle/square: affected with diabetes. AC: Acute onset of type 1 diabetes.

DISCUSSION

The occurrence of T1D among three or more siblings is extremely rare. Although large families with T1D arising from a common ancestor have been reported among Arabs^[13] and in the Netherlands^[14]. However, none have been reported among Nigerians except a patient with T1D whose three siblings also had prediabetes^[15]. An analysis of 767 multiplex Caucasian families showed that fifty-one families had three affected siblings, one family had four affected siblings and two families had five affected siblings^[16]. There has also been a reported case of T1D in three sisters in a Japanese family, whose parents also had T1D^[17]. To our knowledge, no case report has described T1D in three or more siblings in Nigerian patients.

In this case report, three out of four siblings (two males and one female) developed T1D, although neither of the parents had diabetes. Fathers have been known to transmit T1D to their offspring more than mothers^[17,18]. At diagnosis, 4%-7% of children have a father with T1D whereas 1.5%-3% have an affected mother. This tendency of transmitting the disease is amplified if both parents have the disease. This familial T1D has been shown to be associated with increased genetic susceptibility attributed to a higher prevalence of HLA genes among family members^[8,19]. Hence, the T1D risk for siblings depends on genetic background primarily in the HLA region on chromosome 6 in particular^[8], and it depends on siblings sharing the HLA haplotype with the proband^[18]. While HLA-B8 is strongly associated with T1D in Caucasians, the contrary was the case among Nigerians^[20]. Another study^[21] reported a low prevalence of HLA-DR4 among Nigerians with T1D. The incidence and prevalence of T1D is not known in Nigeria although the prevalence was said to be much lower than and occurred at later ages compared to Caucasians^[22]. This rare occurrence makes it difficult to identify families with multiple siblings with T1D.

Regarding the age of diagnoses of our patients, there was a progressive decline in the age of diagnosis and reduced levels of glycemia at diagnosis as assessed by both plasma glucose and HbA1C with subsequent siblings with diabetes. The decreasing age of diagnosis of other siblings compared to the first affected sibling is in keeping with previous reports demonstrating a younger age at diagnosis among affected siblings^[23-25]. The progressive reduction in the age of diagnosis might be due to modification of the genetic susceptibility by environmental factors such as diets, abnormal weight gain or sedentary lifestyles. Previous studies showed that HLA genes related to high disease susceptibility (HLA-DR and HLA-DG genotypes) were associated with earlier onset of diabetes^[26,27]. Also, Harjutsalo *et al*^[16] suggested that genetic effects in such families may be particularly strong. They proposed that a young age at onset in the first child diagnosed with T1D indicated an overall increased lifetime risk for T1D in siblings and that the process leading to diabetes seems more rapid in such siblings. Long-term follow-up studies corroborated this statement^[28,29]. Another explanation that has been proposed is that genetic or environmental factors that precipitate an earlier onset of T1D in the proband are shared with the other siblings and may also increase their risk for T1D.

The mode of presentation at diagnosis in all these three siblings was quite similar. All of them presented with polyuria, polydipsia and weight loss over a similar mean period of time with consequent diagnosis of diabetic ketoacidosis. Although, there was a slight increase in blood sugar of the second sibling compared to the first, there was a reduction in the plasma glucose at presentation in the third sibling who had the lowest blood sugar among the three affected siblings. The first affected sibling

Table 1 Laboratory parameters of the siblings at diagnosis

	Sibling 1	Sibling 2	Sibling 3
Age of diagnosis, yr	15	11	9
BG at presentation, mg/dL BMI, kg/m ²	403 23	448 28.4	337 22.4
Mode of presentation of diagnosis	DKA	DKA	DKA
HbA1C, %	10.7	11.0	10.7
Urinalysis			
Glucose	4+	4+	3+
Ketone	3+	3+	2+
Serum C-peptide levels, pmol/L	< 33.0 (364-1655)	Undetectable	Low
Anti-GAD antibodies, IU/mL (Normal range: 0.0-10.0)	1561.0	1208.8	1054.2
Anti-IA-2 Ab	Increased	Increased	Increased
TPO Ab, U/mL	Normal	Normal	Normal

BG: Blood glucose; BMI: Body mass index; DKA: Diabetic ketoacidosis; HbA1C: Glycated hemoglobin; GAD: Glutamic acid decarboxylase; IA-2 Ab: Insulinoma-associated protein-2 antibody; TPO Ab: Thyroid peroxidase antibody.

presented with the worst clinical symptoms at presentation as he was drowsy. This observation was probably due to greater family awareness of diabetic symptoms, hence earlier presentation to the hospital of the younger siblings.

Although this report showed the possibility of familial clustering of T1D among siblings, our inability to do genetic analysis for proper characterization of the exact loci genes affected was a limitation of this study. Although, one sibling of the four children was not affected by T1D, we could not assess him for the presence of auto-antibodies, which may also predispose him to development of diabetes in the future.

CONCLUSION

This is the first report of familial clustering of T1D in three out of four children in a Nigerian family. This shows that the occurrence of T1D among siblings is possible even in areas where the incidence and prevalence of T1D is rare (as in Nigeria). This report is also unique because neither of the parents had either type 1 or type 2 diabetes. The finding of decreasing age at diagnosis and severity of symptoms at presentation with each subsequent sibling suggested a possible influence of environmental effects on genetic susceptibility and awareness of diabetes symptoms from prior diagnosis.

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Observational Study

Risk factors and urinary biomarkers of non-albuminuric and albuminuric chronic kidney disease in patients with type 2 diabetes

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Abstract

BACKGROUND

A number of recent studies indicate a transformation in the natural course of chronic kidney disease (CKD) in type 2 diabetes (T2D) patients: an increasing prevalence of declined renal function without proceeding to the accompanying elevation of albuminuria. It has been suggested that albuminuric and non-albuminuric CKD patterns could be different in their phenotypes and pathogenic mechanisms.

AIM

To identify the risk factors and biomarkers of albuminuric and non-albuminuric patterns of CKD in patients with T2D.

METHODS

Three hundred sixty patients with T2D duration ≥ 10 years were included in this observational cross-sectional study. The associations of a panel of demographic and clinical characteristics, complications, comorbidities, and metabolic and hematology parameters with albuminuric and non-albuminuric CKD patterns were analyzed. The urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2 (WFDC-2), a marker of tubulointerstitial fibrosis, was determined by ELISA in comparison with healthy controls.

RESULTS

Non-albuminuric CKD was associated with age ≥ 65 years ($P = 0.0001$), female

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sex ($P = 0.04$), diabetes duration ≥ 15 years ($P = 0.0009$), and the use of diuretics ($P = 0.0005$). Male sex ($P = 0.01$), smoking ($P = 0.01$), waist-to-hip ratio >1.0 ($P = 0.01$) and hemoglobin A1c (HbA1c) $> 8.0\%$ ($P = 0.005$) were risk factors for elevated albuminuria not accompanied by a decrease in estimated glomerular filtration rate (eGFR). Duration of diabetes ≥ 15 years and the use of calcium channel blockers were risk factors for albuminuria with decreased eGFR (both $P = 0.01$). In multivariate logistic regression analysis, age, HbA1c, female sex and diuretics were significant predictors for reduced eGFR, while waist-to-hip ratio, HbA1c and male sex were associated with elevated urinary albumin-to-creatinine ratio (UACR). Excretion of nephrin and podocin was increased in patients with albuminuria, regardless of decline in renal function ($P < 0.001$), correlating positively with UACR. The urinary excretion of WFDC-2 was markedly higher in men than in women ($P < 0.000001$). Men with T2D demonstrated increased WFDC-2 levels independently of the CKD pattern (all $P < 0.05$). In T2D women, WFDC-2 excretion was increased in those with reduced renal function ($P \leq 0.01$), correlating negatively with eGFR.

CONCLUSION

The data provide further evidence that albuminuric and non-albuminuric CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

Key words: Diabetes mellitus; Chronic kidney disease; Albuminuria; Glomerular filtration rate; Podocytes; Risk factors; Biomarkers

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Core tip: In this study, we demonstrate the differences in clinical and laboratory characteristics between albuminuric and non-albuminuric phenotypes of chronic kidney disease (CKD) in patients with long-term type 2 diabetes. Different risk factors are found for three different CKD phenotypes. We also show the diversity in the urinary excretion of nephrin and podocin, two slit diaphragm proteins, and of WAP-four-disulfide core domain protein 2, a tubulointerstitial fibrosis marker, between different CKD phenotypes. The results further support the notion that albuminuric and non-albuminuric CKD phenotypes are different in their pathophysiology and clinical characteristics.

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INTRODUCTION

The increasing prevalence of diabetes around the world and changes in diabetes management have transformed the epidemiology of chronic kidney disease (CKD) in recent years. In many countries, including the United States, diabetes is responsible for over 40% of new cases of end-stage renal disease (ESRD), surpassing other causes to become the leading driver of the renal impairment^[1]. Despite the fact that the prevalence of CKD among adult patients with diabetes remains stably high, a transformation in its natural course has been recorded. According to the classical paradigm, albuminuria is an early indicator of diabetic kidney disease. A number of recent studies have documented an increasing proportion of diabetic patients in whom a reduction in renal function develops without a preceding or concomitant increase in albuminuria^[1-3]. This tendency is most evident in type 2 diabetes (T2D); the proportion of the non-albuminuric CKD (NA-CKD) pattern in this type of disease currently ranges from 40% to 70%^[4].

The causes for the shift in the natural course of diabetic kidney disease are not fully understood. Among others, the wide use of renin-angiotensin system blockers; advances in antihyperglycemic, antihypertensive, and hypolipidemic therapy; and

smoking cessation are discussed^[2,5]. New classes of antihyperglycemic agents, including glucagon-like peptide-1 (GLP-1) analogs, dipeptidylpeptidase-4 (DPP-4) inhibitors, and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, have demonstrated a distinct antialbuminuric effect in clinical trials^[6-9]. Accordingly, the growing use of these agents in clinical practice may cause a reduction in the prevalence of albuminuria among diabetic patients.

Presently, little is known about the clinical phenotypes and pathophysiology of albuminuric and NA-CKD patterns in diabetes. A growing body of evidence indicates that these patterns demonstrate significant differences in natural course and outcomes. Even if a more favorable situation in terms of the risk of ESRD, NA-CKD is clearly associated with cardiovascular disease and its risk factors^[4]. Accordingly, the non-albuminuric phenotype might be related to macroangiopathy instead of microangiopathy and/or be the consequence of repeated and/or unresolved episodes of acute kidney injury, even of a mild degree^[10]. When comparing renal biopsy findings associated with normo-, micro-, or macroalbuminuria in T2D patients with glomerular filtration rate (GFR) less than 60 mL/min/1.73 m², typical glomerular changes were revealed mostly in patients with elevated albuminuria. In those with NA-CKD, predominant interstitial and vascular changes were more frequent findings^[11]. It was speculated that non-albuminuric renal impairment represents a different pathway to the loss of renal function compared to albuminuric one.

Podocyte injury has been identified as a pivotal event resulting in proteinuric kidney disease, glomerulosclerosis, and loss of renal function^[12]. During filtration, plasma passes through a sieve consisting of a fenestrated endothelium and a broad basement membrane before it reaches the most unique part, the slit diaphragm, a specialized type of intercellular junction that connects neighboring podocyte foot processes. When podocytes become stressed, irrespective of the causative stimulus, they undergo foot process effacement and loss of slit diaphragms – two key steps leading to proteinuria^[13]. It was demonstrated that not only proteinuria but also tubulointerstitial lesions should be assessed to predict rapid GFR decline in patients with T2D who have overt proteinuria^[14]. Moreover, it was reported that interstitial fibrosis, tubular atrophy and interstitial inflammation, but not glomerular lesions, are significant predictors for renal prognosis in T2D patients with overt proteinuria^[15]. In a recent study, interstitial fibrosis and tubular atrophy score, as well as glomerular basement membrane thickness, were independent predictors for renal replacement therapy initiation in T2D patients^[16]. Taking into account the results of morphological investigations, the assessment of urinary markers of podocyte and interstitial involvement may provide further information on the development of different CKD phenotypes. This study aimed to identify the risk factors, as well as the markers of podocyte and interstitial involvement, in albuminuric and NA-CKD in patients with T2D.

MATERIALS AND METHODS

Ethical issues

The study protocol was approved by the Ethical Committee of the Research Institute of Clinical and Experimental Lymphology – branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences. All patients provided their written informed consent prior to the inclusion.

Design

The design of this observational, single-center, cross-sectional study is presented in **Figure 1**. Adult men and women with T2D duration of at least 10 years from the date of diagnosis were included. Non-diabetic CKD, ESRD, urinary tract infection, ketoacidosis or hyperosmolar state at the time of the survey, treatment with DPP-4 inhibitors, GLP-1 receptor agonists and/or SGLT-2 inhibitors for three months prior to inclusion, malignant neoplasms, inflammatory or autoimmune diseases in the medical history, and a high-protein diet acted as exclusion criteria.

Subjects

Five hundred six potentially eligible T2D patients who met the inclusion criteria were selected. After evaluation for exclusion criteria, 360 patients, 100 men and 260 women, from 43 to 88 years of age (median 66 years), were included in the analysis. Twenty individuals who had no history of diabetes, obesity or cardiovascular disease, including 13 women and 7 men, from 50 to 74 years of age (median 62.5 years), acted as controls in the study of urinary biomarkers.

Methods

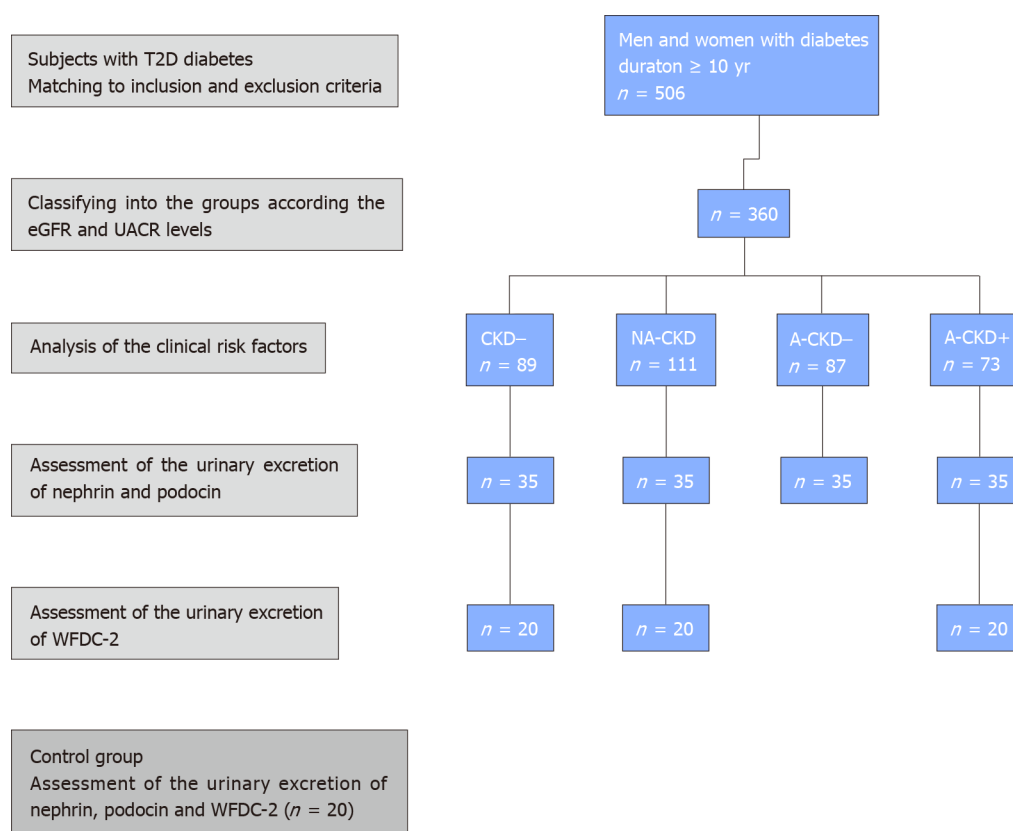


Figure 1 The design of the study. The study was designed as an observational, single-center, cross-sectional study. Adult men and women with type 2 diabetes (T2D) duration of at least 10 years from the date of diagnosis were included ($n = 506$). After evaluation for exclusion criteria, 360 patients were included in the analysis. Patients were divided into four groups according to their estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR) levels. Individuals with $\text{eGFR} \geq 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and $\text{UACR} < 3.0 \text{ mg/mmol}$ were recorded as patients without chronic kidney disease (CKD) signs (CKD- group). Those with $\text{eGFR} < 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and $\text{UACR} < 3.0 \text{ mg/mmol}$ were assigned to the non-albuminuric chronic kidney disease group. Patients with $\text{eGFR} \geq 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and $\text{UACR} \geq 3.0 \text{ mg/mmol}$ were defined as albuminuric with preserved renal function (A-CKD- group). Individuals with $\text{eGFR} < 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and $\text{UACR} \geq 3.0 \text{ mg/mmol}$ comprised the albuminuric CKD group (A-CKD+). All patients underwent clinical examination, which included an evaluation of diabetes control and in-depth screening/monitoring of complications and comorbidities. The set of clinical risk factors was estimated for each CKD pattern. Urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2, a marker of tubulointerstitial fibrosis, was assessed in T2D patients and the control group (20 subjects without a history of diabetes, obesity or cardiovascular disease). CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; NA-CKD: Non-albuminuric chronic kidney disease; T2D: Type 2 diabetes; UACR: Urinary albumin-to-creatinine ratio; WFDC-2: WAP-four-disulfide core domain protein 2; CKD-: The group of individuals with estimated glomerular filtration rate $\geq 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $< 3.0 \text{ mg/mmol}$; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate $< 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $< 3.0 \text{ mg/mmol}$; A-CKD-: Group of patients with estimated glomerular filtration rate $\geq 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $\geq 3.0 \text{ mg/mmol}$; A-CKD+: Group of individuals with estimated glomerular filtration rate $< 60 \text{ mL/min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $\geq 3.0 \text{ mg/mmol}$.

All patients underwent clinical examination, which included an evaluation of diabetes control and in-depth screening/monitoring of complications. Routine laboratory measurements, including glycated hemoglobin A1c (HbA1c), total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and uric acid, were performed on AU480 Chemical Analyzer (Beckman Coulter, United States) with commercially available cartridges. The HbA1c levels were measured by turbidimetric immunoinhibition method. A kinetic enzymatic method was applied for determination the levels of lipids and uric acid. Three fasting and three 2-h postprandial blood glucose values were obtained daily from each patient in three-day series. The measurements were performed by One Touch Verio® (Johnson and Johnson/Lifescan, United States) glucose meter. A complete blood count was performed on a hematology analyzer (Analyticon Biotechnologies AG, Germany). Concentrations of fibrinogen, soluble fibrin monomer complex (SFMC) and D-dimer were evaluated on the hemostasis analyzer system (Instrumentation Laboratory, United States).

The levels of creatinine in serum and urine were determined on AU480 Chemical Analyzer (Beckman Coulter, United States) by modified kinetic Jaffe's method. The estimated GFR (eGFR) was calculated by the CKD-EPI formula (2009). Urinary albumin was determined in three morning urine samples by immunoturbidimetry on AU480 Chemical Analyzer (Beckman Coulter, United States) in accordance with the manufacturer's instructions. The mean albumin concentration (mg) was adjusted to

the excreted creatinine (mmol) and is expressed as the urinary albumin/creatinine ratio (UACR). Urinary excretion of total protein was assessed by colorimetric method with pyrogallol red-molybdate complex on AU480 Chemical Analyzer (Beckman Coulter, United States).

In this study, we assessed the urinary excretion of nephrin and podocin, two podocyte-specific markers, and of WAP-four-disulfide core domain protein 2 (WFDC-2), a marker of tubulointerstitial fibrosis. Both nephrin and podocin are expressed on the surface of podocytes, acting as components of the slit diaphragm complex; accordingly, these molecules are used as markers of dysfunction and injury of podocytes^[17-19]. The increase in urinary excretion of nephrin and podocin in patients with diabetes was reported in a number of studies^[17,20-22]. WFDC-2, also known as human epididymal protein-4 (HE-4), is expressed by myofibroblasts^[23]. Focal and low expression of WFDC-2 is found in the distal convoluted tubule of the kidney^[24]. It was shown that WFDC-2 suppressed the activity of multiple proteases, including serine proteases and matrix metalloproteinases, and specifically inhibited their capacity to degrade type I collagen^[23]. Recently, serum WFDC-2 has been validated as a clinical marker of renal fibrosis^[25,26].

The morning urine samples for the biomarker assay were centrifuged, and the supernatants were separated and stored at -80 °C until analysis. Repeated freeze-thaw cycles were avoided. The concentrations of nephrin, podocin and WFDC-2 in the urine were assessed by ELISA using commercially available kits (Cloud-Clone Corp., United States, catalog/serial No. SEA937Hu/5A788AAD51, SEA938Hu/6E430916C8 and SEA241Hu/8D970EE435, respectively), in accordance with the manufacturer's instructions. The results were adjusted to urinary creatinine and compared to the control.

In-depth screening/monitoring of diabetic complications and associated conditions was performed in all patients. Diabetic retinopathy was diagnosed by ophthalmologist with a comprehensive dilated eye examination. Coronary artery disease was defined as myocardial infarction, unstable angina, coronary revascularization procedure, or transient myocardial ischemia in medical history, and/or abnormal result of exercise ECG testing or stress echocardiography. Chronic heart failure was assessed by New York Heart Association functional classification taking into account the limitation to physical activity, the results of physical examination and echocardiography. Carotid atherosclerosis and peripheral artery disease was verified by duplex ultrasound.

Statistical analysis

The statistical software package Statistics 12.0 (Dell, United States) was used to analyze the results. Quantitative data are presented as medians (lower quartiles; upper quartiles). Frequencies are presented as percentages (%). The normal distribution was determined by the Kolmogorov-Smirnov test. Because most of the quantitative were not distributed normally, non-parametric multiple comparisons of mean ranks were used to assess the statistical significance of differences between groups by continuous characteristics. The statistical significance of differences in discrete parameters between groups was analyzed using the χ^2 test. A difference was defined as significant if the *P* value was less than 0.05. Spearman rank correlation analysis was applied to test the association between variables. To assess the contribution of the investigated parameters to declining eGFR and development of albuminuria, a multiple logistic regression was used. The contribution of the factor was defined as significant if the standard deviation of the coefficient β did not exceed the coefficient β and the *P* value was less than 0.05. To assess the significance of the studied factors, the odds ratio, 95% confidence interval (CI), and *P* value were calculated using MedCalc 18.11.6 (MedCalc Software, Belgium). The influence of the factor was determined to be significant when the boundaries of the 95%CI were located on the same side of 1.0 and the *P* value was less than 0.05.

RESULTS

Clinical and laboratory characteristics of T2D patients with different CKD patterns

Patients were divided into four groups according to their eGFR and UACR levels. The individuals with eGFR ≥ 60 mL/min $\times 1.73$ m² and UACR < 3.0 mg/mmol were recorded as patients without CKD signs (CKD- group). Those with eGFR < 60 mL/min $\times 1.73$ m² and UACR < 3.0 mg/mmol were assigned to the NA-CKD group. Patients with eGFR ≥ 60 mL/min $\times 1.73$ m² and UACR ≥ 3.0 mg/mmol were defined as albuminuric with preserved renal function (A-CKD- group). Finally, the individuals with eGFR < 60 mL/min $\times 1.73$ m² and UACR ≥ 3.0 mg/mmol comprised

the albuminuric CKD group (A-CKD+). The demographic and clinical characteristics of these groups are presented in [Table 1](#).

Women made up a high proportion of NA-CKD patients ($P < 0.001$ compared with the CKD- group), while the proportion of men was highest in the A-CKD group ($P = 0.012$ compared with the CKD- group). Patients from the NA-CKD and A-CKD+ groups were older than CKD- and A-CKD- patients (all $P < 0.01$). Patients with NA-CKD demonstrated the lowest waist-to-hip ratio (WHR) among the examined T2D patients. In contrast, A-CKD- patients had the largest WHR values. There were no differences between the groups in body mass index (BMI). The percentage of current smokers was highest in the A-CKD- group.

Patients with reduced renal function (NA-CKD and A-CKD+ groups) had longer diabetes durations than those without. The prevalence of diabetic retinopathy tended to be higher in A-CKD- and A-CKD+ patients, though the differences between the groups were not statistically significant. Despite the fact that the prevalence of coronary artery disease did not differ between the groups, myocardial infarction occurred more frequently in individuals with albuminuria (A-CKD- and A-CKD+ groups). In contrast, carotid atherosclerosis and a history of cerebrovascular events (stroke or transient ischemic attack) were most prevalent in patients with decreased eGFR (NA-CKD and A-CKD+ groups). The prevalence of peripheral artery disease was highest in A-CKD+ patients.

Most patients in our cohort were insulin-treated ([Table 1](#)). The duration of insulin therapy was longest in NA-CKD patients. Interestingly, the daily insulin dose in this group was lowest. All patients received antihypertensive agents, mostly in combinations. The highest frequency of the use of diuretics was observed among NA-CKD patients, while the highest rate of treatment with dihydropyridine calcium channel blockers (nifedipine or amlodipine) was found in the A-CKD+ group. Statins and antiplatelet agents were more commonly prescribed to NA-CKD and A-CKD+ patients.

Laboratory parameters of T2D patients depending on their CKD status are summarized in [Table 2](#). The highest HbA1c and 2h-postprandial blood glucose levels were observed in the A-CKD- group. The NA-CKD group was characterized by the lowest HbA1c values. Serum uric acid was increased significantly in CKD patients compared to those without, but no dependence on the CKD pattern was observed. As expected, the red blood cell (RBC) count was decreased in patients with reduced renal function (NA-CKD and A-CKD+ groups) compared to those without. The lowest hemoglobin levels were found in the NA-CKD group. The erythrocyte sedimentation rate (ESR) was increased significantly in all CKD groups compared to the CKD- group. No differences in other hematological parameters were revealed. The A-CKD- patients demonstrated significantly increased levels of plasma SFMC compared to CKD- individuals. Fibrinogen and D-dimer levels did not differ between the groups.

Risk factors for CKD patterns

The risk factors for different CKD patterns are presented in [Table 3](#). Age ≥ 65 years, duration of T2D ≥ 15 years, female sex, and the use of diuretics were significant risk factors for NA-CKD. On the other hand, male sex, smoking, WHR > 1.0 and HbA1c $> 8.0\%$ significantly increased the risk of A-CKD-. Diabetes duration ≥ 15 years and the use of dihydropyridine calcium channel blockers were associated with A-CKD+. In multiple logistic regression analysis, age, HbA1c, female sex and treatment with diuretics were significant predictors of decreased eGFR ([Table 4](#)). Meanwhile, WHR, HbA1c and male sex predicted elevated albuminuria ([Table 5](#)).

Urinary biomarkers in T2D patients with different patterns of CKD

The excretion of nephrin and podocin was increased significantly in all diabetic groups compared to control (all $P < 0.05$, [Figure 2](#)). The CKD- and NA-CKD groups did not differ in the levels of urinary excretion of nephrin or podocin. Patients with elevated albuminuria (A-CKD- and A-CKD+ groups) demonstrated an increase in the excretion of both markers compared to the CKD- group (A-CKD-: $P = 0.001$ and $P = 0.006$, respectively; A-CKD+: $P = 0.04$ and $P = 0.002$, respectively) and the NA-CKD group (A-CKD-: $P = 0.000003$ and $P = 0.0003$, respectively; A-CKD+: $P = 0.04$ and $P = 0.00007$, respectively).

The urinary excretion of WFDC-2 in men was 9.2 times higher than in women ($P < 0.000001$). Accordingly, sex differences in marker excretion were taken into account when evaluating the results ([Figure 3](#)). Men of the CKD-, NA-CKD and A-CKD+ groups demonstrated increased excretion of WFDC-2 compared to the nondiabetic control ($P = 0.04$, $P = 0.01$ and $P = 0.009$, respectively). However, there were no significant differences in this marker between diabetic groups. In women, WFDC-2 excretion was increased markedly in the NA-CKD and A-CKD+ groups compared to control ($P = 0.01$ and $P = 0.0007$, respectively) and to patients without CKD ($P = 0.01$

Table 1 Clinical characteristics of type 2 diabetic individuals with different patterns of chronic kidney disease

Parameter	CKD- (n = 89)	NA-CKD (n = 111)	A-CKD- (n = 87)	A-CKD+ (n = 73)
General clinical parameters				
Sex, M/F, n (%)	20/69	13/98	45/42	22/51
Age, yr	22.5/77.5	11.7/88.3 ^{afh}	51.9/48.1 ^{ch}	30.1/69.9 ^e
BMI, kg/m ²	64 (58; 67)	68 (64; 73) ^{bfi}	63 (59; 68) ⁱ	67 (61; 77) ^{ae}
WHR	33.4 (28.7; 36.9)	32.6 (29.4; 37.2)	33.6 (30.1; 38.2)	33.4 (30.0; 36.8)
Smoking, n (%)	0.97 (0.94; 1.03)	0.94 (0.89; 0.99) ^{fh}	1.04 (0.97; 1.11) ^a	0.98 (0.95; 1.07)
Diabetes duration, yr	7 (7.9)	6 (5.4)	18 (20.9) ^a	3 (4.1)
Diabetic complications and comorbidities	15 (12; 19)	18 (15; 25) ^{cd}	15 (13; 20)	18 (14; 22) ^a
Diabetic retinopathy, n (%)				
Arterial hypertension, n (%)	62 (69.7)	74 (66.7)	65 (74.7)	57 (78.1)
Coronary artery disease, n (%)	85 (95.5)	111 (100)	87 (98.9)	73 (100)
Myocardial infarction in anamnesis, n (%)	41 (46.1)	58 (52.3)	46 (52.9)	41 (56.2)
Chronic heart failure (NYHA class III-IV), n (%)	7 (7.9)	19 (17.1)	20 (23.0) ^b	17 (23.3) ^b
Carotid atherosclerosis, n (%)	5 (5.6)	7 (6.3)	11 (12.6)	4 (5.5)
Cerebrovascular event in anamnesis, n (%)	15 (16.9)	51 (45.9) ^c	33 (37.9) ^a	40 (54.8) ^c
Peripheral artery disease, n (%)	6 (6.7)	13 (11.7) ^a	5 (5.8)	11 (15.1) ^a
Treatment	60 (67.4)	84 (75.7)	59 (67.8)	57 (78.1) ^a
Metformin, n (%)				
Sulfonylurea, n (%)	61 (68.5)	64 (57.7)	56 (64.4)	43 (58.9)
Insulin, n (%)	29 (32.6)	31 (27.9) ^g	21 (24.1)	10 (13.7) ^b
Duration of insulin therapy, yr	74 (83.1)	94 (84.7) ^g	76 (87.5)	70 (95.9) ^a
Daily insulin dose, IU	6 (4; 10)	10 (7; 13) ^{cf}	6 (3; 10)	8 (3; 11)
Daily insulin dose, IU/kg	52 (36; 72)	46 (34; 62) ^g	56 (40; 78)	60 (42; 74)
RAS blockers, n (%)	0.60 (0.40; 0.80)	0.55 (0.40; 0.70)	0.60 (0.40; 0.80)	0.63 (0.45; 0.90)
Diuretics, n (%)	67 (75.3)	93 (83.8)	69 (79.3)	61 (83.6)
Calcium channel blockers, n (%)	36 (40.4)	73 (65.8) ^{adg}	38 (43.7)	35 (47.9)
Antiplatelet agents, n (%)	27 (30.3)	38 (34.2) ^g	34 (39.1)	36 (49.3) ^a
Statins, n (%)	46 (51.7)	78 (70.3) ^{bd}	50 (57.5) ^g	57 (78.1) ^{be}
	28 (31.5)	59 (53.2) ^{bd}	31 (35.6) ^g	39 (53.4) ^{be}

^a*P* < 0.05,^b*P* < 0.01,^c*P* < 0.001 *vs* CKD–,^d*P* < 0.05,^e*P* < 0.01,^f*P* < 0.001 *vs* A-CKD–,^g*P* < 0.05,^h*P* < 0.01,

ⁱ*P* < 0.001 *vs* A-CKD+ (χ^2 test for discrete parameters and multiple comparisons of mean ranks for continuous parameters). BMI: Body mass index; WHR: Waist-to-hip ratio; CKD–: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD–: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol.

and *P* = 0.0007, respectively), while no difference between the CKD– group and the nondiabetic control was found.

In diabetic patients, urinary excretion of nephrin and podocin correlated positively with UACR (*r* = 0.47, *P* = 0.000001 and *r* = 0.43, *P* = 0.00001, respectively). Nephrin excretion demonstrated positive relationships with age (*r* = 0.27, *P* = 0.0007), diabetes duration (*r* = 0.31, *P* = 0.0002), and SFMC (*r* = 0.37, *P* = 0.001); at the same time, podocin excretion was related to age only (*r* = 0.27, *P* = 0.0004). No relationships between podocyte-specific markers and eGFR were found (*r* = 0.47, *P* = 0.000001 and *r* = 0.43, *P* = 0.00001, respectively). In women with diabetes, the excretion of WFDC-2 correlated with serum creatinine levels (*r* = 0.45, *P* = 0.001), eGFR (*r* = -0.50, *P* = 0.001) and UACR (*r* = 0.45, *P* = 0.001). In males, WFDC-2 correlated with BMI and WHR (*r* = 0.52, *P* = 0.01 and *r* = 0.53, *P* = 0.04, respectively), but not with UACR (*r* = 0.18, *P* > 0.05) or eGFR (*r* = -0.13, *P* > 0.05).

Table 2 Laboratory parameters of type 2 diabetic individuals with different patterns of chronic kidney disease

Parameter	CKD- (n = 89)	NA-CKD (n = 111)	A-CKD- (n = 87)	A-CKD+ (n = 73)
Renal tests				
Serum creatinine, $\mu\text{mol/L}$	76 (67.3; 86.8)	111 (99.1; 124) ^{cf}	85.8 (76.1; 96.5)	116 (97.8; 144) ^{cf}
eGFR, $\text{mL}/\text{min} \times 1.73 \text{ m}^2$	77 (69; 87)	52 (46; 56) ^{cf}	72 (66; 84) ⁱ	51 (46; 55.8) ^c
UACR, mg/mmol	0.5 (0.3; 0.9)	0.7 (0.4; 1.0) ^{fi}	8.3 (4.8; 36.7) ^c	11.4 (5.6; 42.1) ^c
Urinary protein excretion, mg/day	65 (50; 100)	70 (50; 140)	170 (90; 530) ^{ci}	200 (130; 520) ^{ci}
Biochemistry				
HbA1c, %	8.4 (7.5; 10.1)	8.1 (7.2; 9.5) ^f	9.7 (8.5; 11.2) ^{bg}	8.6 (7.5; 9.8)
HbA1c, mmol/L	68 (58; 87)	65 (55; 80) ^f	83 (69; 99) ^{bg}	70 (58; 84)
Fasting blood glucose, mmol/L	8.9 (6.8; 10.2)	8.8 (6.5; 10.1)	9.5 (8.0; 12.8) ^a	9.6 (7.7; 12.0)
2h-postprandial blood glucose, mmol/L	10.7 (9.0; 13.7)	11.7 (8.9; 14.0)	13.1 (9.9; 15.0) ^a	11.3 (10.0; 14.0)
Total cholesterol, mmol/L	5.1 (4.5; 5.9)	5.1 (4.3; 6.0)	4.9 (4.1; 6.0)	5.3 (4.1; 6.4)
LDL-cholesterol, mmol/L	3.3 (2.7; 3.9)	3.2 (2.5; 4.0)	3.1 (2.5; 3.8)	3.2 (2.5; 4.1)
HDL-cholesterol, mmol/L	1.2 (1.0; 1.4)	1.3 (1.1; 1.5) ^e	1.2 (1.0; 1.3)	1.1 (1; 1.4)
Triglycerides, mmol/L	1.6 (1.3; 2.2)	1.6 (1.1; 2.4)	1.8 (1.2; 2.9)	1.8 (1.3; 2.8)
Uric acid, $\mu\text{mol/L}$	279 (218; 349)	327 (269; 381) ^a	324 (276; 376) ^a	349 (272; 390) ^a
Hematology				
Hemoglobin, g/L	137 (130; 144)	129 (123; 140) ^{bd}	138 (126; 147)	133 (123; 143)
RBC, $\times 10^{12}/\text{L}$	4.8 (4.5; 5.0)	4.5 (4.2; 4.8) ^{ad}	4.7 (4.5; 5.1) ^g	4.5 (4.1; 4.9) ^b
WBC, $\times 10^9/\text{L}$	6.5 (5.7; 8.0)	6.7 (5.7; 7.8)	6.6 (5.3; 7.9)	6.9 (5.7; 8.0)
Platelets, $\times 10^9/\text{L}$	238 (199; 270)	234 (195; 270)	233 (191; 281)	229 (189; 273)
ESR, mm/h	16.5 (10; 23)	22 (15; 31) ^b	22.5 (15.5; 29.5) ^b	23 (18; 33) ^c
Coagulation tests				
Fibrinogen, g/L	4.4 (3.9; 5.5)	4.4 (3.9; 5.1)	4.5 (3.8; 5.7)	4.1 (3.7; 5.1)
SFMCs, mg/dL	5.5 (3.5; 15)	12 (7; 16)	14 (8; 23) ^a	12.5 (7; 21)
D-dimer, ng/mL	263 (235; 303)	287 (239; 351)	271 (232; 304)	290 (254; 363)

^a $P < 0.05$,^b $P < 0.01$,^c $P < 0.001$ vs CKD-,^d $P < 0.05$,^e $P < 0.01$,^f $P < 0.001$ vs A-CKD-,^g $P < 0.05$,

ⁱ $P < 0.001$ vs A-CKD+ (χ^2 test for discrete parameters and multiple comparisons of mean ranks for continuous parameters, estimated glomerular filtration rate, $\text{mL}/\text{min} \times 1.73 \text{ m}^2$). LDL: Low-density lipoprotein; HDL: High-density lipoprotein; UACR: Urinary albumin-to-creatinine ratio; eGFR: Estimated glomerular filtration rate; HbA1c: Hemoglobin A1c; RBC: Red blood cell; WBC: White blood cell; SFMC: Soluble fibrin monomer complex; CKD-: The group of individuals with estimated glomerular filtration rate $\geq 60 \text{ mL}/\text{min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $< 3.0 \text{ mg}/\text{mmol}$; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate $< 60 \text{ mL}/\text{min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $\geq 3.0 \text{ mg}/\text{mmol}$; A-CKD-: Group of patients with estimated glomerular filtration rate $\geq 60 \text{ mL}/\text{min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $\geq 3.0 \text{ mg}/\text{mmol}$; A-CKD+: Group of individuals with estimated glomerular filtration rate $< 60 \text{ mL}/\text{min} \times 1.73 \text{ m}^2$ and urinary albumin-to-creatinine ratio $\geq 3.0 \text{ mg}/\text{mmol}$.

DISCUSSION

Key findings

The results of this study demonstrate the characteristics of different CKD course patterns in patients with long-term T2D. First, by matching a panel of clinical and laboratory parameters of T2D patients who had an increase in albuminuria or a decrease in eGFR, or both deviations, with those without, we identified the risk factors for these CKD patterns. Second, we showed some features in the urinary excretion of biomarkers, reflecting podocyte and interstitium involvement, in patients with T2D and different patterns of CKD. The data provide further evidence that albuminuric and NA-CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

The risk factors for NA-CKD

The development of NA-CKD in patients with T2D was associated with older age (≥ 65 years), female sex, longer diabetes duration (≥ 15 years), and the use of diuretics. Age over 65 years was a risk factor for both albuminuric and NA-CKD patterns in our

Table 3 Risk factors for different patterns of chronic kidney disease in patients with type 2 diabetes

Risk factor	Pattern of CKD		
	NA-CKD (n = 111)	A-CKD- (n = 87)	A-CKD+ (n = 73)
Age ≥ 65 yr	3.16 (1.76-5.70) <i>P</i> = 0.0001	1.00 (0.55-1.80) <i>P</i> = 0.99	1.76 (0.94-3.28) <i>P</i> = 0.08
Duration of diabetes ≥ 15 yr	2.81 (1.53-5.17) <i>P</i> = 0.0009	1.63 (0.89-3.01) <i>P</i> = 0.12	2.32 (1.19-4.53) <i>P</i> = 0.01
Male sex	0.46 (0.21-0.98) <i>P</i> = 0.04	2.32 (1.20-2.48) <i>P</i> = 0.01	1.49 (0.74-3.01) <i>P</i> = 0.24
Female sex	2.19 (1.02-4.69) <i>P</i> = 0.04	0.43 (0.22-0.83) <i>P</i> = 0.01	0.67 (0.33-1.36) <i>P</i> = 0.24
Smoking	0.81 (0.25-2.60) <i>P</i> = 0.72	3.49 (1.31-9.28) <i>P</i> = 0.01	0.56 (0.13-2.34) <i>P</i> = 0.43
WHR >1.0	0.61 (0.22-1.65) <i>P</i> = 0.32	3.64 (1.32-9.99) <i>P</i> = 0.01	1.53 (0.57-4.10) <i>P</i> = 0.40
HbA1c > 8.0%	0.68 (0.38-1.20) <i>P</i> = 0.18	2.67 (1.35-5.27) <i>P</i> = 0.005	1.10 (0.58-2.09) <i>P</i> = 0.76
Treatment with diuretics	2.80 (1.56-5.00) <i>P</i> = 0.0005	1.10 (0.60-2.00) <i>P</i> = 0.76	1.30 (0.70-2.44) <i>P</i> = 0.41
Treatment with calcium channel blockers	1.20 (0.66-2.17) <i>P</i> = 0.56	1.47 (0.79-2.75) <i>P</i> = 0.22	2.23 (1.17-4.25) <i>P</i> = 0.01

The data are presented as odds ratio, 95% confidence interval and *P* value. CKD: Chronic kidney disease; CKD-: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD-: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min $\times 1.73$ m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; HbA1c: Hemoglobin A1c; WHR: Waist-to-hip ratio.

cohort. This finding may be explained by the general tendency for GFR to decrease in elderly patients^[27], as well as the inverse dependence of eGFR on age when calculated using the CKD-EPI formula^[28].

In our patients, female sex was a risk factor for NA-CKD, which is consistent with previous studies^[29,30]. It should also be taken into consideration that in women, who predominated in the NA-CKD group, the CKD-EPI formula gives lower eGFR values than in men when operating with equal creatinine levels. It was recently revealed that in healthy individuals, single-nephron GFR demonstrated no differences between men and women, but the total GFR values in women are typically lower than those in men due to fewer nephrons in the female kidney^[31]. The duration of diabetes for 15 years or more increased the risk of NA-CKD and A-CKD+ phenotypes, affecting eGFR more than albuminuria.

The proportion of patients taking diuretics was highest in the NA-CKD group. The relationships between diuretics and CKD require cautious interpretation. On the one hand, diuretics, especially in high doses, can cause deteriorative effects on the renal tubulointerstitium by provoking metabolic acidosis^[32], activation of the renin-angiotensin system^[33], or hypokalemia^[34]. Recent data indicate that the use of diuretics is associated with adverse renal outcomes, indicated by a decline in eGFR and an increasing risk of renal replacement therapy initiation in CKD patients. It was speculated that reduced GFR can be the result of episodes of lowering blood pressure, volume depletion and related acute renal injury induced by diuretics, especially when used in combination with other antihypertensive agents^[35]. The worsening of renal function in elderly patients with chronic heart failure has been associated with high doses of loop diuretics^[36]. The more frequent use of diuretics in patients with reduced renal function may be attributed to more prevalent and/or advanced arterial hypertension, heart failure or other fluid retention syndromes, which occur before the start of diuretic therapy. In our cohort, no differences in the prevalence of arterial hypertension or congestive heart failure were observed between the groups. The role of diuretics as factors modifying the CKD course requires further research.

Table 4 Logistic regression model for estimated glomerular filtration rate < 60 mL/min × 1.73 m², $\text{logit}(P) = \ln[P/(1-P)]$

Parameter	Coefficient β	95%CI	P value
Constant	-3.5742	-6.1459, -1.0025	0.006
Age, years	+0.0751	0.0413, 0.1089	0.00001
HbA1c, %	-0.2277	-0.3645, -0.0908	0.001
Female sex (1 or 0)	+0.2277	0.0051, 0.5743	0.046
Use of diuretics (1 or 0)	-0.2521	-0.4895, -0.0143	0.04

Area under the receiver operating characteristic curve = 0.7441, P value for Kolmogorov-Smirnov statistics = 2×10^{-11} . CI: Confidence interval; HbA1c: Glycated hemoglobin; logit: Logit-function.

Risk factors for albuminuria not accompanied by eGFR reduction

In our study, male sex, smoking, WHR > 1.0 and HbA1c > 8.0% were identified as risk factors for albuminuria not accompanied by a decrease in eGFR. This CKD pattern was more common in men. In multivariate logistic regression analysis, male sex was a significant risk factor for albuminuria. These data are in agreement with results of other research indicating an increased risk of albuminuria in men with T2D^[37,38].

The percentage of current smokers was highest in the A-CKD- group. The association between albuminuria and smoking has been reported previously^[37,39,40]. It was demonstrated that smoking cessation contributes to the reduction of albuminuria in patients with newly diagnosed T2D^[41]. The direct fibrogenic effect of tobacco smoke in the kidneys has been revealed in experimental CKD^[42]. Other underlying mechanisms for the association between smoking and albuminuria have been proposed, including activation of the sympathetic and renin-angiotensin systems, an increase in blood pressure, changes in intraglomerular hemodynamics, progression of atherosclerotic changes, and activation of vascular-platelet interactions^[43].

As expected, poor glycemic control turned out to be a risk factor for UACR elevation. The albuminuric effect of hyperglycemia has been linked with the accumulation of advanced glycation end-products, which, through the activation of protein kinase C and nuclear factor- κ B, enhance the synthesis of fibrogenic and proinflammatory factors in glomerular and tubular cells^[44]. These changes lead to deterioration of the glomerular endothelium and podocytes^[45] and impair albumin reabsorption in the proximal tubules^[46]. Recent studies have indicated that suppression of autophagy under hyperglycemic conditions promotes podocytopathy and increases the permeability of the glomerular filter^[47,48].

The WHR was identified as another risk factor for albuminuria not accompanied by eGFR reduction. The relationship between abdominal obesity and albuminuria has been shown in a number of studies^[37,49,50]. Hyperproduction of proinflammatory and fibrogenic cytokines, oxidative stress, and imbalances in adipokines are suggested as mechanisms of albuminuric effect in abdominal obesity^[51]. The association between WHR and albuminuria could be mediated by insulin resistance. A study in *db/db* mice, a T2D model, showed that both albuminuria and glomerulosclerosis are related to insulin resistance^[52]. A positive correlation has been found between homeostatic model assessment of insulin resistance index and the UACR values in patients with T2D^[53]. Misregulation of epithelial proteins, such as nephrin and megalin, and activation of the mTOR/S6 kinase pathway seem to be involved in mediating the pathophysiology of insulin resistance, kidney hypertrophy, hyperfiltration and microalbuminuria^[54].

Risk factors for albuminuric CKD

According to our results, diabetes duration and the use of calcium channel blockers were risk factors for albuminuric CKD. If the effect of the disease duration on the risk of CKD is quite natural, the relationships between dihydropyridines and CKD deserve discussion. On the one hand, initially more severe hypertension and the use of several antihypertensive drugs, there could be a combination of factors similar to the effects of diuretics, such as initially more severe arterial hypertension, and the use of several antihypertensive agents, which lead to a higher risk of arterial hypotension and prerenal acute kidney injury^[35]. Another possible mechanism involves the dilatation of the *vas afference* and the intraglomerular hypertension that can be induced by nifedipine, or amlodipine, the most widely used L-type calcium channel blockers^[55-57]. In our patient cohort, nifedipine and amlodipine were the only representatives of the class of calcium channel blockers. Meanwhile, observational studies have shown a reduction in albuminuria when patients were switched from L-

Table 5 Logistic regression model for urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol, $\text{logit}(P) = \ln[P/(1-P)]$

Parameter	Coefficient β	95%CI	P value
Constant	-8.1206	-13.1599, -3.0813	0.002
WHR	+5.1228	0.3920, 9.8535	0.03
HbA1c, %	+0.3570	0.1169, 0.5971	0.004
Male sex, (1 or 0)	+0.6725	0.1920, 1.1531	0.006

Area under the receiver operating characteristic curve = 0.7612, P value for Kolmogorov-Smirnov statistics = 0.00004. CI: Confidence interval; HbA1c: Glycated hemoglobin; logit: Logit-function; WHR: Waist-to-hip ratio.

type calcium channel blockers to T/L-type^[58] or L/N-type ones^[59]. According to meta-analyses, antialbuminuric activity has been shown for nondihydropyridine^[60], dihydropyridine L/N-, L/T-^[61] and T-type^[62] calcium channel blockers.

Urinary excretion of nephrin and podocin

In this study, we found that urinary excretion of nephrin and podocin was increased significantly in individuals with long-term T2D and correlated positively with UACR. These data are in agreement with the notion that podocytopathy is a key factor leading to the development of proteinuria and glomerulosclerosis in diabetic kidney disease^[12]. Elevated excretion of nephrin and podocin, which are expressed in podocytes exclusively, may reflect more severe podocyte injury in albuminuric patients. It was demonstrated that the loss of the podocytes correlates with the levels of proteinuria in diabetic nephropathy^[63]. Thus, increased urinary excretion of nephrin and podocin can be a sign of podocytopathy, which is detected in diabetic kidney disease^[64]. The correlation between these markers and the urinary concentrations of podocytes in diabetes has been shown previously^[65]. In our study, the excretion of nephrin and podocin was increased dramatically in patients with elevated albuminuria, regardless of the concomitant decline in renal function, compared to patients without any signs of CKD or NA-CKD. This may indicate more advanced podocyte injury in T2D patients with albuminuric CKD.

Urinary excretion of WFDC-2

The serum levels of WFDC-2 (HE-4) were validated previously as a marker of tubulointerstitial fibrosis^[25,26]. In this study, we investigated for the first time the urinary excretion of WFDC-2 in individuals with T2D. First, we found that excretion of WFDC-2 in men was markedly higher (approximately 10 times) than in women. These findings could be explained by the sexual differences in the expression of this molecule. Besides the kidneys, in males WFDC-2 is expressed in the epithelial cells of the epididymal and seminal ducts and the glandular epithelium of the prostate, i.e., in the organs that are related to the urinary tract anatomically. In women, WFDC-2 expression is detected in the fallopian tubes, endometrium, and Bartholin's glands^[24], which do not contact directly with the urinary system.

Excretion of WFDC-2 showed different relationships with CKD between men and women. In men with diabetes, excretion of WFDC-2 was increased in all groups, regardless of the presence or pattern of CKD. In women, WFDC-2 excretion was increased in the groups with declined eGFR only. The women had an inverse correlation between WFDC-2 and eGFR values and a direct correlation between WFDC-2 and UACR. At the same time, urinary excretion of WFDC-2 was not associated with excretion of nephrin or podocin. These data are in agreement with previous morphological studies indicating a close relationship between GFR and tubulointerstitial involvement rather than glomerulopathy^[15,66].

Limitations

Our study is not without limitations. First, it is a cross-sectional study that does not prove causality. The natural intraindividual variability in eGFR and UACR values could be a source of some errors in classifying patients into groups. The recruitment of patients at one clinical center and the relatively small sample size could have led to a shift in the results of biomarker assessment with respect to the general diabetic population.

The remarks for clinical practice and future research

In this study, we demonstrate the differences in the clinical and laboratory

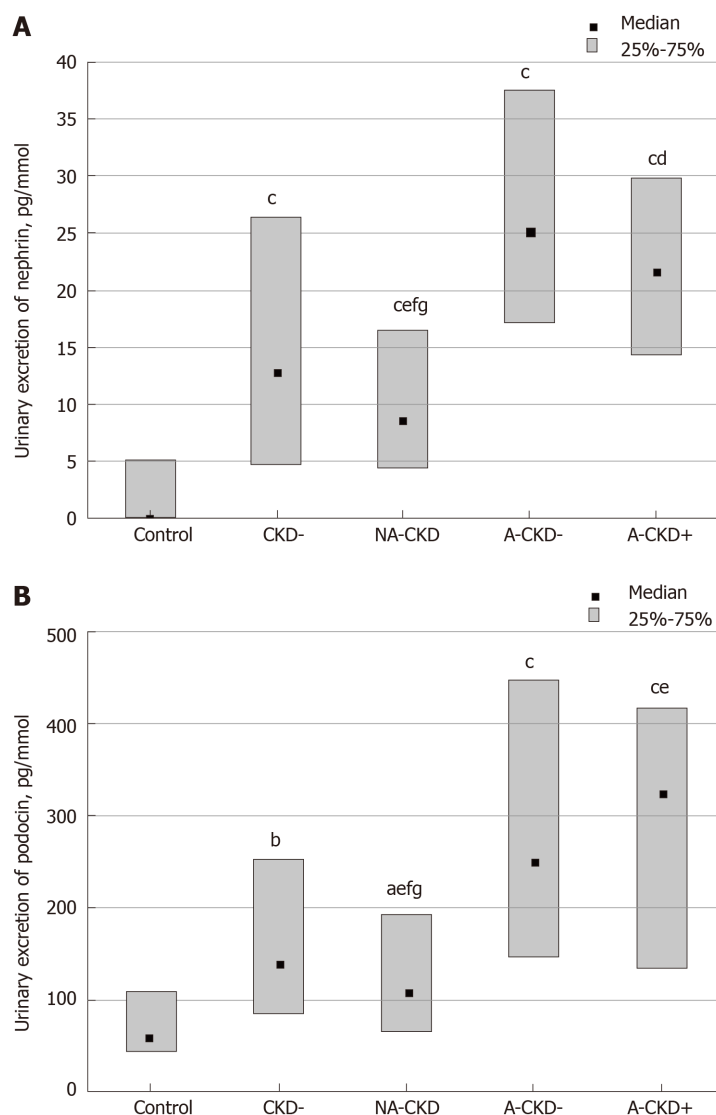


Figure 2 Urinary excretion of podocyte-specific markers in patients with type 2 diabetes and different patterns of chronic kidney disease. A: Nephrin; B: Podocin. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ vs non-diabetic control; ^d $P < 0.05$, ^e $P < 0.01$ vs chronic kidney disease-group; ^f $P < 0.001$ vs albuminuric chronic kidney disease-group, ^g $P < 0.001$ vs albuminuric chronic kidney disease+ group (the test of multiple comparisons of mean ranks). CKD-: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD-: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol.

characteristics of albuminuric and NA-CKD in patients with long-term T2D. We found that female sex, older age, longer diabetes duration and diuretic use were associated with the NA-CKD phenotype. Meanwhile, male sex, smoking, abdominal obesity, and poor glycemic control were risk factors for albuminuria elevation not accompanied by a reduction in eGFR. It should be noted that some of the abovementioned risk factors are modifiable, especially those associated with albuminuria, which is important from clinical point of view. The predominant effect of antihyperglycemic drugs on albuminuria or GFR should be considered when choosing treatment for T2D patients with different CKD phenotypes.

To our knowledge, this is the first study addressing the diversity in urinary biomarkers in T2D patients with different CKD phenotypes. The different patterns of the shifts in the urinary excretion of the biomarkers of podocyte and tubulointerstitial involvement in albuminuric and NA-CKD give further support the notion that these phenotypes differ in their pathophysiology. A significantly more demonstrative increase in the excretion of nephrin and podocin in patients with elevated UACR compared to those without could suggest that albuminuric CKD is

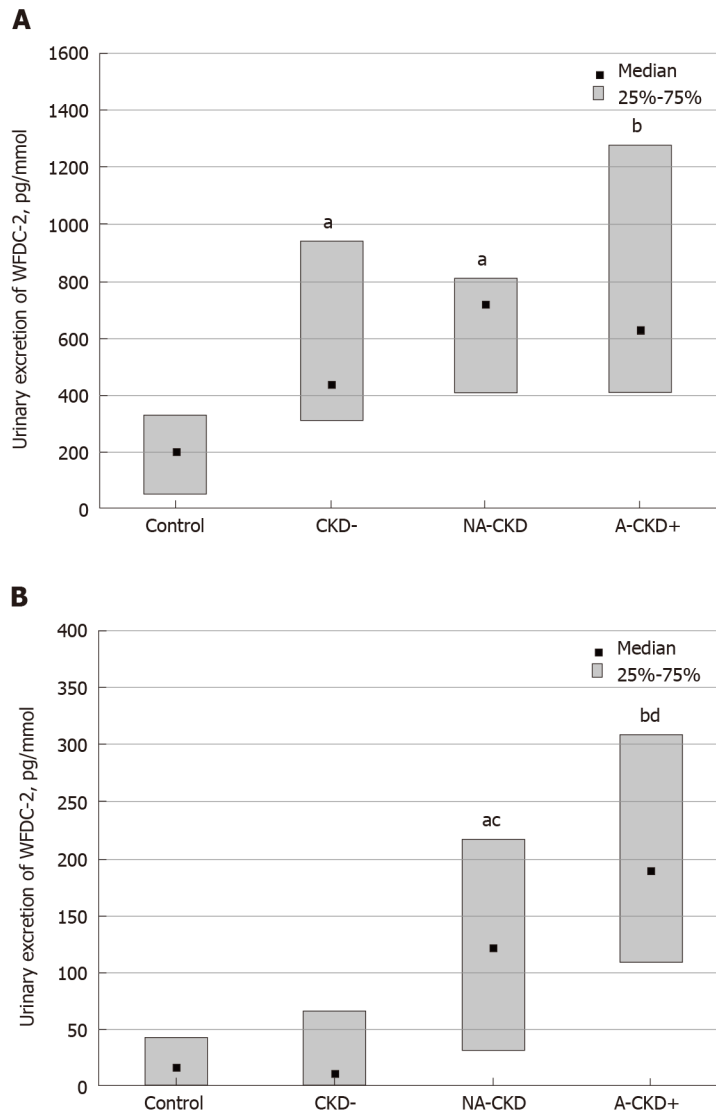


Figure 3 Urinary excretion of WAP-four-disulfide core domain protein 2 in individuals with type 2 diabetes and different patterns of chronic kidney disease. A: Males; B: Females. ^a $P < 0.05$, ^b $P < 0.01$ vs non-diabetic control, ^c $P < 0.05$, ^d $P < 0.01$ vs CKD- group (the test of multiple comparisons of mean ranks). CKD-: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD-: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min \times 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol.

associated with more severe podocyte involvement. The increase in urinary excretion of WFDC-2 in women with T2D and decreased eGFR apparently indicates more advanced tubulointerstitial lesion. The assessment of the predictive value of the studied biomarkers in different phenotypes of CKD is a challenge for future research. Studies of the renoprotective potential of antihyperglycemic, antihypertensive and other therapeutic agents in different CKD phenotypes are urgently needed. In conclusion, the data provide further evidence that albuminuric and NA-CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

ARTICLE HIGHLIGHTS

Research background

A number of researches show the heterogeneity of the natural course of chronic kidney disease (CKD) in patients with diabetes. Moreover, it has been shown that natural course of diabetic kidney disease is being transformed with increasing prevalence of declined renal function not accompanied by elevation of albuminuria. The trend is more evident in patients with type 2

diabetes (T2D). Currently, little is known about the mechanisms that determine the development of the albuminuric or nonalbuminuric phenotype of CKD. It was suggested that an increase in albuminuria may be a consequence of podocytopathy, while a decrease in renal function is associated with the involvement of tubulointerstitium.

Research motivation

The main topic of this study is in-depth clinical characteristics and identification of the risk factors and biomarkers of albuminuric and non-albuminuric CKD phenotypes in patients with T2D. The results may provide further progress in understanding of individual differences in the natural course of diabetic kidney disease and generation differentiated approaches to prevention and treatment of this complication.

Research objectives

The study aimed to identify the risk factors and urinary biomarkers of albuminuric and non-albuminuric CKD in patients with long-term T2D. Wherein, we tested the hypothesis that albuminuric and non-albuminuric CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

Research methods

Three hundred and sixty patients with T2D duration of at least 10 years from the date of diagnosis were included in this observational cross-sectional study. The associations of a panel of demographic and clinical characteristics, complications, comorbidities, and metabolic parameters with albuminuric and non-albuminuric CKD were analyzed. The urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2 (WFDC-2), a marker of tubulointerstitial fibrosis, was determined by ELISA in defined CKD phenotypes.

Research results

In this study we identified the risk factors of three CKD phenotypes in T2D patients. According to our data, non-albuminuric CKD is associated with age ≥ 65 years, female sex, diabetes duration ≥ 15 years, and the use of diuretics. Male sex, smoking, waist-to-hip ratio > 1.0 and HbA1c $> 8.0\%$ are risk factors for elevated albuminuria not accompanied by a decrease in estimated glomerular filtration rate (eGFR). Duration of diabetes ≥ 15 years and the use of calcium channel blockers seem to be risk factors for albuminuria with decreased eGFR. We also found some differences in predictors of decreased eGFR and increased albuminuria. In multivariate logistic regression analysis, age, HbA1c, female sex and diuretics were significant predictors for reduced eGFR, while waist-to-hip ratio, HbA1c and male sex were associated with elevated urinary albumin-to-creatinine ratio (UACR). In accordance with the tested hypothesis, we found the differences in urinary biomarkers of podocyte and tubulointerstitium involvement in patients with different CKD phenotypes. Excretion of nephrin and podocin was increased in patients with albuminuria, regardless of decline in renal function, correlating positively with UACR. At the same time, in women, WFDC-2 excretion was increased in those with reduced renal function, correlating negatively with eGFR.

Research conclusions

To our knowledge, this is the first study addressing the diversity in clinical characteristics and urinary biomarkers in T2D subjects with different CKD phenotypes. The results of this study provide new data on the risk factors and mechanisms of different variants of CKD in patients with long-term T2D. Firstly, by matching a panel of clinical and laboratory parameters of T2D patients who had an increase in albuminuria, a decrease in eGFR, or both deviations, with parameters in T2D patients with normoalbuminuria and preserved renal function, we showed the differences in profiles of the risk factors for CKD phenotypes. According to our data, non-albuminuric CKD phenotype is associated with age, female sex, diabetes duration, and the use of diuretics, whereas male sex, smoking, abdominal obesity and poor glycemic control are risk factors for elevated albuminuria. Secondly, we demonstrated some features in the urinary excretion of biomarkers, reflecting the podocyte and interstitial involvement, in patients with different CKD phenotypes. A significantly more demonstrative increase in the excretion of nephrin and podocin in patients with elevated UACR compared to those without could suggest that albuminuric CKD is associated with more severe podocyte involvement. The increase in urinary excretion of WFDC-2 in women with T2D and decreased eGFR apparently indicates more advanced tubulointerstitial fibrosis. The data provide further evidence that albuminuric and non-albuminuric CKD phenotypes correspond to different pathways of diabetic kidney disease progression. The diversity in the profiles of risk factors should be taken into account by clinicians in the management of diabetes.

Research perspectives

Since our study has a cross-sectional design, it does not prove causality. Accordingly, significance of some identified risk factors needs further confirmation. In particular, the role of abdominal obesity, insulin resistance, diuretics and calcium channel blockers needs to be verified in prospective studies. The assessment of the predictive value of the studied biomarkers in albuminuric and non-albuminuric CKD phenotypes is a challenge for future research. The studies of the renoprotective potential of antihyperglycemic, antihypertensive and other therapeutic agents in different CKD phenotypes are urgently needed.

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Observational Study

Type 1 diabetes loci display a variety of native American and African ancestries in diseased individuals from Northwest Colombia

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Abstract

BACKGROUND

Type 1 diabetes (T1D) is a complex disease with a higher incidence in Europeans than other populations. The Colombians Living in Medellín (CLM) is admixed with ancestry contributions from Europeans, Native Americans (NAT) and Africans (AFR).

AIM

Our aim was to analyze the genetic admixture component at candidate T1D loci in Colombian individuals with the disease.

METHODS

Seventy-four ancestry informative markers (AIMs), which tagged 41 T1D candidate loci/genes, were tested by studying a cohort of 200 Northwest Colombia diseased individuals. T1D status was classified by testing for glutamic acid decarboxylase (GAD-65 kDa) and protein tyrosine-like antigen-2 auto-antibodies in serum samples. Candidate loci/genes included *HLA*, *INS*, *PTPN22*, *CTLA4*, *IL2RA*, *SUMO4*, *CLEC16A*, *IFIH1*, *EFR3B*, *IL7R*, *NRP1* and *RNASEH1*, amongst others. The 1,000 genome database was used to analyze data from 94 individuals corresponding to the reference CLM. As the data did not comply with a normal distribution, medians were compared between groups using the Mann-Whitney *U*-test.

RESULTS

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Both T1D patients and individuals from CLM displayed mainly European ancestry (61.58 *vs* 62.06) followed by Native American (27.34 *vs* 27.46) and to a lesser extent the AFR ancestry (10.28 *vs* 10.65) components. However, compared to CLM, ancestry of T1D patients displayed a decrease of NAT ancestry at gene *EFR3B* (24.30 *vs* 37.10) and an increase at genes *IFIH1* (32.07 *vs* 14.99) and *IL7R* (52.18 *vs* 39.18). Also, for gene *NRP1* (36.67 *vs* 0.003), we observed a non-AFR contribution (attributed to NAT). Autoimmune patients (positive for any of two auto-antibodies) displayed lower NAT ancestry than idiopathic patients at the MHC region (20.36 *vs* 31.88). Also, late onset patients presented with greater AFR ancestry than early onset patients at gene *IL7R* (19.96 *vs* 6.17). An association analysis showed that, even after adjusting for admixture, an association exists for at least seven such AIMs, with the strongest findings on chromosomes 5 and 10 (gene *IL7R*, $P = 5.56 \times 10^{-6}$ and gene *NRP1*, $P = 8.70 \times 10^{-19}$, respectively).

CONCLUSION

Although Colombian T1D patients have globally presented with higher European admixture, specific T1D loci have displayed varying levels of Native American and AFR ancestries in diseased individuals.

Key words: Type 1 diabetes; Genetic admixture; Native American; Idiopathic; Colombia

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Core tip: We have tested the effect of genetic admixture in a set of Colombian patients with Type 1 Diabetes (T1D). We show that, although no differences between T1Ds and Colombians living in Medellín arose globally, there appear to be ancestry differences when looking at specific T1D loci/genes (*e.g.*, genes *EFR3B*, *IFIH1*, *IL7R* and *NRP1*). Also, when comparing patient ancestry according to the presence/absence of T1D-related auto-antibodies or age at onset of the disease, differences were also observed. The most striking differences in ancestry occurred outside the HLA region, which is considered the master risk locus in T1D and for autoimmune diseases overall. This in itself is a striking observation.

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INTRODUCTION

Type 1 diabetes (T1D) is a heterogeneous disease with pathogenic processes and phenotypic characteristics that show marked variation. It is accepted that genetic effects are an important factor for this heterogeneity. *HLA* confers the major genetic susceptibility to T1D, contributing up to 50%; it is located on chromosome 6p21^[1]. In addition, over 50 non-*HLA* genes (so far) increase susceptibility to T1D^[2,3]. Recently, we have identified that *RNASEH1* gene variants associate with T1D in Northwest Colombia^[4]. This gene, which is located on chromosomal region 2p25, has not thus far been associated elsewhere with the disease. A wide geographical variation in the incidence of T1D both among and within countries has been reported^[5]. Incidence of T1D is higher in Europeans^[6-8] than in Latin American countries^[7,8]. Genetic admixture is a factor that influences allelic frequencies in a population; this, in part, may contribute to explaining the differences observed in T1D epidemiology.

Three studies in Latin America have tested the admixture effect on T1D. Two of these were carried out in Brazil^[9,10] and the third in Cuba^[11]. These three studies found that T1D patients are mostly of European descendant and not necessarily different than controls. Thus, one Brazilian study and the one from Cuba reported that patients carried a greater European component than their controls; this observation was established as a risk factor^[9,11].

In Colombia, the admixture process was produced differently in each region of the country. Populations in southern Colombia show higher values of Native American

ancestry (NAT, average 60%), whilst African (AFR) ancestry is more observed in the region of Chocó (average 68%) and the Caribbean coast (average 30%)^[12-14]. On the other hand, northwest Colombia, inhabited by the “paisa” population, exhibits the highest percentage of European ancestry, which ranges in studies from 47-79%^[15-19]. In Colombia, the admixture effect has been examined for some complex diseases such as type 2 diabetes^[20], asthma^[21], cancer^[22,23], dengue patients^[24], Alzheimer’s disease^[17], as well as for cardio-metabolic parameters^[25].

Although much of the work on the admixture effect on several phenotypes has been done in Latin America and Colombia, none has tested this effect on T1D in Colombian patients. Our purpose was to analyze the genetic admixture composition of a set of Colombian T1D patients, by testing previously reported admixture informative markers (AIMs) in the vicinity of previously reported T1D candidate genes/loci. Besides, two chromosomal regions of high relevance to T1D in our population were tested more thoroughly. These loci were *6p21* (*HLA*), which is globally accepted as the T1D master risk locus, and *2p25* (*RNASEH1*), which has been reported solely in Colombia, so far. We inferred individual patient proportions of European, AFR and NAT ancestry components. Although the European component was higher than the two other parental contributions in a global analysis, some loci are clearly non-Europeans in cases *vs* the reference population, or between T1D categories. This study shed light on the genetics of T1D in a Colombian population, and reinforces the importance of including different approaches when looking for T1D genetic architecture. This is suggested by finding no admixture differences in strongly associated T1D loci, such as *HLA* (*IDDM1*) or *IDDM2*. In contrast, a strong genetic admixture effect was observed for other loci not described as high determinants for developing T1D. For instance, this was the case for chromosomal regions *5p13.2* and *10p11.22*.

MATERIALS AND METHODS

Study population

The study group consisted of 200 Colombian individuals with T1D. Their age at onset was < 15 years. Diagnostic criteria were according to the American Diabetes Association^[26]. Patients were considered as “Paisas” according to a self-reported questionnaire asking for their geographical origin back until their great-grandparents. Other questions included gender, age at onset, and other family members with autoimmune diseases.

Patients were identified in the main pediatric endocrinology institutes from Antioquia: Program of Pediatric Endocrinology (Universidad de Antioquia and Hospital San Vicente Fundación), IPS Universitaria, Universidad Pontificia Bolivariana, Instituto Antioqueño de Diabetes and Clínica Integral de Diabetes. This study was approved by the ethics committee of the Faculty of Medicine at Universidad de Antioquia. Informed consent was obtained from patients and their parents before drawing blood samples.

Auto-antibodies testing

Two diabetes-related autoantibodies (AABs) were tested in sera samples from the 200 patients. These AABs were glutamic acid decarboxylase (GAD-65 kDa) and protein tyrosine-like antigen-2 (IA-2), as reported previously^[4]. They were measured using a commercial ELISA-based kit (AESKULISA and LifeSpan BioSciences, Inc) according to the manufacturer's instructions. If a patient presented with at least one of these AABs, he/she was classified as autoimmune (T1AD), or was otherwise classified as idiopathic (T1BD).

Genotyping and admixture estimation

Genomic DNA was isolated from peripheral blood samples using either the phenol-chloroform or salting out protocols. A set of 75 AIMs was tested in 200 T1D patient samples using the Competitive genotyping Allele-Specific PCR technology (KASP™), which was undertaken by the Company LGC Genomics Ltd. Details of this method can be obtained from <https://www.lgcgroup.com/genotyping/>.

The AIMs used have a high discriminatory power ($\delta > 45\%$) among ancestral populations (Supplementary Table S1), which increases the statistical power for estimating individual ancestry. We selected these markers from Latino populations panels reported by Mao *et al*^[27], Galanter *et al*^[28] and Ruiz-Linares *et al*^[29]. The AIMs were distributed throughout the genome, tagging previously reported T1D candidate loci. However, we chose a higher density of markers for chromosome 2 (23 AIMs) where the *RNASEH1* gene is; and for chromosome 6 (18 AIMs) where the *HLA* region

is.

The 1,000 genome database was used to extract genetic information from 94 Colombians living in Medellin (CLM) for the 74 AIMs successfully typed (<ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/>). These population individuals are from the same geographical region as the patients. We calculated allele and genotypic frequencies, and Hardy-Weinberg equilibrium (HWE) using PLINK v. 1.07^[30]. In addition, we considered markers that were not in linkage disequilibrium with each other. We used markers with a genotyping rate higher than 95%, without significant deviations from HWE after a Bonferroni correction.

We estimated individual European, NAT and AFR ancestry proportions using ADMIXTURE software^[31]. The proportions of each component were estimated using a supervised-learning strategy, providing the genotypes of 74 AIMs from reference populations AFR, European and NAT ($k = 3$). We used 74/75 AIMs since one failed the PCR optimization.

To find the parental population allele frequencies, genotypes from 165 Europeans (Utah residents with ancestry from northern Europe and the West, named as CEU), and 165 AFR (Yoruba people in Ibadan, Nigeria, named as YRI) genotyped in the HapMap project were selected, which are deposited in the 1,000 genome database. Since we did not have access to NAT DNA samples or publicly available NAT genotype data on all 74 AIMs, we generated the genotypes of the 74 AIMs for 150 simulated individuals, according to the allele frequencies of NAT previously reported in the panels.

Statistical analysis

Comparison between groups for continuous variables that did not comply with normal distribution was performed using the Mann-Whitney *U*-test. Thus, comparisons of ancestry medians between T1D subtypes (T1AD and T1BD) according to AABs, and individuals with early/late age at onset (*i.e.* ≤ 5 years or > 5 years, respectively) were performed. In addition, these comparisons were also done to the CLM population. We performed these analyses for AIMs distributed across the set of candidate loci and independently for loci at different chromosomes. We ran all statistical analyses and graphs in the R package V3.3.3^[32]. We also tested allelic association of these AIMs between T1D and CLM using PLINK 1.07^[30].

RESULTS

T1D versus CLM, our reference population

One out of 75 AIMs did fail the PCR optimization. Therefore, we tested a total of 74 AIMs in 200 T1D patients from Antioquia, Colombia. AIMs characteristics are shown in [Supplementary Table S1](#). Overall, the rate of genotyping was $> 96\%$ for every AIM, and there was no deviation from the HWE, after Bonferroni correction for multiple testing ($P = 6.75 \times 10^{-4}$). Also, as expected, none of the AIMs was in linkage disequilibrium with each other (data not shown).

The overall ancestral genetic makeup of the 200 T1D children showed a predominant proportion of European ancestry (EUR, Median = 61.58) followed by NAT ancestry (Median = 27.34), and AFR ancestry was found at a lower proportion (Median = 10.28, [Table 1](#) and [Figure 1](#)). [Figure 1](#) presents the ancestry distribution for the 200 T1D children studied here. It can be noticed that the European component is the prominent one. European ancestry ranged from 22% to 93%; the NAT ancestry ranged from 0 to 65%, and the AFR ancestry ranged from 0 to 40%.

Looking at the overall set of AIMs, and also at their distribution in specific loci, it was observed that diseased individuals of EUR ancestry had a median from 61.58-11.56. The lowest value was found for chromosome 5 AIMs ([Table 1](#)). NAT ancestry ranged from 52.18-24.30 in the diseased subjects. The highest value was found for gene *IL7R* AIMs (chromosome 5), and the lowest value was found for gene *EFR3B* AIMs (chromosome 2). The AFR component (AFR) ranged from 20.58 to 0.01. the lowest AFR ancestry was found for gene *IFIH1* AIMs (chromosome 2, [Table 1](#)). The wide ancestry variation across chromosomal regions is noticeable.

Overall, the CLM reference population displayed a very similar ancestry distribution compared to T1D cases. Nonetheless, specific T1D loci presented marked differences between the two groups; one such difference was observed for the gene *EFR3B*, which presented with higher NAT in the CLM population ($P = 0.02$), suggesting a protective role for developing T1D ([Table 1](#)). Also, at gene *IFIH1*, T1D patients presented with lower European ancestry ($P = 0.05$), at the expense of a higher NAT component than in CLM ([Table 1](#)). Other differences between T1D and CLM were observed for the *IL7R* and *NRP1* genes (Chromosomes 5 and 10, respectively) as

Table 1 Genetic ancestry of type 1 diabetes patients compared to Colombians living in Medellin control population

Chromosomal region	Ancestry	T1D, median (IQR)	CLM, median (IQR)	P value ¹
Overall AIMs	EUR	61.58 (52.84-69.85)	62.06 (49.67-73.74)	0.675
	NAT	27.34 (21.36-34.05)	25.46 (16.12- 32.90)	0.106
	AFR	10.28 (4.0-16.83)	10.65 (6.05-16.75)	0.575
Chr2_EFR3B	EUR	60.27 (34.05-80.79)	47.88 (34.04-76.43)	0.189
	NAT	24.30 (0.01-51.24)	37.10 (1.61-62.49)	0.02
	AFR	7.17 (0.01-25.05)	0.01 (0.01-15.67)	0.06
Chr2_CTLA4	EUR	46.24 (16.82-69.52)	58.26 (30.82-82.79)	0.167
	NAT	27.96 (0.04-41.31)	25.01 (0.01-45.16)	0.829
	AFR	20.54 (0.01-41.72)	11.78 (0.01-36.59)	0.183
Chr2_RNASEH1	EUR	56.91 (31.33-73.91)	58.80 (32.75-78.43)	0.482
	NAT	27.41 (11.69-46.36)	24.81 (0.01-48.84)	0.241
	AFR	8.37 (0.01-28.24)	13.04 (0.01-23.87)	0.430
Chr2_IFIH1	EUR	42.42 (0.01-77.63)	52.01 (16.33-82.16)	0.05
	NAT	32.07 (0.01-56.74)	14.99 (0.01-41.35)	0.246
	AFR	14.99 (0.01-32.31)	17.83 (0.01-42.31)	0.181
Chr5_IL7R	EUR	11.56 (1.04-41.14)	24.75 (7.20-46.98)	7.0×10^{-3}
	NAT	52.18 (3.74-98.06)	39.18 (0.04-52.80)	1.0×10^{-4}
	AFR	15.21 (3.0-35.75)	33.89 (11.23-59.94)	1.56×10^{-5}
Chr6_MHC	EUR	51.35 (32.92-70.32)	55.86 (32.61-71.21)	0.76
	NAT	23.28 (8.92-40.0)	21.61 (5.83-43.13)	0.835
	AFR	18.87 (1.63-36.08)	19.53 (0.3-34.11)	0.660
Chr10_NRP1	EUR	63.32 (23.70-63.32)	0.03 (0.001-20.34)	2.2×10^{-16}
	NAT	36.67 (7.93-36.67)	0.003 (0.001-7.93)	2.2×10^{-16}
	AFR	0.03 (0.001-30.91)	94.23 (53.94-99.99)	2.2×10^{-16}

¹Data from Mann-Whitney *U* test. AIMs: Ancestry informative markers; IQR: Interquartile range; CLM: Colombians living in Medellin from 1,000 genomes database; Chr: Chromosome; EUR: European; NAT: Native American; AFR: African; T1D: Type 1 diabetes.

follows.

Chromosome 5 AIMs (gene *IL7R*) showed less European ($P = 7.0 \times 10^{-3}$) and less AFR ancestries (1.56×10^{-5}) in diseased subjects than the CLM population; consequently, T1D patients had more NAT ancestry than CLM subjects at this chromosomal region ($P = 1.0 \times 10^{-4}$, Table 1). Regarding chromosome 10 AIMs (gene *NRP1*), it was observed that T1D patients presented a high European component compared to CLM (63.32 *vs* 0.03, Table 1). Conversely, patients presented an almost zero AFR component for this chromosomal region compared to CLM (0.03 *vs* 94.23, Table 1). Consequently, T1D patients displayed a predominance of NAT ancestry at this locus compared to CLM (36.67 *vs* 0.003, Table 1).

An exploratory association analysis showed that, after adjusting for admixture, seven markers were associated with T1D (Supplementary Table S2 and Table 2). The most significant findings were located on chromosomes 5 and 10 ($P = 5.56 \times 10^{-6}$ and 8.70×10^{-19} , respectively). It is interesting that only one MHC marker (*rs2395656*) presented an association with the disease, and this happened with less strength in its association ($P = 0.04$) than markers at chromosomes 5 and 10 (Table 2).

Ancestral components considering T1D subtypes (according to autoimmunity and age at onset)

We stratified the T1D sample according to the presence (T1AD, autoimmune) or absence (T1BD, idiopathic) of diabetes-related AABs; we also stratified the patient group according to their age at onset, *e.g.*, early (≤ 5 years) or late (> 5 years). We found that 78% ($n = 156$) of the patients had at least one T1D specific autoantibody (GAD-65 and IA-2), while the 22% remaining ($n = 44$) were negative for these two antibodies. T1AD average age at onset was 8.25 years, whilst for T1BD it was 7.22. We did not find significant differences between men and women within these two groups (data not shown).

Over thirty percent ($n = 61$, 30.5%) of T1D individuals developed the disease before

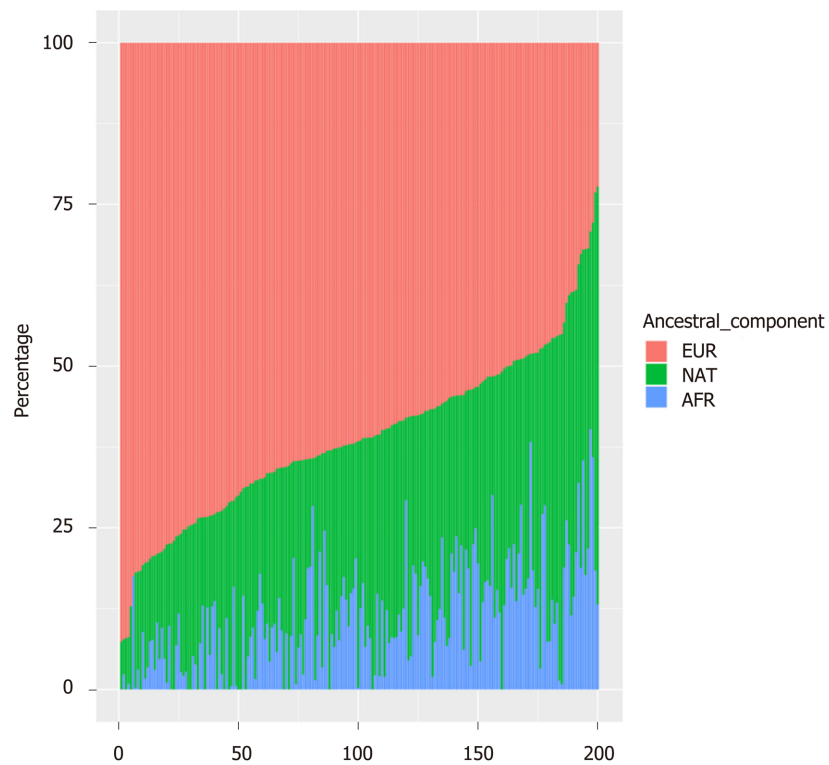


Figure 1 Ancestry proportions of 200 type 1 diabetes patients from Colombia. EUR: European; NAT: Native American; AFR: African.

the age of 5 years, with an average age at onset of 2.66 years. The remaining sample (69.5%, $n = 139$) presented with an age at onset after 5 years, with a mean for this category of 10.28 years. As in the stratification by AABs, we did not find significant differences between men and women within the age at onset categories (data not shown). Regarding the autoimmune category, comparisons among ancestral genetic composition led to the identification of no differences for the 74 AIMs taken together (Table 3). However, looking at individual loci, it was observed that *MHC* AIMs present with lower NAT ancestry in the autoimmune subgroup ($P = 0.019$, Table 3).

In addition, when comparing diseased individuals in the autoimmune categories to CLM population, it was observed that gene *EF3B* AIMs present differences in their ancestral components (Supplementary Table S3). Thus, autoimmune patients presented with less NAT ancestry ($P = 0.032$), whilst T1D idiopathic category presented with higher AFR ancestry ($P = 0.016$). Regarding the age at onset categories, it was observed that the AFR ancestry is significantly higher in the late onset subgroup at gene *IL7R* AIMs ($P = 0.023$, Table 4). Comparing these two categories to the CLM population showed no significant differences for either the overall set of AIMs nor specific loci (Supplementary Table S3).

DISCUSSION

T1D incidence differences among countries, mainly related to European *versus* non-Europeans, led us to assess whether our T1D patients had a predominantly European ancestral component or other. Our analyses were based on 74 AIMs located on previously reported T1D loci/genes. AIM deltas (δ s) between the NAT, European and AFR populations indicated that they were appropriate discriminators. We found that T1D patients from northwest Colombia are predominantly of European ancestry, followed by NAT and AFR components. Proportion estimates of the three parental populations for this sample were consistent with those reported in previous studies for Colombians, but using different sets of markers^[13,16,19,20,29].

We also compared T1D children to CLM. Analyzing the overall set of AIMs found no statistically significant differences in the ancestral genetic component between the two groups. Comparable results were obtained by Gomes *et al*^[10] in Sao Paulo-Brazil; they noted that the European component predominated in both T1D patients and controls, followed by AFR and NAT ancestry; however, no significant differences

Table 2 Significant findings in an exploratory association analysis

CHR	SNP	A1	MAF		OR ^a	95%CI	P value ¹	EMP1
			T1D	CLM				
2	rs798364	A	0.18	0.27	0.62	0.40-0.96	0.034	0.035
2	rs1606237	T	0.33	0.26	1.55	1.04-2.30	0.031	0.031
5	rs700164	T	0.56	0.35	2.38	1.62-3.48	5.56 × 10 ⁻⁶	3.0 × 10 ⁻⁶
6	rs9378428	C	0.38	0.48	0.61	0.42-0.89	0.010	0.018
6	rs2523747	G	0.42	0.30	1.68	1.13-2.51	0.010	0.012
6	rs2395656	G	0.23	0.28	0.63	0.41-0.98	0.040	0.041
10	rs3123687	G	0.15	0.86	0.04	0.02-0.08	8.70 × 10 ⁻¹⁹	1.0 × 10 ⁻⁶

¹Odds ratio and *P* value adjusted for genetic admixture. This table extracts the significant findings shown in [Supplementary Table S2](#). CHR: Chromosome; A1: Minor allele; MAF: Minor allele frequency; CI: Confidence interval; EMP1: Empirical *P* value obtained by permutation tests; OR: Odds ratio; SNP: Single nucleotide polymorphism; T1D: Type 1 diabetes; CLM: Colombians living in Medellin.

between cases and controls were observed. For the contrary, a study conducted in ten Brazilian cities showed that T1D patients presented a higher percentage of European component than the healthy population^[9]. Similarly, a study by Diaz-Horta *et al*^[11] found a higher proportion of European component in cases than in controls. Even more, they found a risk association with the European ancestry.

Further analysis disaggregating the candidate loci tested led us to find a different ancestry composition for *MHC* AIMS. Lower NAT ancestry was observed in T1AD compared to T1BD patients (Table 3). Ancestry variation at the HLA region has been reported for Latin American populations. However, such variation has shown an excess of the AFR component in these populations, including CLM^[16,33,34]. It has been suggested that the excess of the AFR component in the HLA region in Latin America is due to a positive selection orchestrated by the presence of infectious agents during the process of the conquest. The European conquerors brought to America, African and European diseases such as smallpox, measles, and influenza, which caused massive epidemics and were responsible for the extinction of many native populations^[34]. Given this historical background, these AFR fragments could obtain a selective advantage, since the AFR populations have the most diverse repertoire in HLA^[35,36]. However, the ancestry variation observed here shows that the European component is higher in autoimmune (T1AD) than T1BD, in combination with lower NAT in T1AD than T1BD (Table 3).

Another gene with remarkable findings is *IFIH1*. This observation is of particular interest to our population, since we had found in the past that SNP *rs10930046*, which is located at *IFIH1*, associates with T1D in our population^[37]. This SNP has been reported as a rare variant in European populations (MAF = 0.02) related to Psoriasis^[38]. Interestingly, we found in our previous study that this variant MAF = 0.3^[37]. Therefore, such an allele frequency difference could have been speculatively explained by random genetic drift, involving over-representation of European chromosomes with such variants at the time of conquering Colombia. However, in the present study, evidence suggests that this allele frequency difference between populations might be a NAT contribution.

It is worth mentioning that *IFIH1* AIMS presented wide values for the AFR component comparing autoimmune to idiopathic patients (14.99 *vs* 0.01, Table 3), without reaching statistical significance. This was the case since the interquartile range overlapped between these two autoimmune categories. Neither gene *CTLA4* nor *RNASEH1* AIMS revealed significant contributions to T1D, either looking to the overall set of AIMS or in any of the loci/genes analyzed. Regarding *CTLA4*, this observation makes sense when related to our previous finding of no association of this gene variant with T1D^[37]. However, a different situation holds for the *RNASEH1* gene.

RNASEH1 gene variants have thus far been associated with T1D only in the northwest Colombia population and not elsewhere in the world^[4]. It has not even been reported in GWA studies using large sample sizes, albeit mostly of European origin^[3]. Analyzing a larger sample size of T1D patients from this region in Colombia will allow us to conclude whether there really is an ancestry effect related to *RNASEH1* gene variants in T1D.

Unexpectedly, we found that ancestry for chromosomes 5 and 10 were sharply different between T1D patients and the CLM population (Tables 1 and 3). The former

Table 3 Genetic ancestry for type 1 diabetes patients stratified according to autoimmunity

Chromosomal region	Ancestry	T1AD, median (IQR)	T1BD, median (IQR)	P value ¹
Overall AIMs	EUR	61.92 (53.57-70.89)	59.79 (49.70-68.03)	0.333
	NAT	27.07 (20.82-33.48)	29.16 (21.57-37.06)	0.312
	AFR	10.19 (2.88-16.01)	11.54 (5.55-17.80)	0.344
Chr2_EFR3B	EUR	60.28 (36.24-83.66)	59.79 (32.34-80.01)	0.551
	NAT	25.16 (0.01-51.24)	21.92 (0.01-45.95)	0.979
	AFR	4.53 (0.01-23.32)	14.41 (0.01-27.36)	0.119
Chr2_CTLA4	EUR	53.34 (16.82-70.53)	41.16 (16.62-60.86)	0.231
	NAT	26.28 (4.55-41.31)	32.69 (9.35-50.25)	0.343
	AFR	20.54 (0.01-41.73)	19.47 (0.01-42.52)	0.764
Chr2_RNASEH1	EUR	58.96 (37.72-73.87)	47.50 (26.38-78.10)	0.506
	NAT	26.92 (14.19-45.66)	31.59 (0.01-51.98)	0.885
	AFR	6.85 (0.01-28.24)	11.71 (0.01-27.86)	0.657
Chr2_IFIH1	EUR	41.90 (0.01-76.72)	42.42 (0.01-67.93)	0.708
	NAT	32.56 (0.01-55.56)	27.39 (2.01-56.89)	0.985
	AFR	14.99 (0.01-42.31)	0.01 (0.01-36.48)	0.654
Chr5_IL7R	EUR	13.27 (1.02-43.73)	9.19 (1.77-33.67)	0.555
	NAT	50.94 (38.08-98.60)	53.12 (31.82-95.13)	0.984
	AFR	14.68 (3.17-33.94)	17.98 (2.75-41.01)	0.634
Chr6_MHC	EUR	52.31 (37.26-72.43)	45.73 (19.25-67.03)	0.087
	NAT	20.32 (7.12-37.06)	31.88 (17.65-44.62)	0.019
	AFR	18.50 (3.21-36.04)	21.13 (0.53-36.56)	0.905
Chr10_NRP1	EUR	63.32 (28.61-69.09)	63.31 (6.89-63.32)	0.092
	NAT	36.67 (7.93-36.67)	36.67 (7.93-59.79)	0.282
	AFR	0.25 (1e-05-26.0)	0.52 (1e-05-37.80)	0.848

¹Mann-Whitney *U* test. AIMs: Ancestry informative markers; IQR: Interquartile range; Chr: Chromosome; EUR: European; NAT: Native American; AFR: African; T1AD: Autoimmune type 1 diabetes; T1BD: Idiopathic type 1 diabetes.

involves chromosomal region 5p13.2 (*IL7R*)^[39]. This region was assessed with only one AIM, which clearly discriminates between NAT and non-NAT (Supplementary Table S1). As shown in Table 1, the T1D ancestry observed for this locus is confidently greater for NAT, at the expense of the two other ancestries. It is also apparent that AFR ancestry at this locus contributes to late onset of the disease (Table 4). Such results, in turn, should be taken with caution since this AIM does not clearly discriminate between EUR and AFR (Supplementary Table S1). Therefore, we cannot rule out the possibility that this effect is of European origin.

The second striking finding involves chromosomal region 10p11.22 (gene *NRP1*)^[40]. Although the opposite ancestry contributions between T1D and CLM are evident (Table 1), it is worth keeping in mind that the only AIM (*rs3123687*) used for this locus is highly informative for AFR and non-AFR ancestries (*i.e.*, either EUR or NAT). Given this information, we are aware that the conclusion regarding greater NAT contribution in our study could eventually go towards greater EUR ancestry. Therefore we can only tell that the difference observed is non-AFR, but are not able to define whether it is European or NAT.

The actual SNPs reported as associated with disease in these two genes (*IL7R* and *NRP1*) have not yet been tested in the sample presented here. However, a test of association using the AIMs analyzed here, after adjusting for the admixture effect, revealed that AIM *rs700164* associates with affected status (5.56×10^{-6} , Supplementary Table S2 and Table 2) and that similarly *rs3123687* strongly associates with the disease ($P = 8.07 \times 10^{-19}$, Supplementary Table S2 and Table 2) for *IL7R* and *NRP1* genes, respectively. A verification of this finding should be performed using the transmission disequilibrium test (TDT). The TDT is not susceptible to population structure issues, such as admixture. This analysis is to be done for the actual SNPs, as the parents for the patients presented here are available. Such association analyses should include choosing gene variants from the genetic variability in this set of patients, and should also consider the LD blocks observed in this population.

No ancestry differences were found overall when comparing T1AD to idiopathic (T1BD) (Table 3). T1AD, whose etiology and pathology are better characterized, has a

Table 4 Genetic ancestry for type 1 diabetes patients stratified according to age at onset

Chromosomal region	Ancestry	Early age at onset, ≤ 5 yr	Late age at onset, > 5 yr	P value ¹
Overall AIMs	EUR	62.06 (54.50-71.38)	61.12 (51.66-68.68)	0.420
	NAT	25.40 (18.14-33.55)	27.75 (21.86-34.35)	0.345
	AFR	11.09 (3.90-17.25)	10.19 (3.88-16.91)	0.927
Chr2_EFR3B	EUR	57.65 (34.05-82.17)	62.45 (42.04-80.67)	0.419
	NAT	25.16 (1.27-52.11)	24.30 (0.01-51.73)	0.607
	AFR	5.02 (0.01-27.29)	7.37 (0.01-23.32)	0.941
Chr2_CTLA4	EUR	58.49 (24.63-78.80)	46.09 (12.90-66.30)	0.180
	NAT	26.28 (3.98-39.82)	30.19 (4.55-42.10)	0.362
	AFR	18.05 (0.01-37.80)	20.68 (0.01-41.72)	0.701
Chr2_RNASEH1	EUR	56.84 (44.50-72.18)	56.91 (28.02-74.15)	0.672
	NAT	27.22 (0.01-31.22)	28.03 (8.15-48.58)	0.472
	AFR	3.38 (0.01-31.22)	8.62 (0.01-27.20)	0.917
Chr2_IFIH1	EUR	41.35 (0.01-66.94)	42.42 (4.44-76.73)	0.126
	NAT	33.05 (0.67-57.49)	29.05 (0.01-53.09)	0.339
	AFR	14.57 (0.01-42.31)	1.01 (0.01-42.31)	0.796
Chr5_IL7R	EUR	9.38 (0.33-41.70)	12.86 (1.73-43.11)	0.338
	NAT	53.83 (39.76-99.60)	50.94 (30.21-88.75)	0.197
	AFR	6.17 (1.01-29.03)	19.96 (9.6-37.36)	0.023
Chr6_MHC	EUR	51.67 (32.23-63.29)	52.07 (34.26-71.84)	0.596
	NAT	30.57 (13.47-43.21)	26.71 (7.69-37.39)	0.480
	AFR	15.03 (4.46-36.05)	20.52 (1.08-36.73)	0.118
Chr10_NRP1	EUR	63.32 (23.70-63)	63.32(43.07-69.08)	0.357
	NAT	36.68 (7.93-36.67)	36.67 (7.93-36.68)	0.797
	AFR	0.16 (0.001-39.1)	0.38 (0.001-63.3)	0.498

¹Mann-Whitney *U* test. AIMs: Ancestry informative markers; IQR: Interquartile Range; Chr2: Chromosome 2; Chr6: Chromosome 6; EUR: European; NAT: Native American; AFR: African.

higher incidence in Europe^[6]; on the contrary, T1BD is reported mainly in AFR and Asian countries^[26]. Our results are different from those by Piñero-Piloña *et al*^[41], who reported a high incidence of T1BD in Mexican patients, whose predominant ancestral component was NAT. Our cohort presents a majority of autoimmune cases (78%) and, as described here, their predominant ancestry is of European contribution.

However, looking at chromosomal regions along the analysis stratified by age at onset of T1D, we found that patients with a late onset of the disease have a greater AFR component, which was more marked on chromosome 5 (Table 4). This suggests that AFR ancestry could be a risk factor for developing the disease at a late age in our population (over 2/3 of the sample had age at onset > 5 years), which can modify the metabolic phenotype of patients, and influence the risk of late complications of diabetes^[42].

Our study has an important limitation regarding the number and location of the AIMs. Thus, chromosomes 5 and 10 were tested with just a few such markers. It will be worth testing more AIMs nearby these two loci to further examine the differences revealed. Also, the reference population we used (CLM from the 1,000 genome database), although supposedly unaffected and older than our patients, were typed by a different method from the one we used to type our T1D cases. Nonetheless, both groups share comparable genetic ancestries.

Our study's strength is its population choice. As described, the northwest Colombia population is the one with a greater European component in the country^[15-19]. Thus, our results make much more sense regarding the overall European contribution, together with the apparent unexplored NAT input to T1D, in addition to certain contributions of the AFR ancestry for late age at onset.

In conclusion, this study describes the ancestral genetic composition of 200 T1D patients from an admixed population from northwest Colombia. Consistently, we found a predominant proportion of European followed by NAT ancestry. No statistical difference was observed in the distribution of the proportions of ancestral genetic components between T1D patients and the CLM reference population. A variation in chromosomal segments derived from the parental populations was

observed when comparing individuals with T1AD *versus* T1BD, and those who had an early (≤ 5 years) or late (> 5 years) age at onset of the disease. These results demonstrate that the study of the genetic admixture provides new perspectives in the delineation of the genetic architecture underlying autoimmune diseases. Finally, performing a novel study in this sample, including unbiased distribution of AIMs through the whole genome, could help find undetected loci in previous studies, which would contribute to complete the T1D genetic architecture for our population. This will also contribute to making approaches, such as the polygenic risk score, become more accurate for these types of populations.

ARTICLE HIGHLIGHTS

Research background

Type 1 diabetes (T1D) is described as a disease predominantly in white populations. Subtypes of the disease are also more frequent in different ethnicities. Thus, the autoimmune form of the disease is observed more frequently in Caucasian countries, whilst the idiopathic form is more frequently observed in African and Asian countries. The patients included in this study are from Northwest Colombia. This is an admixed population originated by a three ethnic contribution. This population has been described as the most European in the country, followed by the Native American ancestry, and with its least significant component being African contribution.

Research motivation

In this study, we looked at the genetic ancestry of a set of 200 diseased subjects from Northwest Colombia. We were interested in describing whether their global ancestry, as well as some specific genomic regions, were of which particular ancestry. Only a few of these types of studies have been reported in Latin American populations, and none have occurred in Colombia.

Research objectives

We aimed at describing the ancestry composition of a cohort of Colombian patients with T1D. This description included both global analysis as well as specific tests on loci/genes previously related to the disease.

Research methods

We studied 200 diseased subjects from Northwest Colombia. We tested 75 admixture informative markers (AIMs) distributed through a set of previously reported genes (or chromosomal regions) associated with T1D. The disease was classified as either autoimmune or idiopathic in the study subjects. This was done by testing two disease-related auto-antibodies (AABs). If at least one such AAB was present, then the disease was classified as autoimmune. We also classified the age at onset of the disease as early (≤ 5 years) or late (> 5 years). The reference population of Colombians living in Medellin (CLM) was compared to the set of patients presented here. We applied appropriate statistical tests given the non-normality of the data obtained.

Research results

Seventy eight percent of the patients presented at least one AAB. Over two thirds (69.5%) of the subjects developed the disease after 5-years-old. There were no significant differences between genders among the affected individuals. Seventy four AIMs were successfully tested (one failed the PCR optimization). It was observed that both the diseased and CLM groups were predominantly of European ancestry (61.58 *vs* 62.06), followed by Native American (24.30 *vs* 37.10) and African ancestries (10.28 *vs* 10.65). In addition, specific genes such as *EFR3B*, *IFIH1*, *IL7R* and *NRP1* displayed differential Native American or African rather than European contributions. In addition, we found that autoimmune patients displayed lower Native American ancestry than idiopathic cases.

Research conclusions

Our study shows that diseased individuals from Northwest Colombia are predominantly of European ancestry, followed by native American and African ancestries. Also, other European contributions were found for specific genes in our study.

Research perspectives

MHC is expected to play the strongest role in T1D susceptibility. However, this was not the observation in our study. Our results suggest that different loci effect sizes might be at play in our admix population. This is inferred from the observation of the significance strength observed for MHC ancestry compared to other loci. Therefore, it would be worth testing AIMs in this sample (expanded with extra individuals from the same region in Colombia) throughout the whole genome. This way, it would be feasible to reveal differences in local ancestry either for known or unknown loci associated with T1D in our population. This would help complete the genetic architecture of the disease, particularly for our population. In turn, this would contribute to the knowledge of the disease biology, and would also make this sample population appropriate for applying approaches such as the polygenic risk score.

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**ORIGINAL ARTICLE****Basic Study**

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Basic Study

Influence of 10-(6-plastoquinonyl) decyltriphenylphosphonium on free-radical homeostasis in the heart and blood serum of rats with streptozotocin-induced hyperglycemia

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Abstract

BACKGROUND

It is known that under conditions of tissue tolerance to insulin, observed during type 2 diabetes mellitus (DM2), there is an increased production of reactive oxygen species. Moreover, the free radicals can initiate lipid peroxidation (LPO) in lipoprotein particles. The concentration of LPO products can influence the state of insulin receptors, repressing their hormone connection activity, which is expressed as a reduction of the glucose consumption by cells. It is possible that reduction in glucose concentration during administration of 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) to rats with DM2 may be related to the antioxidant properties of this substance.

AIM

To establish the influence of SkQ1 on free-radical homeostasis in the heart and blood serum of rats with streptozotocin-induced hyperglycemia.

METHODS

To induce hyperglycemia, rats were fed a high-fat diet for 1 mo and then administered two intra-abdominal injections of streptozotocin with a 7-d interval at a 30 mg/kg of animal weight dose with citrate buffer equal to pH 4.4. SkQ1 solution was administered intraperitoneally at a 1250 nmol/kg dose per day. Tissue samples were taken from control animals, animals with experimental hyperglycemia, rats with streptozotocin-induced glycemia that were administered SkQ1 solution, animals housed under standard vivarium conditions that were administered SkQ1, rats that were administered intraperitoneally citrate buffer equal to pH 4.4 once a week during 2 wk after 1-mo high-fat diet, and animals that were administered intraperitoneally with appropriate amount of solution without SkQ1 (98% ethanol diluted eight times with normal saline solution). To determine the intensity of free radical oxidation and total

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antioxidant activity, we used the biochemiluminescence method. Aconitate hydratase (AH), superoxide dismutase, and catalase activities were estimated using the Hitachi U-1900 spectrophotometer supplied with software. The amount of citrate was determined by means of the Natelson method. Real-time polymerase chain reaction was carried out using an amplifier ANK-32.

RESULTS

It was found that the mitochondrial-directed antioxidant elicits decrease of biochemiluminescence parameter values that increase by pathology as well as the levels of primary products of LPO, such as diene conjugates and carbonyl compounds, which indicate intensity of free radical oxidation. At the same time, the activity of AH, considered a crucial target of free radicals, which decreased during experimental hyperglycemia, increased. Apparently, increasing activity of AH influenced the speed of citrate utilization, whose concentration decreased after administering SkQ1 by pathology. Moreover, the previously applied antioxidant during hyperglycemia influenced the rate of antioxidant system mobilization. Thus, superoxide dismutase and catalase activity, as well as the level of gene transcript under influence of SkQ1 at pathology, were changing to the direction of control groups values.

CONCLUSION

According to the results of performed research, SkQ1 can be considered a promising addition to be included in antioxidant therapy of DM2.

Key words: Diabetes mellitus; Free radical oxidation; Antioxidants; 10-(6-plastoquinonyl) decyltriphenylphosphonium

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Core tip: The results of this research suggest that the mitochondria targeted antioxidant 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) might be a potential substance for incorporation into the antioxidant therapy of type 2 diabetes mellitus. The ability of this compound to lower the intensity of free-radical processes, acting as the key component of the pathogenesis of the type 2 diabetes mellitus, serves as the basis for this conclusion. Thus, after the introduction of SkQ1 to the animals with streptozotocin induced hyperglycemia, the values of the biochemiluminescence parameters reflecting the free-radical oxidation intensity, the concentration of diene conjugates and carbonyl products of protein oxidation, aconitate hydratase activity, and citrate content approached those of control values. At the same time, the activity level of the antioxidant enzymes superoxide dismutase and catalase approached those of normal values.

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INTRODUCTION

Type 2 diabetes mellitus (DM2) is a pandemic metabolic disease and is becoming a serious problem for health protection due to the global increase of its prevalence. Endocrinopathic complications, such as nephropathy, retinopathy, brain angiopathy, myocardial angiopathy, and lower limbs angiopathy, prove to be the major reason for incapacity, disability, or patient mortality. It is known that diabetic cardiomyopathy develops in patients with insulin resistance and DM regardless of diagnosed coronary heart disease or hypertension^[1].

The crucial target is vascular endothelium, which is affected by a number of metabolic, hemodynamic, and immunologic factors that characterize the development of the disease^[2]. It has been shown in DM2 that the delivery of fatty acids to the myocardium is intensified and glycolysis is slowed down^[3]. At the same time, the

concentration of reactive oxygen species (ROS) have a damaging effect on the lipids of cardiomyocyte membranes and contribute to the mitochondrion mechanism malfunction and, as a result, to the inhibition of ATP elaboration. This leads to calcium imbalance in cardiomyocytes and results in muscular relaxation and contraction. Hyperglycemia as well contributes to the development and progression of fibrotic degeneration of cardiomyocytes due to the increased deposition of collagen in the interstitium^[4].

Taking into account the most important factors of free radical oxidation activation in the pathogenesis of DM, as well as in the development of its complications, antioxidant therapy should be considered among the modern medical endocrinopathy treatment technologies. In this context, there is an urgent need for effective substances that would protect cellular structures from oxidative stress.

Ten-(6'-plastoquinonyl) decyltriphenylphosphonium (SkQ1) is an aromatic cation (triphenylphosphonium) conjugated with a 10-12 atom aliphatic compound as well as plastoquinone, which is an active molecular component of this substance^[5].

Several Sk-compounds with modified lipophilic and antioxidant parts were synthesized and tested by varying the length of the aliphatic linker. All these compounds have abbreviated names derived from the surname of Russian academician Skulachev VP (Sk), the letter to designate ubiquinone (Q), and the letter or numerical symbol to designate the modification.

When used in nanoconcentrations, this substance participates in the ROS balance regulation as it has the ability to neutralize free radicals (FR), including OH radicals in aqueous solutions. This may protect cells from apoptosis and necrosis induced by ROS^[6].

The goal of this study was to evaluate the influence of SkQ1 on biochemiluminescence (BCL) parameters, which reflect the free radical processes reactions rate, the total activity of the antioxidant system (AOS), the level of primary lipid peroxidation (LPO) products such as diene conjugates (DC), the activity of aconitase, which is the sensitive target of FR action and citrate content, the degree of protein oxidative modification, the activity of superoxide dismutase (SOD) and catalase, and the level of their genes' transcripts in heart and blood serum of the rats with hyperglycemia induced by the administration of streptozotocin (STZ).

MATERIALS AND METHODS

Experimental animals

To conduct the study, laboratory rats were selected of nursery rat males from Federal State-financed Organization of Health Service "Voronezh Hygiene and Epidemiology Center". The animals were divided through stratified randomization by their weight and age. The rats used for the study weighed 200-250 g and aged 3-5 mo. The experimental animals were kept for 14 d before the start of the study under the following conditions: 12-h light day, temperature 21-25 °C, and food *ad libitum*. The experiment was carried out in accordance with European legislation on the protection of animals (Directive 2010/63/EC).

Hyperglycemia induction in animals

Hyperglycemia was induced by feeding rats a high-fat diet for 1 mo, followed by two intra-abdominal injections of STZ with a 7-d interval at a 30 mg/kg of animal weight dose with citrate buffer equal to pH 4.4^[7].

Hyperglycemia in rats was verified by measuring glucose level in the blood serum using a glucose oxidase test. The blood was collected twice with a day-interval from caudal vein after the second administration of STZ and 1-d food deprivation. The reagent kit used for the study was purchased from Vital Diagnosticum, Saint-Petersburg, Russia.

At 2 wk after STZ administration, drugged animals were devitalized for further research. The laboratory rats were divided into four groups: Group 1 ($n = 20$), animals housed under standard vivarium conditions (control group); group 2 ($n = 20$), animals with STZ injection-induced hyperglycemia; group 3 ($n = 12$), animals with STZ-induced glycemia that were administered SkQ1 solution intraperitoneally at a 1250 nmol/kg dose per day, starting from the second week; and group 4 ($n = 8$), animals housed under standard vivarium conditions that were administered with SkQ1 at a 1250 nmol/kg dose per day, during the second week of conducting the experiment. Group 2 also included rats ($n = 8$) that were administered intraperitoneally with appropriate aliquot quantity of citrate buffer equal to pH 4.4 once a week during 2 wk after 1-mo high-fat diet. Group 3 included animals ($n = 8$) that were administered intraperitoneally with appropriate amount of solution without SkQ1 (98% ethanol

diluted eight times with normal saline solution).

Preparation of materials for the study

To obtain tissue homogenate, heart tissue sample was homogenized in triple amount of cooled medium (0.1 mol/L Tris-HCl buffer (pH 7.8) containing 1 mmol/L EDTA, 1% beta-mercaptoethanol) and centrifuged for 10000 g for 15 min. The serum was obtained from venous blood collected in test tubes without anticoagulant. For this purpose, the blood was thermostated at a temperature of 37 °C until phase immiscibility, and supernatant fluid was centrifuged for 4000 g for 10 min. The obtained serum was used for further examination.

Evaluation methods of the intensity of free radical oxidation

The free-radical oxidation and total antioxidant status processes intensity was measured by applying the Fe²⁺-induced biochemical luminescence method. The principle of the method is based on catalytic degradation of peroxide by transition valence metal ions (Fe²⁺) in accordance with the Fenton reaction. Catalytic degradation leads to the formation of FR that enter free-radical oxidation initiation process in the examined biotic substrate. Recombination of RO₂ radicals results in unstable tetroxide formation that causes liberation of light quantum when it degrades. Biochemoluminescence kinetic curve was recorded for 30 seconds using BCL-07 with software (Medozons OOO, Nizhny Novgorod, Russia), and the following parameters were measured: Chemicoluminescence light sum (S) and flash intensity (I_{max}) that characterized free-radical oxidation intensity and slope of curve magnitude (tgα₂) depicting total antioxidant status.

The medium for estimating BCL/L contained 0.4 mL of 0.02 mol/L potassium phosphate buffer (pH 7.5); 0.4 mL of 0.01 mol/L FeSO₄, and 0.2 mL of 2% H₂O₂ solution that had been added immediately prior to measurement. The test material had been added in an amount of 0.1 mL prior to measurement.

The amount of DC was measured by means of spectrophotometric method at 233 nm^[32].

The oxidative modification of proteins valuation method is based on the interaction between oxidized amino acid residues and 2,4-dinitrophenylhydrazine (2,4-DNPH) that forms 2,4-DNPHs^[10]. Protein load was identified by the biuret test.

The concentration of DC was analyzed spectrophotometrically at 233 nm^[8].

For the analysis of protein oxidative modification (POM), we used the method based on the interaction between oxidized amino acid residues and 2,4-DNPH using the Hitachi U-1900 spectrophotometer (Hitachi High-Technologies, Tokyo, Japan)^[9].

Enzyme analysis

Aconitate hydratase (AH) activity was estimated using the Hitachi U-1900 spectrophotometer supplied with software at 233 nm in the medium that contained 0.05 mmol/L of Tris-HCl-buffer (pH 7.8) and 4 mmol/L of sodium citrate (PanReac, Barcelona, Spain)^[10].

The amount of citrate was determined by means of the Natelson method^[11].

SOD activity was determined by the nitroblue tetrazolium recovery rate inhibition in the non-enzymatic system of phenazine methosulfate (PMS) and NADH.

The incubation medium, with a total volume of 3 mL, contained 0.1 M phosphate buffer (pH 7.8), 0.33 mmol/L EDTA, 0.41 mmol/L NBT, 0.01 mmol/L PMS, and 0.8 mmol/L NADH. The activity was measured spectrophotometrically according to the extinction augmentation after 5 min using the Hitachi U-1900 spectrophotometer at 540 nm^[12].

Catalase activity was determined at a wavelength of 410 nm using the method based on the ability of hydrogen peroxide to form stable colored complex with ammonium molybdate^[13].

The amount of the enzyme that was required for the conversion of 1 mM of substrate per min at 25 °C was defined as the enzyme unit (E). Biuret method was used to determine the protein content.

Total RNA extraction

Total RNA was isolated from heart tissues and blood cells of experimental animals using Extran RNA reagent kit (Syntol Company, Moscow, Russia). The severity of RNA degradation was determined *via* 1% denaturing agarose gel electrophoresis. The amount of RNA was determined by measuring the absorption at a wavelength of 260 nm using a Hitachi U-1900 spectrophotometer.

A process of reverse transcription

M-MuLV Reverse Transcriptase (Fermentas, Vilnius, Lithuania) was used to carry out reverse transcription. DNA, or complementary mRNA, was obtained *via* Oligo(dT) 18

Primer. The reaction was carried out at 40 °C for 1 h with the following inactivation of reverse transcriptase at 70 °C per 15 min. Ready-to-use cDNA was used for real-time amplification.

A real-time PCR amplification

The primers selected *via* a database of a web-based system Universal Probe Library (Universal Probe Library Assay Design Center) were applied to amplify the region of a gene.

PCR amplification was based on reagent kits containing the SYBR Green I (Syntol Company). Real-time PCR was carried out using an amplifier ANK-32 (Syntol Company) according to the following pattern: Per 5 min at 95 °C, then 40 cycles: 95 °C 15 s, 60 °C 15 s, 72 °C 30 s. The next step was to analyze the threshold cycle value obtained by PCR amplification. Among other scientific experiments, there was also a negative control for: (1) An impurity of foreign DNA set components; and (2) A purity level of sample preparation for the amplification. Negative control consisted of a separate test tube at each process of amplification with an equal amount of water instead of DNA test sample.

Reagents used in the research

During the study, the following chemicals were applied: STZ, Tris-HCl, citrate, nitroblue tetrazolium, PMS, NADH (Sigma, St Louis, MO, United States), SkQ1 synthesized according to the following method^[5], EDTA (Reanal, Budapest, Hungary), and other chemicals of “chemically pure” or “analytically pure” grade purchased from Russian manufacturers.

Outcome analysis

Experiments were done in at least 8-20 biological and two analytical replicates. The results were compared with the control. The data were statistically analyzed using a software package STATISTICA 6.0 with numerical variables – arithmetical mean (M), mean error (m), and statistical significance level (*P*). Normal distribution data were compared by applying Student's *t*-test for Bonferroni correction in independent samples^[14]. Significance level was set at ^a*P* ≤ 0.0167 and ^b*P* ≤ 0.0167.

RESULTS

The study showed that SkQ1 administration lowered glycemic level by 2.5 times, which was initially upregulated 2.7 times relative to the control (Table 1). The glucose level of the animals of the fourth experimental group was not significantly different from the stated value. Moreover, glucose concentration was within the stated value in rats of the second experimental group having been administered citrate buffer and of the third group having been administered an aliquot of 12% ethanol.

It is also established that S of BCL value increased by 2.2 times in rats' hearts under pathological conditions and in blood serum by 2.1 times. *I*_{max} BCL increased by 2.5 and 2.0, respectively, compared to the control group, demonstrating increased intensity of free radical oxidation. It is known that the processes of glucose autooxidation and its metabolic intermediates, glycosylation of protein and end-product accumulation of its modification, sorbitol exchange mobilization, glucose utilization *via* hexosamine pathway, and protein kinase C (PKC) activation may be free radical oxidation sources in DM2^[15].

The values of BCL tgα₂ increased in rats' hearts with pathology by 2.1 times and in blood serum by 2.3 times (Table 2), indicating compensatory mechanisms implementation and general antioxidant potential organism mobilization during the development of experimental hyperglycemia.

The administration of SkQ1 led to the S of BCL values decrease in rats' hearts by 1.3 times and in blood serum by 1.6 times and *I*_{max} BCL by 1.5 and 1.6, respectively (Table 2). BCL tgα₂ parameter decreased in rats' hearts by 1.5 times and in blood serum by 1.4 times (Table 2).

No statistically significant BCL parameters changes in rats of the fourth experimental group and the animals belonging to the second experimental group that have been administered citrate buffer and to the third group that have been administered aliquot of 12% ethanol compared with control have been observed (Table 2).

The data received as a result of a research conducted upon the mitochondrial-directed antioxidant and its effect on biochemical luminescence parameters correspond with the results of the assessment of DC level and POM products. It has been shown that the level of DC in the heart and blood serum of animals of the third group has declined 1.4 and 1.7 times, correspondingly, in comparison with the

Table 1 Glucose concentration in rats' blood

	Glucose concentration in rats' blood, mmol/L	
	The 9 th d after STZ administration	The 11 th d after STZ administration
Control group	5.09 ± 0.081	5.41 ± 0.088
Animals with experimental hyperglycemia induced by streptozotocin	10.01 ± 0.166 ^a	14.6 ± 0.241 ^a
SkQ1 is administered to animals with pathology	6.04 ± 0.099 ^b	5.87 ± 0.096 ^b
SkQ1 is administered to animals at control group	5.1 ± 0.083	5.30 ± 0.086

^a*P* ≤ 0.0167 compared with control;^b*P* ≤ 0.0167 compared with pathology. SkQ1: 10-(6-plastoquinonyl) decyltriphenylphosphonium; STZ: Streptozotocin.

pathology (Table 2). It was found that the concentration of primary products of LPO in the heart and blood serum of rats from the second group increased 2.4 and 3.3 times, correspondingly (Table 2). The progression of hyperglycemia with animals injected with STZ went along with the increase in POM products content: 2.7 times in the heart and 6.3 times in blood serum in comparison with the control group. After the injection of rats with pathology with SkQ1 carbonyl compounds, the level decreased 1.5 times in the heart and 2.0 times in blood serum in contrast with the pathology (Table 2).

DC and carbonyl compounds content in the heart and blood serum of rats under the standard mode of the vivarium and injected with SkQ1 and of animals from the second experimental group injected with citrate buffer and the third group that were administered with aliquot of ethanol with mass fraction of 12% did not change significantly in comparison with the control level (Table 2).

It is known that FR may have aconitase as a target of action. Decline in activity of aconitase is interrelated with the accumulation of citrate that, in turn, is an effective low molecular weight antioxidant due to its chelating properties in relation to ions Fe²⁺[16].

It has been established that in case of hyperglycemia, specific activity of aconitase declines 1.9 times in the heart and 2.3 times in blood serum of rats in comparison with the control level (Figure 1). The slowdown in activity of aconitase within the pathology is clearly connected with intensification of ROS formation and the induction in the development of oxidative stress. It was found that FR participate directly in the oxidation and inactivation of the aconitase iron-sulfur cluster. The long-term exposure of FR leads to the dismantlement of [4Fe-4S] cluster, carbonylation, and degradation of the enzyme.

As a result of SkQ1 exposure, the specific activity of the given enzyme increased 1.4 times in the heart and 1.8 times in blood serum in comparison with the data received from experimental hyperglycemia (Figure 1). It appears that the administration of protector in the bodies of animals with pathology facilitated a decline in the level of ROS and, as a result, a decrease in the damage degree of the molecule of the enzyme and a change in aconitase activity towards control.

Along with the development of the examined pathology, citrate content increased 2.1 times in the heart and 2.5 times in blood serum (Table 2). Presumably, it was mediated by the inhibition of aconitase. In turn, citrate accumulation could be an adaptive process with the development of oxidative stress at pathology, due to the fact that citric acid chelates iron ions and decreases the Fenton's reaction rate. As a result, the possibility of hydroxyl radical formation declines.

It was found that the injection of SkQ1 leads to the decline of citrate content by 1.5 times in the heart and by 1.3 times in blood serum (Table 2). Presumably, the antioxidant effect provided by SkQ1 was manifested by the decline in the level of oxidative stress and by damage degree of the aconitase molecule. As a result, the activity of the given enzyme increased and led to the increase in citrate utilization rate.

The administration of SkQ1 to the animals of the fourth experimental group did not cause any statistically significant changes in aconitase activity and in heart and blood serum citrate levels in comparison with the control group. The administration of citrate buffer to the animals of the second group and of 12% ethyl solution to the third group did not cause such changes either.

The experiments showed that pathological conditions caused the increase in SOD and catalase specific activities in comparison with the control group of the animals. They were increased by 2.5 times in the heart and by 2.2 and 2.3 times in the blood serum, respectively (Figure 2 and Figure 3 respectively). It was noted that SOD gene

Table 2 Parameters reflecting the level of free radical processes and general antioxidant system activity

Parameters		Experiment conditions			
		Control group	Animals with experimental hyperglycemia induced by streptozotocin	SkQ1 is administered to animals with pathology	SkQ1 is administered to animals at control group
Biochemoluminescence measurements					
Light sum, mV × c	Heart	485.01 ± 7.912	1062.76 ± 17.124 ^a	818.76 ± 13.611 ^b	478.33 ± 7.971
	Blood serum	307.70 ± 5.141	654.14 ± 10.114 ^a	410.53 ± 6.713 ^b	302.33 ± 5.012
Maximum flash intensity, mV	Heart	51.53 ± 0.851	126.93 ± 1.987 ^a	83.10 ± 1.374 ^b	47.33 ± 0.779
	Blood serum	25.20 ± 0.412	52.23 ± 0.864 ^a	33.67 ± 0.559 ^b	22.15 ± 0.362
Angle tangent of slope of kinetic curve of biochemoluminescence pathway	Heart	7.10 ± 0.101	14.94 ± 0.241 ^a	10.10 ± 0.165 ^b	7.05 ± 0.112
	Blood serum	13.33 ± 0.223	30.43 ± 0.498 ^a	21.71 ± 0.359 ^b	12.93 ± 0.183
Content of conjugated dienes, μmol/mL	Heart	17.70 ± 0.281	41.90 ± 0.687 ^a	29.76 ± 0.492 ^b	17.22 ± 0.267
	Blood serum	7.55 ± 0.122	24.83 ± 0.401 ^a	15.00 ± 0.247 ^b	7.33 ± 0.113
Level of carbonyl compounds, nmol/L/mg of protein	Heart	0.072 ± 0.001	0.199 ± 0.003 ^a	0.136 ± 0.002 ^b	0.070 ± 0.001
	Blood serum	0.021 ± 0.0003	0.154 ± 0.002 ^a	0.076 ± 0.001 ^b	0.019 ± 0.0003
Citrate content, nmol/L	Heart	0.201 ± 0.003	0.43 ± 0.007 ^a	0.28 ± 0.004 ^b	0.204 ± 0.003
	Blood serum	0.57 ± 0.0097	1.45 ± 0.021	1.07 ± 0.016 ^b	0.59 ± 0.0093

^a*P* ≤ 0.0167 compared with control;^b*P* ≤ 0.0167 compared with pathology. SkQ1: 10-(6-plastoquinonyl) decyltriphenylphosphonium.

transcript and catalase gene transcript blood cell levels increased by 2.5 and by 1.6 times, respectively, and their heart levels increased by 2.3 and by 4.0 times, respectively (Figure 4). These enzyme activity changes in the case of hyperglycemia can probably be considered as a compensatory reaction to intensifying free-radical processes.

The administration of SkQ1 to the animals caused a decrease in SOD and catalase activities in comparison with pathological conditions. It was found that the impact of SkQ1 leads to the decrease in SOD and catalase heart and blood serum specific activities in comparison with the second group of animals. Thus, SOD heart and blood serum specific activities were decreased by 1.4 times and by 1.6 times, respectively (Figure 2), and catalase heart and blood serum activities were decreased by 1.5 and 1.4 times, respectively (Figure 3).

The identified studied enzyme activity is connected with the decrease in the number of their gene transcripts. Thus, the concentration of SOD1 and CAT gene products decreased by 1.5 and 1.3 times in the blood cells and by 1.4 and 1.3 times in the heart (Figure 4). Apparently, the reduction of free radical formation and, consequently, the reduction of oxidative stress, caused indirectly by SkQ1 antioxidant effect, were accompanied by decreased pressure on AOS, which was reflected in the approximation of SOD and catalase activities to the control values.

In comparison with the first group, the rats of the fourth experimental group, the rats of the second experimental group, to which citrate buffer was administered, and the rats of the third group, to which the aliquot of 12% ethyl alcohol solution was administered, did not have any statistically significant changes in SOD and catalase activities as well as in SOD and CAT gene transcript levels.

DISCUSSION

It is known that the inclusion of high fat content in rat diet contributes to the occurrence of animal tolerance to insulin^[17]. Small dose administration of STZ leads to the moderate decline in insulin production, which is similar to a later stage of DM2^[18]. Thus, the administration of STZ causes a significant basal increase in the blood glucose level. It also causes glucose tolerance, increase in the glycated hemoglobin level, significant reduction of insulin concentration, and resistance.

It should be noted that under conditions of tissue tolerance to insulin, observed

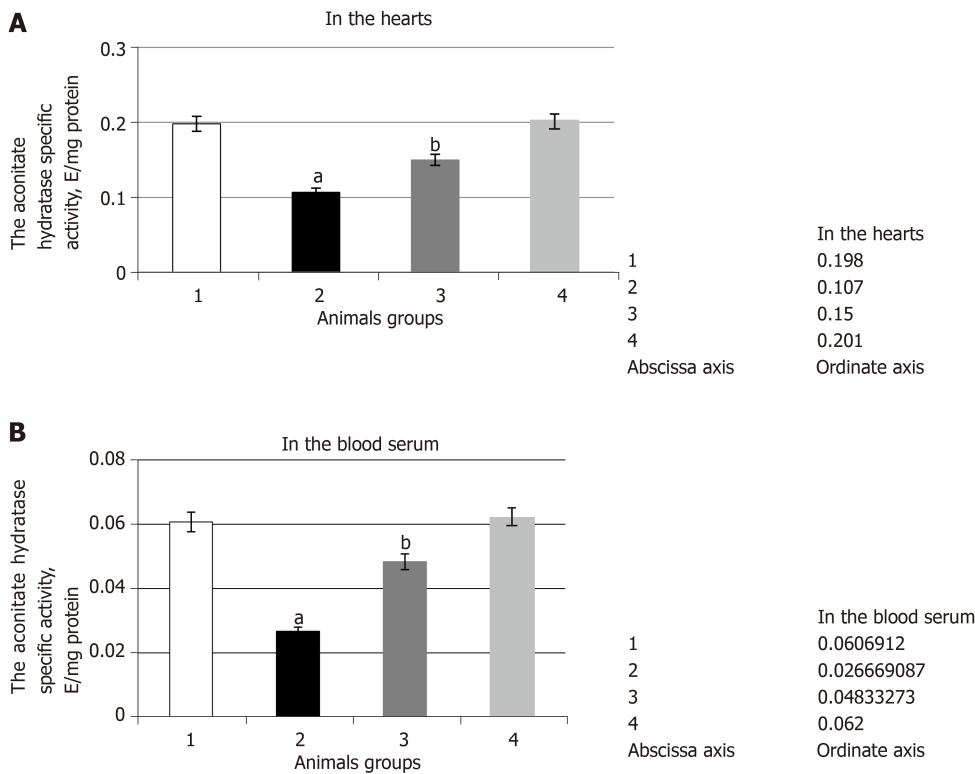


Figure 1 The aconitate hydratase specific activity, E/mg protein in the rats' hearts and the blood serum during the development of experimental hyperglycemia. A: The aconitate hydratase specific activity, E/mg protein in the rats' hearts during the development of experimental hyperglycemia; B: The aconitate hydratase specific activity, E/mg protein in the blood serum during the development of experimental hyperglycemia. 1: Control group; 2: Animals with experimental hyperglycemia induced by streptozotocin; 3: 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) is administered to animals at pathology; 4: SkQ1 is administered to animals at control group. ^a $P \leq 0.0167$ compared with control; ^b $P \leq 0.0167$ compared with pathology. SkQ1: 10-(6-plastoquinonyl) decyltriphenylphosphonium.

during DM2, there is an increase in superoxide production by mitochondria^[19], cytochrome P450, xanthine oxidase, and PKC-dependent NADPH oxidase activation. Moreover, the FR, which are generated during glucose autooxidation or glycosylation end products, can initiate LPO in lipoprotein particles^[20]. The concentration of LPO products through an increase in hydrophilic hydrocarbon tails contents, in turn, can lead to formation of membrane pores and membrane stiffening through downregulation of unsaturated fatty acids, and thus it can influence state of insulin receptors, repressing their hormone connection activity, which is expressed as a reduction of the glucose consumption by cells^[21]. It is possible that reduction in glucose concentration during administration of SkQ1 to rats with DM2 may be related to the realization of antioxidant properties of this substance^[22].

It is well known that during several pathological states, including DM, LPO activation occurs and can lead to a number of defects, structural changes in membranes, and cell metabolic disturbance in particular. At the same time, ROS, including LPO products, act as the main POM inducers^[23].

The use of SkQ1 may contribute to an inhibition of accumulation of LPO molecular products and a normalization of the structural condition of lymphocytes' membranes and their apoptosis level during oxidative stress^[24].

Reviewing past literature, it is well known that through administration of SkQ1, the rate of development of such diseases as cataract, retinopathy, glaucoma, osteoporosis, hypothermia, and renal ischemia decreases^[25]. Furthermore, in animal experiments, SkQ1 efficiency of correction of the several neurodegenerative states was shown^[26]. The effect of SkQ1 led to acceleration of the end of the inflammatory phase, the formation of granulation tissue, vascularization, and epithelization of a wounded area^[27]. Moreover, it was shown that in OXYS rats of an experimental model of accelerated aging, which is a famous animal model of human age-related macular degeneration, SkQ1 reduces clinical features of retinopathy^[28].

According to the results obtained, it was found that administration of the antioxidant compound is accompanied by reduction in concentration of primary LPO products in both blood serum and heart closer to its control values.

During the experiment, it was found that an increase in activity of SOD and

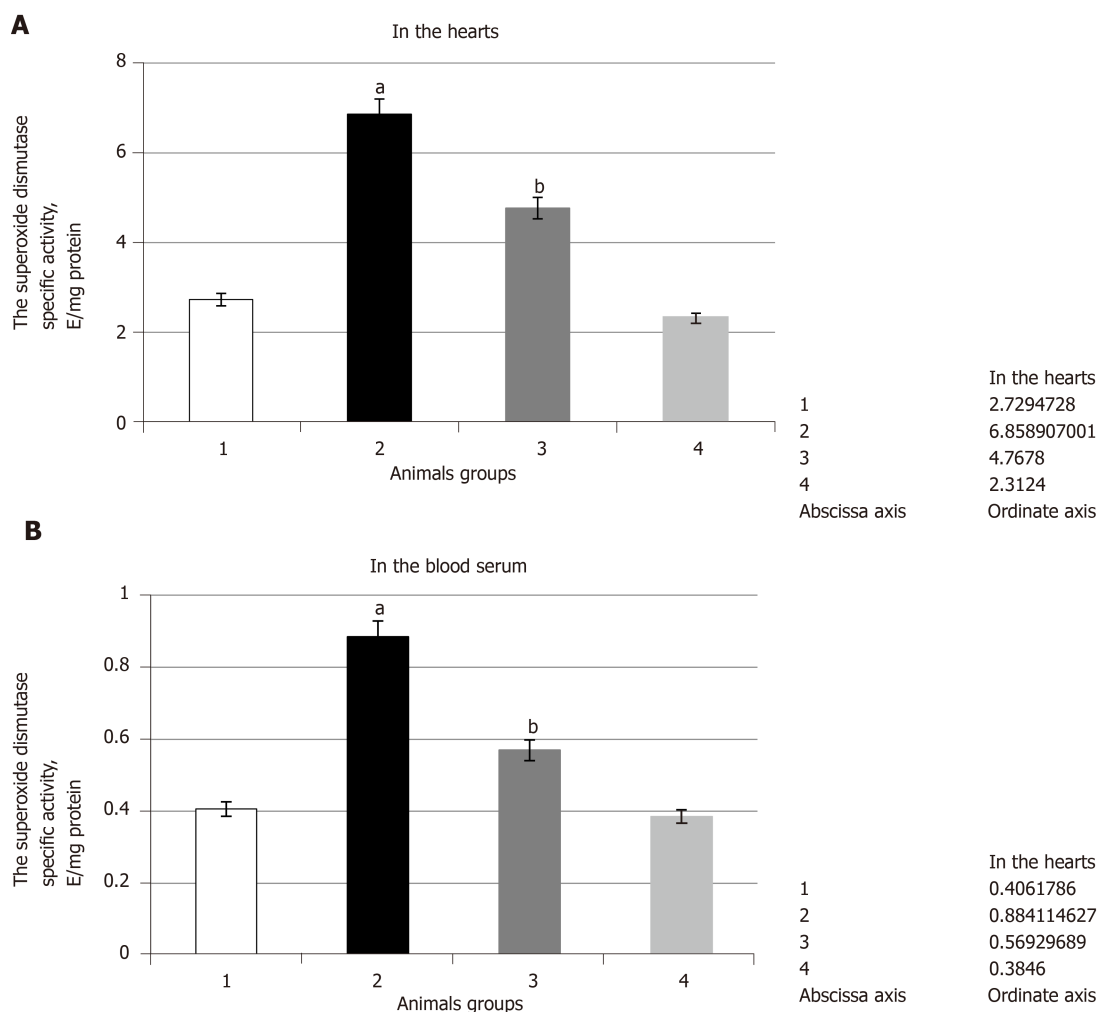


Figure 2 The superoxide dismutase specific activity, E/mg protein in the rats' hearts, and the blood serum during the development of experimental hyperglycemia. A: The superoxide dismutase (SOD) specific activity, E/mg protein in the rats' hearts during the development of experimental hyperglycemia; B: The SOD specific activity, E/mg protein in the blood serum during the development of experimental hyperglycemia. 1: Control group; 2: Animals with experimental hyperglycemia mellitus induced by streptozotocin; 3: 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) is administered to animals at pathology; 4: SkQ1 is administered to animals at control group. ^a $P \leq 0.0167$ compared with control; ^b $P \leq 0.0167$ compared with pathology. SkQ1: 10-(6-plastoquinonyl) decyltriphenylphosphonium.

catalase in rats of the DM2 group may be related to induction of the synthesis of these enzymes under conditions of the oxidative stress formed in pathology.

Thus, according to the data provided by the literature, the regions reacting with nuclear factor kappa B (NF- κ B) were discovered in the coding regions of all three SOD genes^[29]. NF- κ B is a redox-sensitive transcription factor and acts as a regulator of genes, playing a role of the actual defendant to injurious effects on a cell. It should be noted that H_2O_2 , generated in the reaction that is catalyzed by Cu, Zn-SOD on the endosomal surface, can cause oxidation-reduction NF- κ B activation. NF- κ B activation leads to an increase in Cu, Zn-SOD expression^[30].

It is evident that an increase in the dismutation rate of a superoxide anion radical leads to the accumulation of hydrogen peroxide. It is known that the amount of catalase is controlled by the presence of substrate^[31].

It is stated that superoxide anion radicals activate various signaling systems. It includes Keap1 - Nrf2, which is responsible for the induction of a significant number of genes, including antioxidant enzyme genes^[32]. Besides, c-jun N-terminal kinase, which is the main inducer activity of forkhead homeobox type O (FOXO) transcription factors, can be activated during hyperglycemia that contributes to the development of oxidative stress. FOXO can also be considered as messengers during the development of oxidative stress, since their activity is regulated by H_2O_2 and depends on the state of the cell^[33].

It is known that protection against oxidative stress of human cardiac fibroblasts after myocardial infarction is mediated by expression of antioxidant enzymes regulated by FOXO, such as SOD, catalase, and peroxidase^[34]. In addition, FOXO3a, a

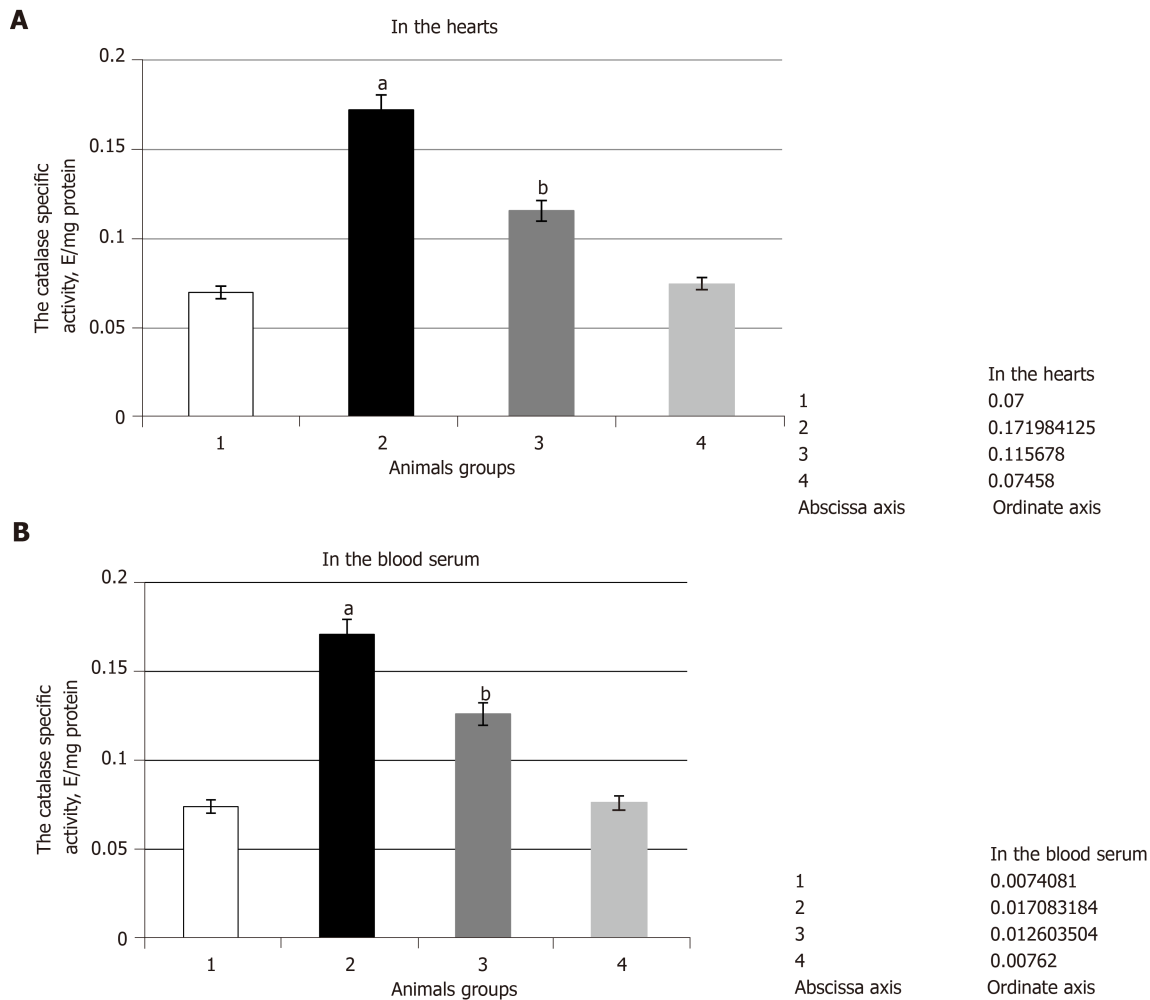


Figure 3 The catalase specific activity, E/mg protein in the rats' hearts, and the blood serum during the development of experimental hyperglycemia. A: The catalase specific activity, E/mg protein in the rats' hearts during the development of experimental hyperglycemia; B: The catalase specific activity, E/mg protein in the blood serum during the development of experimental hyperglycemia. 1: Control group; 2: Animals with experimental hyperglycemia induced by streptozotocin; 3: 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) is administered to animals at pathology; 4: SkQ1 is administered to animals at control group. ^a $P \leq 0.0167$ compared with control; ^b $P \leq 0.0167$ compared with pathology. SkQ1: 10-(6-plastoquinonyl) decyltriphenylphosphonium.

member of the FOXO family, induces catalase gene expression in human cells^[35]. Moreover, it activates the expression of Mn-SOD by regulating the SOD2 gene after exposure to resting cells with hydrogen peroxide^[36].

The increase in the activity and level of transcripts of the studied antioxidant enzymes in pathology probably could be related to the considered molecular genetic mechanisms of the redox regulation of gene expression activity under conditions of excessive generation of ROS. A decrease in the intensity of free radical oxidation under the influence of SkQ1 led to a change in the studied parameters towards control values.

In conclusion, the results of this research suggest that the mitochondria targeted antioxidant SkQ1 might be a perspective substance for incorporation into the antioxidant therapy of DM2. The experimentally revealed ability of this compound to lower the intensity of free-radical processes, acting as the key component of the pathogenesis of the DM2, may serve as the reason for this conclusion. Thus, after the introduction of SkQ1 to the animals with STZ induced hyperglycemia, the values of the BCL parameters reflecting the free radical oxidation intensity, the concentration of DC and carbonyl products of protein oxidation, AH activity, and citrate content changed towards the control values. At the same time, the activity level of the antioxidant enzymes SOD and catalase changed to the direction of normal values.

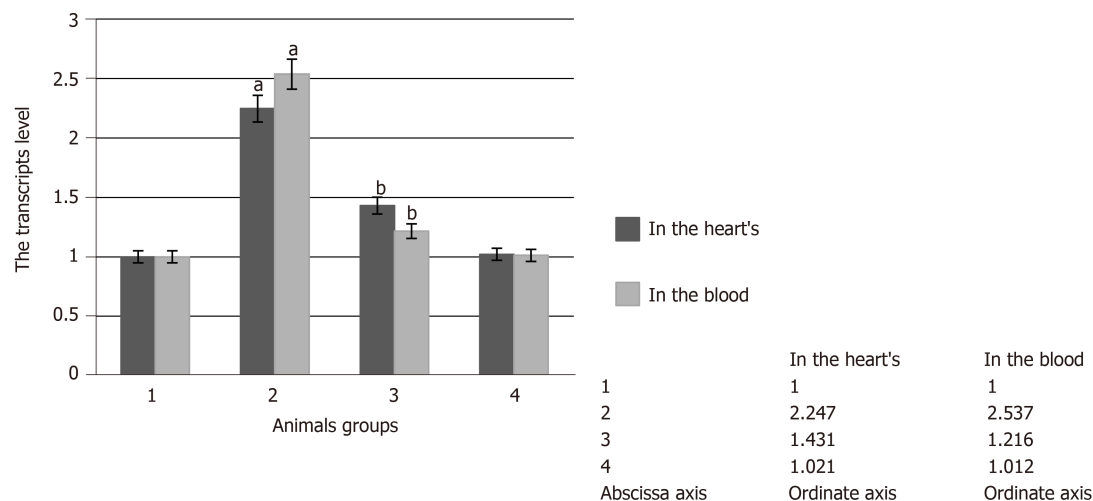
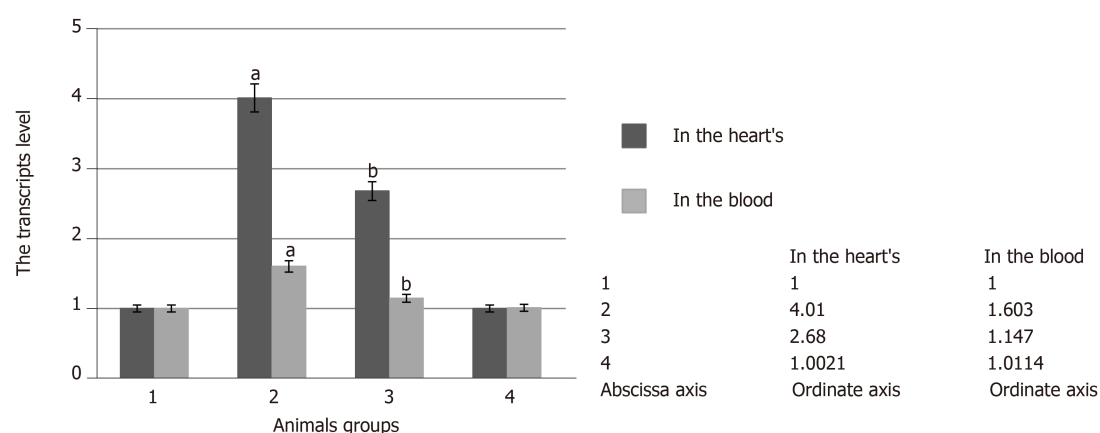
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B


Figure 4 The relative level of the superoxide dismutase and catalase genes transcripts in the rats' hearts and the blood cells during the development of experimental hyperglycemia. A: The relative level of the superoxide dismutase genes transcripts in the rats' hearts and the blood cells during the development of experimental hyperglycemia; B: The relative level of the catalase genes transcripts in the rats' hearts and the blood cells during the development of experimental hyperglycemia. 1: Control group; 2: Animals with experimental hyperglycemia induced by streptozotocin; 3: 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) is administered to animals at pathology; 4: SkQ1 is administered to animals at control group. ^a $P \leq 0.0167$ compared with control; ^b $P \leq 0.0167$ compared with pathology. SkQ1: 10-(6-plastoquinonyl) decyltriphenylphosphonium.

ARTICLE HIGHLIGHTS

Research methods

All treatments of the experiment were consistent with the requirements of the European legislation on the protection of animals (Directive 2010/63/EU).

The laboratory rats were divided into four groups: Group 1 ($n = 20$), animals housed under standard vivarium conditions (control group); group 2 ($n = 20$), animals with streptozotocin (STZ) injection-induced hyperglycemia; group 3 ($n = 12$), animals with STZ-induced glycemia that were administered with 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) solution intraperitoneally at a 1250 nmol/kg dose per day, starting from the second week; and group 4 ($n = 8$), animals housed under standard vivarium conditions that were administered with SkQ1 at a 1250 nmol/kg dose per day, during the second week of conducting the experiment. Group 2 also included rats ($n = 8$) that were administered intraperitoneally with appropriate aliquot quantity of citrate buffer equal to pH 4.4 once a week during 2 wk after 1-mo high-fat diet. Group 3 included animals ($n = 8$) that were administered intraperitoneally with appropriate amount of solution without SkQ1 (98% ethanol diluted eight times with normal saline solution). The experimental unit was a single animal. Rats were kept in laboratory, and they were divided through stratified randomization by their weight and age.

Hyperglycemia was induced by feeding rats a high-fat diet for 1 mo. STZ was administered intra-abdominally at a 30 mg/kg of animal weight dose with citrate buffer equal to pH 4.4. Hyperglycemia was verified by measuring the glucose level in the blood serum with a glucose oxidase test. SkQ1 solution was administered intraperitoneally at a 1250 nmol/kg dose per day. Appropriate aliquot quantity of citrate buffer equal to pH 4.4 was administered intraperitoneally.

To conduct the study, laboratory rats were selected of nursery rat males from Federal State-financed Organization of Health Service "Voronezh Hygiene and Epidemiology Center". The rats used for the study weighed 200-250 g and aged 3-5 mo. Rats were kept at 12-h light day, room temperature, and access to water and food *ad libitum* for 2 wk before the study. Type of housing – plastic. Bedding material – sawdust. Number of cage companions – two rats per cage. The total number of animals used in experiment – 76 rats. The number of animals in each experimental group: Group 1 ($n = 20 + 8$); group 2 ($n = 20 + 8$); group 3 ($n = 12$); and group 4 ($n = 8$).

The number of animals was necessary for obtaining statistically significant results. The animals were divided through stratified randomization by their weight and age. The rats used for the study weighed 200-250 g and aged 3-5 mo.

The order in which the animals in the different experimental groups were treated and assessed: Group 2 compared with group 1; group 3 compared with group 2; and group 4 compared with group 1. Weight gain, increased water intake, and slowness in rats of the second group were seen. SkQ1 administration lowered glycemic level by 2.5 times, which was initially upregulated 2.7 times relative to the control. The glucose level of the animals of the fourth experimental group was not significantly different from the stated value. Moreover, glucose concentration was within the stated value in rats of the second experimental group, having been administered citrate buffer and of the third group, having been administered aliquot of 12% ethanol. The unit of analysis was group of animals.

Experiments were done at least in 8-20 biological and two analytical replicates. The results were compared with the control. The data were statistically analyzed using software package STATISTICA 6.0 with numerical variables – arithmetical mean (M), mean error (m), and statistical significance level (P). Normal distribution data were compared by applying Student's t -test for Bonferroni correction in independent samples. Significance level was set at $^aP \leq 0.0167$ and $^bP \leq 0.0167$.

Research results

It was found that influence of the mitochondrial-directed antioxidant elicits decrease of biochemiluminescence (BCL) parameters values that increase by pathology as well as the level of primary products of lipid peroxidation such as diene conjugates and carbonyl compounds, which indicate intensity of free radical oxidation. At the same time, the activity of aconitate hydratase (AH), considered as a crucial target of FR, which was decreasing during experimental hyperglycemia, increased. Apparently, increasing activity of AH influenced the speed of citrate utilization, whose concentration was decreasing after administering SkQ1 by pathology. Moreover, the previously applied anti-oxidant administration during hyperglycemia influenced the rate of antioxidant system mobilization. Thus, superoxide dismutase and catalase activity as well as the level of gene transcript under influence of SkQ1 at pathology were changing towards control groups values.

Research conclusions

The results of this research suggest that the mitochondria targeted antioxidant SkQ1 might be a perspective substance for incorporation into the antioxidant therapy of type 2 diabetes mellitus (DM2). The experimentally revealed ability of this compound to lower the intensity of free-radical processes, acting as the key component of the pathogenesis of the DM2, may serve as the reason for this conclusion. Thus, after the introduction of SkQ1 to the animals with STZ induced hyperglycemia, the values of the BCL parameters reflecting the free radical oxidation intensity, the concentration of diene conjugates and carbonyl products of protein oxidation, the AH activity, and citrate content changed towards the control values. At the same time, the activity level of the antioxidant enzyme SOD and catalase changed to the direction of normal values.

Research perspectives

The effects of SkQ1 on the stage of compensatory response occurrence in pathology may contribute to decrease of the degree of oxidative stress, normalization of antioxidant system functioning, and blocking of the development of decompensation, characterized by the inhibition of protective systems.

Thus, the mitochondria targeted antioxidant SkQ1 might be considered a perspective substance for incorporation into the antioxidant therapy of DM2.

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Correlating the global increase in type 1 diabetes incidence across age groups with national economic prosperity: A systematic review

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Abstract

BACKGROUND

The global epidemiology of type 1 diabetes (T1D) is not yet well known, as no precise data are available from many countries. T1D is, however, characterized by an important variation in incidences among countries and a dramatic increase of these incidences during the last decades, predominantly in younger children. In the United States and Europe, the increase has been associated with the gross domestic product (GDP) per capita. In our previous systematic review, geographical variation of incidence was correlated with socio-economic factors.

AIM

To investigate variation in the incidence of T1D in age categories and search to what extent these variations correlated with the GDP per capita.

METHODS

A systematic review was performed to retrieve information about the global incidence of T1D among those younger than 14 years of age. The study was carried out according to the PRISMA recommendations. For the analysis, the incidence was organized in the periods: 1975-1999 and 2000-2017. We searched the incidence of T1D in the age-groups 0-4, 5-9 and 10-14. We compared the incidences in countries for which information was available for the two periods. We obtained the GDP from the World Bank. We analysed the relationship between the incidence of T1D with the GDP in countries reporting data at the national level.

RESULTS

We retrieved information for 84 out of 194 countries around the world. We found a wide geographic variation in the incidence of T1D and a worldwide increase

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during the two periods. The largest contribution to this increase was observed in the youngest group of children with T1D, with a relative increase of almost double when comparing the two periods (P value = 2.5×10^{-5}). Twenty-six countries had information on the incidence of T1D at the national level for the two periods. There was a positive correlation between GDP and the incidence of T1D in both periods (Spearman correlation = 0.52 from 1975-1999 and Spearman correlation = 0.53 from 2000-2017).

CONCLUSION

The incidence increase was higher in the youngest group (0-4 years of age), and the highest incidences of T1D were found in wealthier countries.

Key words: Type 1 diabetes; Incidence; Children; Age categories; Gross domestic product per capita

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Core tip: Currently, there is information on the incidence of T1D of 43.3% of the 194 countries of the world, of which only 44 countries have national coverage information. We found a wide geographic variation in the incidence of T1D and a worldwide increase in the two periods (1975-1999 and 2000-2017). Comparing the two periods, the relative increase in the incidence occurred in the 0-4 group (1.9 times), followed by the 5-9 group (1.8 times) and 10-14 group (1.4 times). There was a positive correlation between GDP per capita, and the incidence of T1D, where wealthier countries have higher values of incidence.

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INTRODUCTION

Type 1 diabetes (T1D) is one of the most common endocrine diseases in childhood and adolescence. Additionally, the diagnosis of T1D has increased considerably in adults^[1]. According to the International Diabetes Federation (IDF), it was estimated that more than 86000 children were living with T1D in 2015 around the world^[2]. There is a wide geographic variation in the incidence of T1D, both among countries and within the different regions in these countries. In North America and Europe, the incidence varies between 4 and 41 per 100000. The countries that report the highest rates are Switzerland, Finland, Norway, the United Kingdom and Sardinia, with values > 20 per 100000 per year. In contrast, T1D is rare in Asian countries, such as China, where the incidence is approximately 0.1 case per 100000 people each year^[3-5]. In Latin America, according to IDF, it is estimated that 45100 children younger than 15 years have T1D^[2]. (Table 1)

There are differences in the incidence rates among age categories (0-4, 5-9, 10-14) in almost all countries. According to DIAMOND^[6], for the period between 1991-1996, it was noted that the incidence increased with age; children between 5 and 9 years had 1.62 times the risk of children 0-4 years, *i.e.*, a 62% excess risk, and the 10-14 age group had 1.93 times the risk of the 0-4 age group. Recently, there have been signs suggesting that this trend is changing. Records of the Patterson *et al*^[7] between 1999 and 2008, showed that the incidence was highest in the youngest age group (0 to 4 years), with an increase of 5.4% compared to 4.3% in the 5-9 age group and 2.9% in the 10-14 age group.

In addition, the IDF has suggested the existence of a relationship between income level and the incidence of T1D^[2]. In the United States, where the incidence of T1D in different socio-economic groups was studied, it was found that there was a higher incidence of T1D in the highest income groups^[8]. The same pattern occurs in Europe, where it was shown that the incidence of T1D correlates strongly with the gross domestic product (GDP). GDP is most commonly used to measure the size of a

Table 1 Incidence of type 1 diabetes in individuals aged 0-14 years

Country	Area	Study period	Inc	Asce %	InfoSource	Datacollection	Ref.
Algeria	Oran	1990-1999	8.60	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Argentina	Cordoba	1991-1992	7.00	90.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Avellaneda	1990-1996	6.30	94.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Tierra del Fuego	1993-1996	10.30	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Corrientes	1992-1999	6.60	95.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Australia	NW ^a	2000-2011	23.6	97	PBDR	H	Hayne <i>et al</i> ^[18]
Austria	NW	2004-2008	17.50	97.2	PBDR	P	Patterson <i>et al</i> ^[19]
Bahamas	NW	2001-2002	10.10	NA	MBR	P	Peter <i>et al</i> ^[20]
Barbados	NW	1990-1993	2.00	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Belarus	Gomel, Minsk	1997-2002	5.60	100.0	PBDR	NA	Zalutskaya <i>et al</i> ^[21]
Belgium	Antwerp	2004-2008	15.90	94.9	PBDR	P	Patterson <i>et al</i> ^[19]
Bosnia and Herzegovina	Tuzla Canton	1995-2004	6.93	100.0	PBDR	P	Tahirović <i>et al</i> ^[22]
	Republic of Srpska	1998-2010	8.13	100.0	PBDR	P	Radosevic <i>et al</i> ^[23]
Brazil	São Paulo ^a (Bauru)	1986-2015	12.8	97.7	PBDR	P	Negrato <i>et al</i> ^[24]
	Rio Grande do Sul (Passo Fundo)	1996-1999	7.00	82.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Bulgaria	Eastern	1989-1994	6.80	99.9	PBDR	P	Patterson <i>et al</i> ^[7]
	Varna	1990-1999	8.10	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Western	1990-1999	10.70	99.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Canada	Toronto	1976-1978	9.00	97.2	PBDR	P, H	Ehrlich <i>et al</i> ^[25]
	Manitoba	1985-1993	20.65	95.0	PBDR	P	Blanchard <i>et al</i> ^[26]
	Prince Edward Island	1990-1993	24.50	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Alberta (Edmonton)	1990-1996	23.30	85.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Calgary	1990-1999	20.60	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Québec	1989-2000	15.34	NA	PBDR	P	Legault <i>et al</i> ^[27]
	Newfoundland and Labrador	1987-2010	38.68	NA	PBDR	P, H	Newhook <i>et al</i> ^[28]
Chile	IX Region	1980-1993	1.37	97.0	PBDR	P	Larenas <i>et al</i> ^[29]
	Santiago of Chile (Communes of Metropolitan region)	2000-2005	6.30	100.0	PBDR	P	Torres-Avilés <i>et al</i> ^[30]
China	NW	1988-1994	0.47	93.0	PBDR	P, H	Yang <i>et al</i> ^[31]
	Zhejiang ^a	2007-2013	2.02	94.6	PBDR	H	Wu <i>et al</i> ^[32]
	Beijing ^a	1995-2010	1.7	NA	PBDR	H	Gong <i>et al</i> ^[33]
	Shanghai ^a	1997-2011	3.1	90	PBDR	H	Zhao <i>et al</i> ^[34]
Colombia							

	Bogotá	1990-1990	3.80	97.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Cali	1995-1999	0.50	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Croatia	NW ^a	2004-2012	17.2	96.69	PBDR	H	Rojnic Putarek <i>et al</i> ^[35]
Cuba	NW	1990-1999	2.30	62.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Cyprus	NW	1990-2009	12.34	50.0	PBDR	NA	Skordis <i>et al</i> ^[36]
Czech Republic	NW	2004-2008	19.30	97.4	PBDR	P	Patterson <i>et al</i> ^[19]
Dem. People's Republic of Korea	NW ^a	2012-2014	3.1	NA	PBDR	P or H	Kim <i>et al</i> ^[37]
Denmark	NW	2004-2008	25.10	99.2	PBDR	P	Patterson <i>et al</i> ^[19]
Dominican Republic	NW	1995-1999	0.50	53.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Egypt	Alexandria, Damanhour	1992-1992	8.00	NA	OPD	NA	Arab <i>et al</i> ^[38]
	Northern ^a	1996-2011	1.93	NA	MBR	H	El-Ziny <i>et al</i> ^[39]
Estonia	NW	1983-2006	13.09	98.0	PBDR	P, H	Teeäär <i>et al</i> ^[40]
Ethiopia	Gondar	1995-2008	0.33	NA	MBR	P	Alemu S <i>et al</i> ^[41]
	Jimma	2002-2008	0.33	NA	MBR	P	Alemu S <i>et al</i> ^[41]
Fiji	NW ^a	2001-2012	0.93	NA	PBDR	H	Ogle <i>et al</i> ^[42]
Finland	NW	2006-2011	62.42	NS	OPD	NA	Harjutsalo <i>et al</i> ^[43]
France	Frache-Comté	1980-1998	7.01	80.6	PBDR	H	Mauny <i>et al</i> ^[44]
	Aquitaine, Lorraine, Normandia Basse, Normandia Haut	1990-1994	8.50	97.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Aquitaine	1998-2004	12.20	NA	OPD	NA	Barat <i>et al</i> ^[45]
	Languedoc- Roussillon ^a	2000-2010	16.2	NA	PBDR	NA	Trellu <i>et al</i> ^[46]
Georgia	NW	1998-1999	4.60	NA	OPD	NA	Arab <i>et al</i> ^[38]
Germany	NW ^a	2004-2008	22.9	97	PBDR	H	Bendas <i>et al</i> ^[47]
Greece	NW	1992-1992	6.03	NA	PBDR	P	Dacou-Voutetakis <i>et al</i> ^[48]
Hungary	18 of 19 countries (All, less Budapest)	2004-2008	18.30	98.7	PBDR	P	Patterson <i>et al</i> ^[19]
Iceland	NW	1989-1994	13.50	100.0	PBDR	P	Patterson <i>et al</i> ^[7]
India	Madras	1991-1994	11.00	90.0	PBDR	H	Ramachandran <i>et al</i> ^[49]
Iran (Islamic Republic of)	Fars	1991-1996	3.68	100.0	PBDR	P	Pishdad ^[50]
Ireland	NW ^a	2008-2013	28.3	96,8	PBDR	P	Roche <i>et al</i> ^[51]

Israel	NW ^a ; Population: Arabs	2004-2010	9.14	NA	PBDR	P	Blumenfeld <i>et al</i> ^[52]
	NW ^a ; Population: Jews	2004-2010	13	NA	PBDR	P	Blumenfeld <i>et al</i> ^[52]
Italy	Apulia ^a	2001-2013	17.99	NA	PBDR	P	Di Ciaula ^[53]
	Friuli-Venezia Giulia ^a	2010-2013	17.55	NA	MBR	H	Valent <i>et al</i> ^[54]
	Abruzzo ^a	1999-2008	14.30	95	PBDR	H	Altobelli <i>et al</i> ^[55]
	Veneto ^a	2006-2013	17.00	NA	PBDR	H	Marigliano <i>et al</i> ^[56]
	NW-39.7% population	1990-2003	12.55	NA	PBDR	P	Bruno <i>et al</i> ^[57]
Japan	NW ^a	2005-2012	2.14	NA	PBDR	H	Onda <i>et al</i> ^[58]
Jordan	NW	1992-1996	3.33	95.0	NS	P, H	Ajlouni <i>et al</i> ^[59]
Kuwait	NW ^a	2011-2013	41.7	96.7	PBDR	H	Shaltout <i>et al</i> ^[60]
Latvia	NW	1990-1999	7.40	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Libyan Arab Jamahiriya	Benghazi	1991-1999	9.00	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Lithuania	NW	2004-2008	14.20	NA	PBDR	P	Patterson <i>et al</i> ^[19]
Luxembourg	NW	2004-2008	19.00	100.0	PBDR	P	Patterson <i>et al</i> ^[19]
Malta	NW	2006-2010	23.87	100.0	PBDR	P	Formosa <i>et al</i> ^[61]
Mauritius	NW	1990-1994	1.30	67.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Mexico	NW	2000-2010	5.93	NA	PBDR	H	Gómez-Díaz <i>et al</i> ^[62]
Montenegro	NW ^a	1997-2011	18.6	100	PBDR	H	Samardžić <i>et al</i> ^[63]
Netherlands	NW ^a	1999-2011	25.2	NA	PBDR	H	Fazeli <i>et al</i> ^[64]
New Zealand	NW	1999-2000	18.00	95.0	PBDR	NA	Campbell-Stokes <i>et al</i> ^[65]
Norway	NW ^a	2004-2012	32.7	NA	PBDR	H	Skrivarhaug <i>et al</i> ^[66]
Oman	NW	1993-1995	2.59	96.0	PBDR	P	Soliman <i>et al</i> ^[67]
Pakistan	Karachi	1990-1999	0.50	51.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Papua New Guinea	NW	1996-2000	0.08	NA	MBR	P	Ogle <i>et al</i> ^[68]
Paraguay	NW	1990-1999	0.90	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Peru	Lima	1990-1994	0.50	67.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Poland	NW	1989-2004	11.23	NA	PBDR	P	Jarosz-Chobot <i>et al</i> ^[69]

Portugal	Krakow and the Lesser Poland ^a	2004-2011	15.87	NA	PBDR	H	Wojcik <i>et al</i> ^[70]
	Podlasie, Silesia, Łódzkie, Pomorskie, Bydgoszcz ^a	2005-2012	20.22	NA	PBDR	H	Chobot <i>et al</i> ^[71]
	Algarve	1990-1994	14.60	87.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Portoalegre	1990-1994	21.30	93.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Coimbra	1990-1999	9.60	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Qatar	Madeira Island	1990-1999	6.90	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	NW	1992-1996	11.40	NA	OPD	NA	Al-Zyoud <i>et al</i> ^[72]
Republic of China (Taiwan)	NW ^a	2003-2010	5.45	NA	PBDR	H	Lin <i>et al</i> ^[73]
	NW ^a	2002-2011	9.6	96.2	PBDR	H	Serban <i>et al</i> ^[74]
Russian Federation	Novosibirsk	1990-1999	6.90	93.5	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Moscow	1996-2005	12.07	94.0	PBDR	P	Pronina <i>et al</i> ^[75]
Rwanda	capital and 6 regions ^a	2004-2011	2.7	NA	MBR	H	Marshal <i>et al</i> ^[76]
	Eastern Province	1986-1997	12.30	100.0	PBDR	NA	Kulaylat <i>et al</i> ^[77]
Saudi Arabia	Al-Madinah (North West)	2004-2009	30.88	NA	PBDR	P	Habeb <i>et al</i> ^[78]
	Belgrade	2000-2004	12.90	NA	OPD	NA	Vlajinac <i>et al</i> ^[79]
Serbia	NW	1992-1994	2.42	92.2	PBDR	P	Lee <i>et al</i> ^[80]
	NW	1999-2003	13.60	100.0	PBDR	P	Patterson <i>et al</i> ^[19]
Singapore	NW	1992-1994	2.42	92.2	PBDR	P	Lee <i>et al</i> ^[80]
	NW	1999-2003	13.60	100.0	PBDR	P	Patterson <i>et al</i> ^[19]
Slovakia	NW	1999-2003	13.60	100.0	PBDR	P	Patterson <i>et al</i> ^[19]
	NW	1998-2010	13.83	100.0	PBDR	P	Radosevic <i>et al</i> ^[23]
Slovenia	NW	1998-2010	13.83	100.0	PBDR	P	Radosevic <i>et al</i> ^[23]
	NW	1998-2010	13.83	100.0	PBDR	P	Radosevic <i>et al</i> ^[23]
Spain	Madrid	1985-1988	10.60	90.0	PBDR	H	Serrano Ríos <i>et al</i> ^[81]
	Cáceres	1988-1999	16.67	99.2	PBDR	H	Lora-Gómez <i>et al</i> ^[82]
Sudan	Badajoz	1992-1996	17.23	95.0	PBDR	P	Morales-Pérez <i>et al</i> ^[83]
	Navarre ^a	1975-2011	13.2	NA	PBDR	H	Forga <i>et al</i> ^[84]
Sweden	Catalonia	2004-2008	12.10	97.6	PBDR	P	Patterson <i>et al</i> ^[19]
	Castilla y León ^a	2000-2013	10.8	NA	PBDR	H	Vega <i>et al</i> ^[85]
Switzerland	Biscay ^a	1990-2013	10.7	99.1	PBDR	H or P	Fernández-Ramos <i>et al</i> ^[86]
	Gezira	1990-1990	5.00	100.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
TFYR Macedonia	Khartoum	1991-1995	10.10	97.0	PBDR	NA	Elamin <i>et al</i> ^[87]
	NW ^a	2007-2011	42	99	PBDR	N	Rawshani <i>et al</i> ^[88]
Thailand	NW	2004-2008	13.10	91.3	PBDR	P	Patterson <i>et al</i> ^[19]
	NW	2004-2008	5.80	100.0	PBDR	P	Patterson <i>et al</i> ^[19]
Thailand	North-eastern	1996-2005	0.58	NA	MBR	H	Panamonta <i>et al</i> ^[89]
	North-eastern	1996-2005	0.58	NA	MBR	H	Panamonta <i>et al</i> ^[89]

Tunisia							
	Beja, Monastir, Gafsa	1990-1994	6.69	96.0	PBDR	P	Ben Khalifa <i>et al</i> ^[90]
	Kairoan	1991-1993	7.60	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Beja	1990-1999	7.70	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Gafsa	1990-1999	8.50	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
	Monastir	1990-1999	5.80	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Turkey							
	NW ^a	2011-2013	10.8	99	PBDR	H	Yeşilkaya <i>et al</i> ^[91]
Ukraine							
	NW	1985-1992	8.10	NA	OPD	NA	Timchenko <i>et al</i> ^[92]
United Kingdom							
	NW	1991-2008	19.32	NA	PBDR	P	Imkampe <i>et al</i> ^[93]
United Republic of Tanzania							
	Dar es Salaam	1982-1991	0.92	NA	MBR	P	Swai <i>et al</i> ^[94]
United States of America							
	Olmsted, Minnesota ^a	1994-2010	19.9	NA	MBR	H	Cartee <i>et al</i> ^[95]
	Five areas ^a	2002-2013	19.5	98,9	PBDR	H	Mayer-Davis <i>et al</i> ^[96]
Uruguay							
	Montevideo	1992-1992	8.30	97.0	PBDR	P or H	DIAMOND <i>et al</i> ^[6]
Uzbekistan							
	NW ^a	1998-2014	2.48	100	PBDR	H	Rakhimova <i>et al</i> ^[97]
Venezuela (Bolivarian Republic of)							
	Caracas	1990-1994	0.10	NA	PBDR	P or H	DIAMOND <i>et al</i> ^[6]

Update of the publications that report the incidence of type 1 diabetes from population-based studies.

^aUpdated studies. Area and NW: Study at the national level. ASCE%: Percentage of completeness between primary and secondary sources of registers. PBDR: Registration of population-based data; MBR: Medical-based record; OPD: Other population denominators; NS: Non-specified; P: Prospective - incident cases collected prospectively-; H: Historical -incident cases collected retrospectively-; NA: Information not available.

country's economy. However, there have been conflicting results. For example, an ecological study carried out in North Rhine-Westphalia, Germany showed that the risk of T1D was higher in children living in socially disadvantaged areas^[9].

In a previous systematic review, we identified T1D incidence in 80 out of 194 countries and found significant associations between the geographical variation of incidence and a series of economic, climatic and environmental, and health conditions factors^[10]. Among these factors, GDP per capita was highly correlated with the 0-14-year incidence of T1D (Spearman Correlation = 0.72, P value = 9.05×10^{-14}).

Here, we focus on three age categories (0-4, 5-9, 10-14) and two periods (1975-1999 and 2000-2017). We searched, through a systematic review of the literature, the global variation in the incidence of T1D in these age categories and periods. We then searched to what extent these variations correlated with the GDP per capita in these countries.

MATERIALS AND METHODS

In this study, we updated the review on the global T1D incidence published by Diaz-Valencia *et al*^[10] with new papers. Once the incidence data were obtained through the systematic review, we conducted an exploratory ecological analysis. Following the procedures mentioned by Morgenstern^[11] for ecological studies, we analysed the relations of population rates of T1D incidence and the average GDP of these countries, retrieved from the World Bank database. This analysis was divided into two periods (1975-1999 and 2000-2017).

We extracted information on the incidence of T1D in children under 14 years from population-based studies conducted in clearly defined geographical areas at the local, regional or national level, published in original articles in English, Spanish or French, following the PRISMA recommendations. The databases used for the literature search

were Medline and Thomson Reuters (Web of Knowledge). Additionally, we explored Google Scholar. This study followed the protocol search deposited in the International Prospective of Systematic Reviews with the Registration Number: CRD42012002369. **Figure 1** presents the flow diagram of the bibliographic search.

During this systematic review, several procedures were standardized to minimize the possibility of incurring biases in the identification of literature, selection and the interpretation of evidence. To reduce potential biases during the design and execution of the systematic review, a team was created; initially, this team was formed by a senior expert researcher (AJV) and a researcher (PAD). During the update of this systematic review, the team consisted of two researchers (NGL and PAD). The initial search was undertaken between November 2011 and January 2014 and the update between January and June 2017. For this systematic review a query equation was used, which implemented the same strategy as that validated by Diaz-Valencia *et al*^[10] (Supplementary material). To build the original query equation, we performed an exploratory search, from which 92 references were selected that reported on the incidence of T1D. From these 92 references, we analysed the MeSH terms and incorporated them into a preliminary search equation. Using this equation, we excluded the MeSH terms of references that did not report the incidence of T1D in the search equation. We repeated this process until the search equation included the 92 references used initially.

For all query equations, studies were excluded if (1) The main objective was not to study the incidence of T1D (*e.g.*, genetic factors, complications, treatments); (2) The study was not population-based and instead it was performed in selected groups, such as studies based on volunteer subjects or people belonging to a specific health insurance organization; (3) The study did not report using the World Health Organization (1985 or 1999) or American Diabetes Association (1997 or 2011) diagnosis criteria; (4) The study described the incidence of T1D as a general topic, with no description by year and age at diagnosis; (5) We could not translate the article; or (6) The full text of the article was unavailable.

Quality assessment

The quality of the included studies was evaluated independently by 2 reviewers (NGL-PAD) using the evaluation criteria proposed by Loney *et al*^[12] as an external validation. We also implemented an internal quality assessment. The external validation consisted of eight criteria, (1) Was the target population clearly described? (2) Were cases ascertained either by survey of the entire population or by probability sampling? (3) Was the response rate > 70%? (4) Were the non-responders clearly described? (5) Was the sample representative of the population? (6) Were data collection methods standardized? (7) Were validated diagnostic criteria or approaches used to assess the presence/absence of disease? and (8) Were the estimates of incidence given with confidence intervals? An article's score was obtained by adding up the number of criteria it satisfied. Every satisfied criterion was given 1 point. There was no cut-off score for rating low-quality studies; we arbitrarily considered 0-4, 5-6 and 7-8 points as high, medium and low risk of bias, respectively.

The internal validation was based on 5 criteria. (1) The percentage of completeness between primary and secondary sources of registers. A percentage greater than 90 scored a 1, less than 90 scored a 0.5 and unavailable information scored a 0; (2) Information source: If the data came from the registration of population-based data, it was assigned a 1; if the data came from medical-based records or population denominators it was assigned a 0.5; and if the source was non-specified, it scored a 0; (3) Data collection: If the cases were collected prospectively (P) or retrospectively (H) was assigned a 1; and if the information was not available, it scored a 0; (4) Clear criterion for diagnosis scored a 1; And (5) if the study was population-based, it scored a 1. We arbitrarily considered 0-3, 3.5 and 4-5 as high, medium and low risk of bias, respectively.

Data collection

Two reviewers (Diaz-Valencia PA and Gomez-Lopera N) extracted and reached agreement on the data from included articles using a standard data collection form. We included in this systematic review the most updated and comprehensive data. In each of the articles analysed, we extracted the following information: (1) Identification of the study: Authors, title, journal, year of publication; (2) Period and country of study: Countries were categorized by region according to the United Nations^[13]; (3) Geographical coverage of the study: Nationwide (when the study was conducted across the whole nation) or local (when it was restricted to a region, city or geographically defined population); (4) Incidence rates expressed as new cases per 100000 people (both sexes) per year in the age categories 0-4, 5-9, 10-14, and 0-14. The rates were retrieved from either tables or graphs. If we found incidence values in the

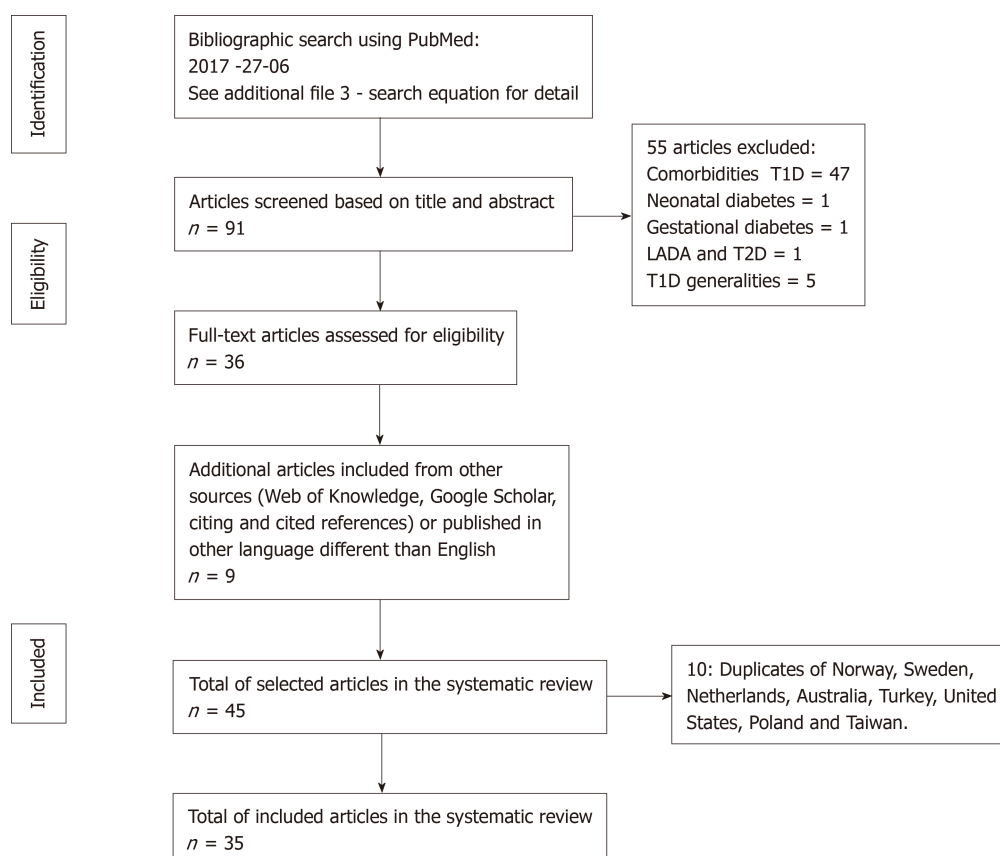


Figure 1 Flow diagram of the bibliographic search strategy. T1D: Type 1 diabetes; T2D: Type 2 diabetes; LADA: Latent autoimmune diabetes in adults.

graphics, we extracted them using GraphClick^[14]. This program allows the user to automatically retrieve the original data from the x and y coordinates of images. Efforts were made to obtain the value of incidence of T1D for each country at the national level. When no information was retrieved at the national level, local studies were considered. In the database, we identified the level of coverage as national or local; (5) The incidence information from two periods was searched: The first was between 1975-1999, and the second was between 2000-2017. We based this separation on a bimodal trend observed in the years of the publications identified in the previous systematic review^[10]; And (6) We collected the percentage of completeness/ascertainment when available.

GPD per capita

The GDP per capita was used to carry out an exploratory ecologic analysis of the relationship between the change in the incidence of T1D and the differences in socio-economic levels during two periods (1975-1999 and 2000-2017). The World Bank database^[15] was used to extract the information for GDP per capita that indicated the relationship between the total value of all the goods and services generated during a year by the economy of a nation or state and the number of its inhabitants in that year. For each study period, we calculated the average of the values of the T1D incidence. In addition, for homogeneity in our analysis, we only chose countries with data at the national level.

Statistical analysis

We presented all the collected data graphically on maps that contain the information obtained from countries at the national level, in two timeframes (1975-1999 and 2000-2017) using the software Tableau^[16]. We compared the incidence of T1D for countries that have information from 1975-1999 and 2000-2017 at the national level in the categories of ages 0-4, 5-9 and 10-14, comparing the means of paired samples.

We performed a correlation to analyse the relationship between the change in incidence of T1D and the change in GDP per capita using the Spearman test and linear regression models to predict the change in the incidence of T1D according to change in the GDP per capita by countries at the national level. Model assumptions for linear models were checked by visual inspection of the residuals. We used the

program R version 3.3.3^[17] to perform the statistical analysis and create graphics related to the study. In all cases, we considered that a *P* value less than 0.05 was statistically significant.

RESULTS

This systematic review of the literature presented information available at the global level on the incidence of T1D and retrieved data for 84 countries, representing 43.3% of the 194 countries of the world. We included 35 additional studies from the previous systematic review^[10]. Among these 35 new papers, we retrieved information for 25 countries; some of them reported data at the national and others at the local level (Figure 1). Updated studies were identified by superscript letters (a) in Table 1. It was possible to update the information published by Diaz-Valencia *et al.*^[10] for 21 countries and obtain data for 4 additional countries: Fiji, Turkey, Rwanda and Republic of China (Taiwan). Of the 84 countries, data were collected at the national level for 44 and the local level for 40.

The median study quality score for studies reporting on the incidence of T1D was high in both cases, with a mean quality score of 7.18 of 8 possible [standard deviation (SD): 0.80] using the external validation, and with a mean quality score of 4.37 of 5 possible [standard deviation (SD): 0.71] using the internal validation. All studies described the target population in detail and used validated diagnostic criteria to assess the presence of disease. Most studies used standardized data collection methods and reported estimates with their accompanying confidence intervals. We found 93.94% concordance between the internal and external validation.

We observed a wide geographical variation in the incidence of T1D at the global level (Table 1). In general, the incidence of T1D was highest in Europe (> 15 per 100000 per year), followed by North America, Australia, Asia, Central and South America. In children from 0-14 years-old, the lowest incidence at the national level (< 1 per 100000 per year) occurred in Thailand; Papua, New Guinea; Fiji; the Dominican Republic; and Paraguay. In contrast, the highest incidence at the national level occurred in Finland, Sweden, Norway and Kuwait, with 62.42, 42, 32.7 and 41.7 per 100000 inhabitants per year, respectively.

We retrieved and compared 26 countries that had information at the national level regarding the incidence of T1D for the periods 1975-1999 and 2000-2017 in individuals from 0-14 years (Figures 2 and 3). In general, an increase in the incidence of T1D is noted at the global level. In the 26 countries, these values were almost double. For example, in Kuwait, the incidence value was 22.3 for the period of 1992-1997^[98] and 41.7 for the period of 2011-2013^[60] (equivalent to a ratio of 1.86).

Additionally, we analysed three distinct categories for age in 15 countries that had information at the national level in the two periods considered in this study (Table 2). We observed an increased in the incidence. In absolute numbers, the period 1975-1999 showed that the incidence increased with age, where the lowest incidence was found in children under the age of 5 years and the highest in children older than 10 years. In the period 2000-2017, there was a higher incidence in the category of 5-9 years, followed by 10-14, and the lowest was found in 0-4. However, comparing the two periods, the relative increase in the incidence of T1D occurred in the 0-4 group (1.9 times), followed by the 5-9 group (1.8 times) and 10-14 group (1.4 times). We performed an extra analysis of all countries reporting incidence values for each age category without taking into account whether they reported the incidence at the local or national level, finding equivalent results (data not shown).

GDP per capita

In general, there was a positive correlation between GDP per capita and the incidence of T1D. A positive correlation was found for the relation between the relative change in T1D incidence and the relative change in GDP for the countries reporting data at national level (Spearman correlation 0.35) (Figure 4).

Analysing the two periods, we found a positive correlation between incidence of T1D and GDP per capita among 26 countries (Spearman correlation = 0.52 between 1975-1999 and Spearman correlation = 0.53 between 2000-2017). Excluding Finland and Switzerland because of their extreme values in T1D incidence and GDP per capita, respectively, we retrieved a Spearman correlation = 0.69 between 1975-1999 and Spearman correlation = 0.62 between 2000-2017 (Figure 5). From the linear regression model, including the 26 countries, it was suggested that for 1991 the country-to-country variation in GDP explained 9% of the country-to-country variation in incidence (adjusted *R*² of the model 0.09), while, for the year 2006, it was 17% (adjusted *R*² of 0.17) (Table 3). We performed the same analysis excluding Finland and

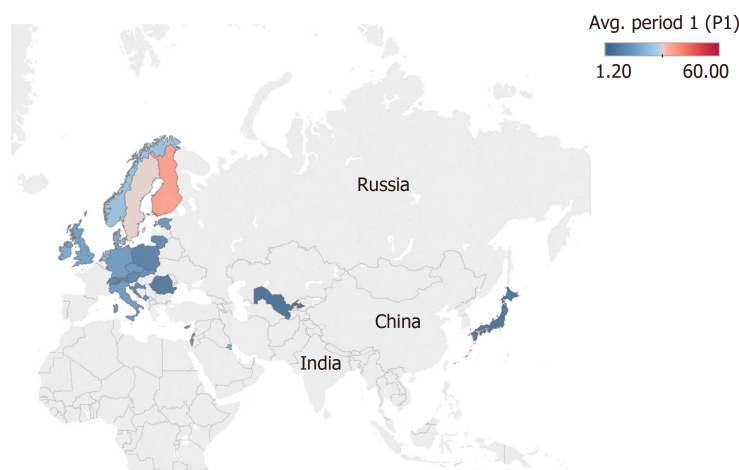


Figure 2 Map showing the mean incidence of type 1 diabetes in 26 countries from 1975-1999. The colour scale represents the level of incidence ranging from 1.20 in blue to 60 per 100000 individuals in red aged 0-14 years.

Switzerland. We found for 1991 that the change in the GDP explained 44% the change in incidence of T1D (adjusted R^2 of the model 0.44), while, for 2006, it was 22% (adjusted R^2 of 0.22) (Table 4).

DISCUSSION

In this study, we updated our previous results on the global incidence of T1D in individuals aged 0-14 years and its variation over time. We analysed the trend in two periods for age categories and GDP per capita. In general, there was a wide geographic variation in the 84 countries for which the incidence of T1D was reported. This variability could be explained to some extent by ethnic differences in allele and haplotype frequencies of risk alleles between populations, for example, in the HLA region, which explains almost 50% of the genetic component of the disease^[99]. There has been a strong association between a high frequency of pre-disposition for HLA haplotypes and a high incidence of T1D. For example, research in the United States, based on the presence of two high-risk haplotypes of HLA-DR3/DR4, revealed that Caucasians have a higher risk of developing T1D than other ethnic groups^[100]. It has been demonstrated that unlike Europeans, DR susceptibility alleles in Asian populations (whose incidence is lower) are in strong linkage disequilibrium with DQ neutral alleles or even protectors, and it is believed that these effects contribute to the low incidence of T1D in these populations^[101,102].

Although there is a consensus on the effect of the genetic susceptibility to T1D between different ethnic groups, these differences cannot fully explain the global variability and the increase in incidence. In this study, we observed an increase in the incidence of T1D worldwide when comparing the periods 1975-1999 and 2000-2017 (Figures 2 and 3). The mechanisms behind the enigma in the increase in the incidence are unknown. However, the mechanism have been attributed to external factors, such as those related to the environment and lifestyle, which may be involved in the epidemiology of the disease^[103].

We also observed an increase in the incidence of T1D in all age categories (0-4, 5-9 and 10-14). In the period 1975-1999, the incidence increased with age, with a peak in children aged 10-14 years. This pattern could be attributed to the onset of puberty with resistance to insulin; therefore, the demand for insulin secretion increases^[104]. In contrast, for the period 2000-2017, there appeared to be an increasing number of patients in the 5-9 age group and a greater relative rise in the 0-4 age group. The mechanisms underlying the increased incidence of T1D in the youngest children are unknown but have largely been attributed to environmental influences^[55].

The environment may act in diverse ways, either by enhancing autoimmunity or modulating normal mechanisms of protection against the development of the disease^[105]. It can be speculated that the plausible causes of temporal changes in the incidence of T1D are attributed to environmental factors, such as social, economic, dietary and health-related factors, which have changed rapidly over the last century.

An example of these changes is socio-economic factors. This study analysed the relationship between one socio-economic indicator (GDP per capita) and the incidence of T1D and found that the highest incidences of the disease were reported in wealthier

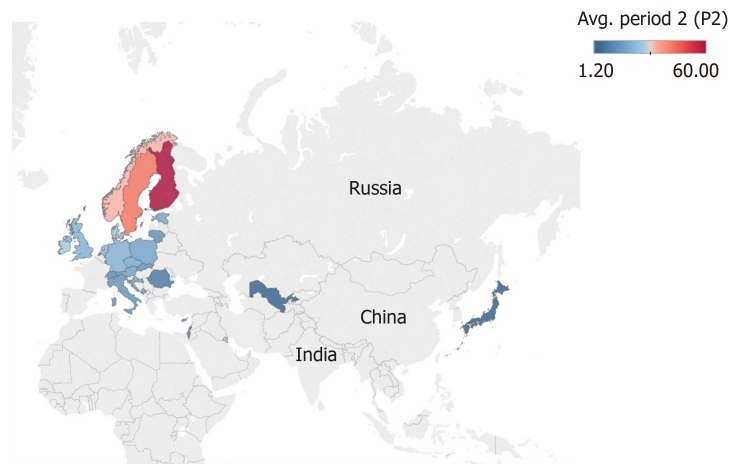


Figure 3 Map showing the mean incidence of type 1 diabetes in 26 countries from 2000-2017. The colour scale represents the level of incidence ranging from 1.20 in blue to 60 per 100000 individuals in red aged 0-14 years.

countries. This same pattern was found by Patterson *et al*^[106] in a study conducted throughout Europe. Similarly, studies conducted at the country level in Sweden^[107], United Kingdom^[108], and Italy^[109] described similar associations between T1D and the socio-economic variables. The influence of changes in GDP per capita on the incidence of the disease was higher for the average year 1991 than that after 2006. We wonder if this behaviour could be explained because 2006 was before the financial crisis of 2008 that provoked a fall in the economies of all regions^[15].

The geographical associations between the socio-economic situation and the incidence of T1D could be attributed to spatial patterns in the composition of the population, which leads to differences in lifestyle and diet. A positive relationship has been demonstrated between the prosperity of the nation, as measured by GDP, with body mass index (BMI)^[110]. Recently, the overload and accelerator hypothesis has been proposed for the increase in body size, BMI and insulin resistance, as well as risk factors for developing T1D^[111]. This hypothesis suggests that the increase in BMI and a more sedentary lifestyle cause resistance to insulin, which leads to β -cells being overworked. This process results in apoptosis and increased production of antigens, which triggers an autoimmune response. Therefore, individuals with a genetic predisposition to T1D will develop an autoimmune response, further accelerating the loss of β -cells. Although an increase in weight contributes to insulin resistance, another consequence of overweight is the storage of ectopic fat with glucotoxicity associated with inflammation resulting in an inhibition of gene expression of insulin, which is also involved in the process of apoptosis of β -cells^[112].

Additionally, the relationship of the socio-economic level with the incidence of T1D could be explained by the improvement of the standards of hygiene, low rates of infection in childhood and low social contact in early childhood, which are possibly experienced in wealthy countries. This theory is known as the hygiene hypothesis, proposed by Gale^[113] in 2002. This hypothesis suggests that changes in hygiene and infection patterns in early childhood alter the development of the immune system and the normal mechanisms of protection against autoimmunity. A study in non-obese diabetic mice, showed that there was a 40%-50% increase in the incidence of T1D when the animals were raised in environments free of pathogens^[114]. In general, there has been an inverse trend between the incidence of infectious diseases and the incidence of autoimmune and allergic diseases^[115].

Other environmental factors potentially related to national economic prosperity must be mentioned. One of these is the nutritional component that has undergone major changes in many developed countries. Early nutrition seems to modulate the development of T1D, for example, the absence or short duration of breastfeeding and early introduction of cow's milk formulae are thought to be risk factors for this disease^[116]. Also, rapid weight gain in infancy, associated with improper feeding, increases the risk of developing T1D^[117]. Other possible factors that experiment in wealthier countries are a higher degree of urbanization, which are associated with an increased incidence of T1D, supporting the hygiene hypothesis^[10]. In addition, there are differences in caesarean deliveries between low- and high-income countries, where wealthier countries have high levels of caesarean use without medical indication^[118]. Delivered by caesarean section are at slightly increased risk of T1D, and it has been postulated that differences in the gut microbiota of these children

Table 2 Summary values for the comparison of the incidence of type 1 diabetes for countries with nationwide data by age category in the periods analysed in the study

	0-4 yr		5-9 yr		10-14 yr	
Period	1975-1999	2000-2017	1975-1999	2000-2017	1975-1999	2000-2017
Mean	6.68	12.59	11.92	21.99	14.04	19.54
95%CI	(4.49, 8.87)	(9.23, 15.96)	(7.95, 15.96)	(14.80, 29.18)	(9.72, 18.38)	(15.09, 23.99)
T student	-6.31		-4.58		-3.22	
P value	0.00002		0.00043		0.006	
Ratio periods	1.9		1.8		1.4	

CI: Confidence Interval.

compared with those born by normal vaginally delivery^[103]. Also, the wealth of countries is associated with environmental pollution. An association between air pollution and T1D incidence has been described. Researchers proposed that chemical and air pollutant exposures have multiple effects that may directly affect the risk of T1D^[119].

However, a single environmental factor or interaction between factors, has not been identified that could explain these changes in the incidence of T1D. Moreover, there are complex interactions between genetic and environmental factors that remain to be discovered. More epidemiological studies of T1D are needed to develop new hypotheses about the genetic and environmental factors that trigger the disease, which should be further tested.

Currently, there is information on the incidence of 43.3% of the 194 countries of the world, of which only 44 countries have national coverage information; most of them are European. Despite the efforts of the DIAMOND project^[6] to describe the incidence of T1D at a global level, there is little information for countries in Africa, Central and South America. Moreover, the data are not entirely representative for some countries. To extrapolate this information for the whole country, there would be a substantial bias, as there may be variability within large nations in both the genetic component and environmental exposures that trigger the disease.

Another aspect to consider is the lack of continuity of the epidemiological studies. Only 21 countries have updated incidence rates in the systematic review conducted between 2014 and 2017. Moreover, this lack of continuity implies a limitation for our study since we retrieved available information to conduct comparisons in two periods, 1975-1999 and 2000-2017; only 26 countries had data at the national level, and age category data are even less available. These 26 countries are mainly from Europe ($n = 23$), and Asia ($n = 3$). Regrettably, we do not have information to conduct comparison in the two periods for countries in Africa, Oceania, and America. It is very important to generate more studies on the epidemiology of T1D. This approach will contribute to understanding the dynamic changes in the disease, which, together with studies in basic sciences such as genetics, could identify the factors that modify the risk to the disease and could probably slow down the current increase in the incidence of T1D.

Limitations of this study are worth noting. Although several procedures were standardized during this systematic review to minimize the possibilities of incurring biases in the identification of literature, selection and interpretation of evidence, we cannot rule out having missed relevant studies also due to publication bias. For example, studies that were published in languages other than English, Spanish or French were not included. An important limitation that is shared by all ecological studies is the possibility of making an ecological fallacy. The implication is that the associations we found are only at the group level, and we cannot assume that they are inferred to each individual in these groups. For example, our results do not necessarily imply that all wealthy countries have a higher incidence of T1D, and our findings only reveal potential associations between GPD and population rates of T1D incidence at the group level. Another limitation of our study is the heterogeneity of the different countries included in the statistical analysis. In addition, available secondary sources of GPD data may not have the same accuracy for all countries, leading to imprecise correlation with the incidence of T1D.

We found a wide geographic variation in the incidence of T1D and a worldwide increase in the two periods considered in this study. The greatest increase was observed in the youngest group of children with T1D (0-4 years), with a relative increase of almost double (P value = $2.47 \times e^{-0.5}$). Finally, there was a positive

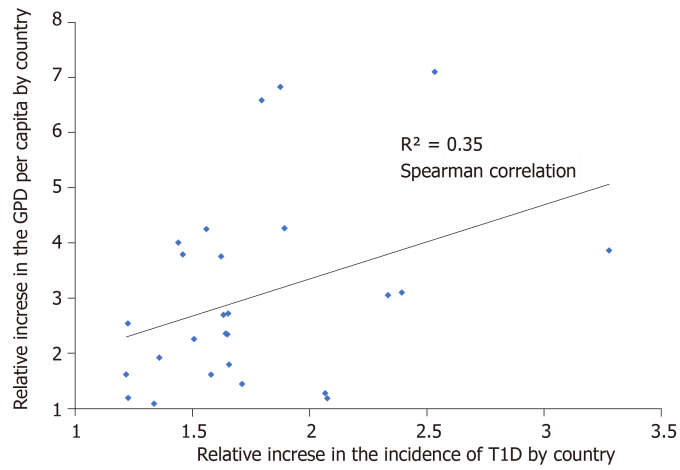


Figure 4 Observed relation between the ratios of the increase in incidence and gross domestic product in 26 countries.

correlation between the socio-economic level, as measured by GDP per capita, and the incidence of T1D, where wealthier countries have higher values of incidence.

Table 3 Models of change in the incidence of type 1 diabetes for 26 countries with nationwide data according to the change in gross domestic product

Model GDP per capita	Coefficient	Est	CI 2.5%	CI 97.5%	SE	t value	P value
Year 1991: Residual standard error: 6.63 Adjusted R ² : 0.09509; F-statistic: 3.312 on 1 and 21 DF; P value: 0.08307	Intercept	8.99	4.63	13.36	2.1	4.28	0.0003
	GDP per capita	0.0002	0.0000	0.0005	0.0001	1.8000	0.0830
Year 2006: Residual standard error: 8.6; Adjusted R ² : 0.176; F-statistic: 5.698 on 1 and 21 DF. P value: 0.02647	Intercept	13.5	7.34	19.66	2.96	4.56	0.0002
	GDP per capita	0.0000	0.0000	0.0000	0.0000	2.39	0.0260

Est: Estimator; CI: Confidence Interval; SE: Standard Error; Df: Degree freedom; GDP: Gross domestic product.

Table 4 Models of change in the incidence of type 1 diabetes for countries with nationwide data according to the change in gross domestic product, excluding Finland and Switzerland

Model GDP per capita	Coefficient	Est.	CI 2.5%	CI 97.5%	SE	t value	P value
Year 1991: standard residual error: 5.08; adjusted R ² : 0.44. F statistic: 19.37. 22 DF. P value: 0.0002	Intercept	5.82	2.27	9.36	1.71	3.40	0.002
	GDP Per capita	0.0005	0.0003	0.0008	0.0001	4.1220	0.0002
Year 2006: Standard residual error: 8.08; adjusted R ² : 0.22; F statistic: 7.62. 22 DF. P value: 0.01	Intercept	13.57	8.16	18.98	2.61	5.21	0.00003
	GDP Per capita	0.0002	0.00004	0.0003	0.0001	2.76	0.01

Est: Estimator; CI: Confidence Interval; SE: Standard Error; Df: Degree freedom; GDP: Gross domestic product.

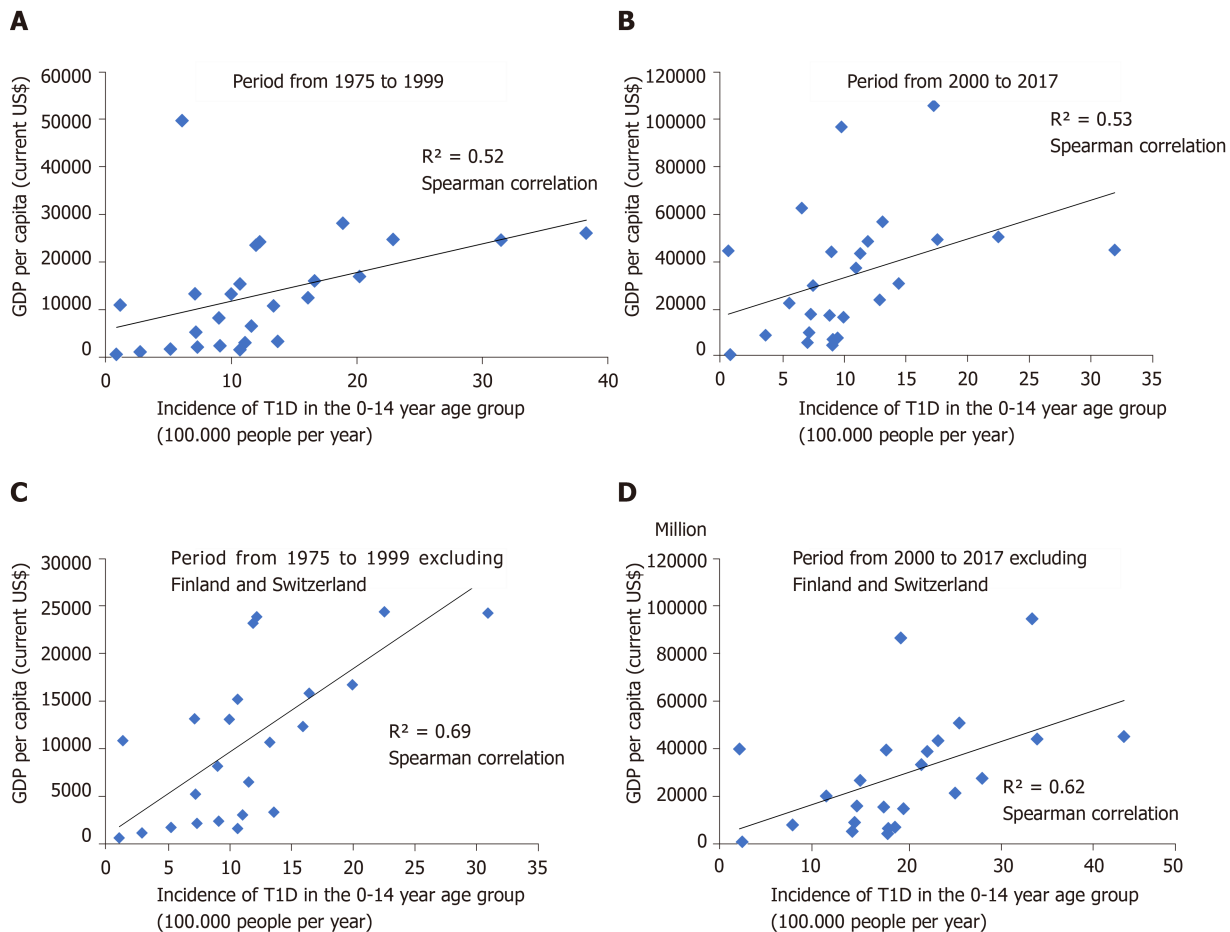


Figure 5 Correlation between incidence of type 1 diabetes and gross domestic product per capita for countries with information at the national level in two periods. A: Analysis for 26 countries period from 1975 to 1999; B: Analysis for 26 countries, period from 2000 to 2017; C: Analysis excluding Finland and Switzerland, period from 1975 to 1999; D: Analysis excluding Finland and Switzerland, period from 2000 to 2017.

ARTICLE HIGHLIGHTS

Research background

Type 1 Diabetes (T1D) is a complex disease resulting from the interplay of genetic, epigenetic, and environmental factors. There is a dramatic increase in the incidence of T1D, predominantly in younger children (0-4 years old) worldwide. The cause of this increase is still under study.

Research motivation

This work updates the current knowledge on the global incidence of T1D across age categories and its variation over time. The increase of incidence of T1D has been associated with socioeconomic factors, such as gross domestic product (GDP). However, there have been conflicting results about the relationship between income level and the incidence of T1D.

Research objectives

We searched the global variation in the incidence of T1D in the age categories and two periods (1975-1990 and 2000-2017). We then searched to what extent these variations correlated with the GDP per capita in these countries.

Research methods

We updated through a systematic review, our previous results on the global incidence of T1D in individuals aged 0-14 years. We first retrieved the incidence of T1D data in different age categories (0-4, 5-9, 10-14, 0-14) and divided the incidence information into two periods (1975-1999 and 2000-2017). Then, we conducted an exploratory ecological analysis about the relations of population rates of T1D incidence and the average GDP of these countries. Comparisons of means, correlations, linear regression were made.

Research results

We retrieved incidence data for 84 countries, most of them are European. We observed an increase in the incidence of T1D worldwide when comparing the periods 1975-1999 and 2000-2017. We also observed an increase in the incidence of T1D in all age categories (0-4, 5-9 and 10-

14). In the period 1975-1999, the incidence increased with age, with a peak in children aged 10-14 years. For the period 2000-2017, there appeared to be an increasing number of patients in the 5-9 age group and a greater relative rise in the 0-4 age group. Also, we found that the highest incidences of the disease were reported in wealthier countries.

Research conclusions

We found a wide geographic variation in the incidence of T1D and a worldwide increase in the two periods considered in this study, especially in younger children (0-4 years old); showing an early age at onset. Also, we confirmed that there was a positive correlation between the socio-economic level and the incidence of T1D. More studies are required to elucidate the interaction between environmental, immunological and genetic factor.

Research perspectives

This study showed the enormous differences in surveillance and epidemiological reports of T1D worldwide. Most of the countries retrieved from the systematic review are European and few studies were carried out in Central and Latin America, Central Asia and Sub-Saharan Africa. It is very important that the scientific community generates more studies on the epidemiology of T1D that contribute to understanding the changes in the dynamics of the disease.

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