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EDITORIAL

mTOR: Driving apoptosis and autophagy for neurocardiac complications of diabetes mellitus

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Abstract

The World Health Organization estimates that diabetes mellitus (DM) will become the seventh leading cause of death during the next two decades. DM affects approximately 350 million individuals worldwide and additional millions that remain undiagnosed are estimated to suffer from the complications of DM. Although the complications of DM can be seen throughout the body, the nervous, cardiac, and vascular systems can be significantly affected and lead to disorders that include cognitive loss, stroke, atherosclerosis, cardiac failure, and endothelial stem cell impairment. At the cellular level, oxidative stress is a significant determinant of cell fate during DM and leads to endoplasmic reticulum stress, mitochondrial dysfunction, apoptosis, and autophagy. Multiple strategies are being developed to combat the complications of DM, but it is the mechanistic target of rapamycin (mTOR) that is gaining interest in drug development circles especially for protective therapies that involve cytokines and growth factors such as erythropoietin. The pathways of mTOR linked to mTOR complex 1, mTOR complex 2, AMP activated protein kinase, and the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) complex can ultimately influence neuronal, cardiac, and vascular cell survival during oxidant stress in DM through a fine interplay between apoptosis and autophagy. Further understanding of these mTOR regulated pathways should foster novel strategies for the complications of DM that impact millions of individuals with death and disability.

Key words: Apoptosis; Autophagy; Cardiac disease; Diabetes mellitus; Erythropoietin; Metformin; Oxidative stress; Neurodegeneration; Mechanistic target of rapamycin; Vascular disease

Core tip: The pathways of mechanistic target of rapamycin (mTOR) linked to mTOR complex 1, mTOR complex 2, AMP activated protein kinase, and tuberous sclerosis 1/tuberous sclerosis 2 complex can offer novel strategies for the complications of diabetes mellitus to prevent death and disability for the millions of individuals afflicted with this disorder.

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THE GROWING THREAT FROM DIABETES MELLITUS

The incidence of diabetes mellitus (DM) throughout the world is increasing at an exponential rate such that the World Health Organization predicts that DM will be the seventh leading cause of death by the year 2030[1]. In 2013, greater than a million deaths were attributable to DM that is believed to affect 347 million individuals throughout the world. In the United States, 21 million individuals are diagnosed with DM and another 8 million individuals are estimated to suffer from DM but are currently undiagnosed[2]. Reduced activity, increased body weight, and poor nutritional intake are considered significant factors that can lead to adult onset DM[3,4]. Duration of obese-years rather than body mass index can become a significant risk for developing DM[5].

DM is defined as being either non-insulin dependent (type 1) or insulin dependent (type 2)[6,7]. Type 1 DM occurs in approximately 5%-10% of DM patients and is an autoimmune disorder with the presence of alleles of the human leukocyte antigen class Ⅱ genes within the major histocompatibility complex. Destruction of pancreatic β-cells with inflammatory infiltration of the islets of Langerhans results in lost insulin production and regulation. About 90% of patients with type 1 DM have increased titers of autoantibodies (type 1A DM). The remaining 10% of type 1 DM individuals do not have serum autoantibodies and are considered to have maturity-onset diabetes of the young that can be a result of β-cell dysfunction with autosomal-dominant inheritance (type IB DM). Type 2 DM occurs in approximately 80%-90% of individuals with DM greater than the age of 40. Although approximately 10% of individuals with type 2 DM may have elevated serum autoantibodies similar to type 1 DM, type 2 DM represents a progressive deterioration of glucose tolerance with early β-cell compensation that has cell hyperplasia followed by a decrease in β-cell mass. Insulin resistance ensues as well as impairments in insulin secretion. Insulin resistance also may be a component of type 1 DM in some patients. Defective insulin secretion can result from impaired β-cell function, chronic exposure to free fatty acids and hyperglycemia, as well as the absence of inhibitory feedback through plasma glucagon levels.

CLINICAL IMPLICATIONS OF DM IN THE NERVOUS, CARDIAC, AND VASCULAR SYSTEMS

As a disease that affects all systems of the body, DM can lead to multiple clinical impairments especially in the nervous, cardiac, and vascular systems. DM results in cognitive loss not only through vascular disease and stroke[8], but also during chronic neurodegenerative disorders such as Alzheimer's disease[9,10]. Insulin resistance similar to its occurrence in DM also has been reported in patients with Alzheimer's disease, suggesting that degenerative disorders such as Alzheimer's disease could be mediated in some patient populations by impaired cellular metabolism[11]. DM also results in neuropsychiatric disorders[12,13], retinal disease[14-16], and peripheral nerve disorders[17]. In the cardiac system, DM can lead to sympathetic nerve dysfunction[18], cardiac fibrosis[19,20], ischemic reperfusion injury[21], cardiomyocyte injury[22], and cardiac hypertrophy[23]. DM also can significantly impact endothelial cells either in the brain or elsewhere in the body. Exposure to elevated glucose levels can result in endothelial cell senescence[24], dysfunctional mobilization of endothelial progenitor cells from the bone marrow[25], injury to the neuroglialvascular unit[14], loss of angiogenesis[26], and endothelial cell injury and loss[27-33].

During DM, oxidative stress is an important driver of cell injury[4,6,34-39]. In murine animal models of type 2 DM, oxidative stress can lead to elevated glutathione levels and increased lipid peroxidation[23]. "Highly-oxidized glycated" low density lipoproteins that can occur in DM lead to oxidative and endoplasmic reticulum stress in human retinal capillary pericytes. Subsequently, mitochondrial dysfunction and cell death with apoptosis and autophagy ensues[15]. Exposure of glucolipotoxicity caused by elevated plasma glucose and lipid levels to pancreatic β-cells promotes oxidative stress with cytochrome c release, caspase activation, and apoptosis[40]. Advanced glycation end products (AGEs), entities that promote complications in DM[41], lead to the release of reactive oxygen species (ROS) and caspase activation[37]. In addition, high fat diets[42] as well as free fatty acids have been shown to release ROS, lead to mitochondrial DNA damage, and impair pancreatic β-cell function[43]. In cardiomyocytes[20,22,44], neurons[8,15,30,45,46], and endothelial cells[14,25,27-29,47], exposure to elevated glucose levels foster oxidant stress mechanisms that can impair cellular function and lead to cell death. In clinical studies, patients with type 2 DM display serum markers of oxidative stress with ischemia-modified albumin[48]. Interestingly, elevations in serum glucose can increase antioxidant enzyme levels in human endothelial cells, suggesting that some cells may initiate a reparative process against oxidative stress injury[49]. Of note, chronic hyperglycemia is not necessary to lead to oxidative stress injury, since even brief periods of hyperglycemia generate ROS[50]. Clinical correlates support these experimental studies to show that both acute glucose swings as well as chronic hyperglycemia can trigger oxidative stress mechanisms during type 2 DM[51].

NOVEL STRATEGIES FOR DM WITH MECHANISTIC TARGET OF RAPAMYCIN

Numerous cellular pathways can lead to oxidative stress during DM. As a result, multiple therapeutic avenues are being pursued to develop therapy against

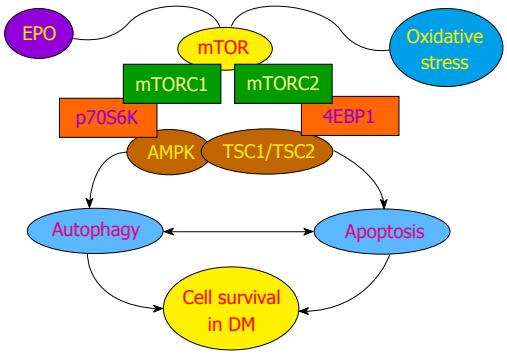

Figure 1 Cellular signaling of mechanistic target of rapamycin in diabetes mellitus. mTOR is a component of the protein complexes mTORC1 and mTORC2 with two important targets of p70S6K and 4EBP1 that promote mTOR kinase activity. mTOR signaling is controlled by AMPK that oversees the activity of TSC1/TSC2, an inhibitor of mTORC1. During periods of oxidative stress in DM, EPO uses mTOR to protect cell survival from programmed cell death injury. For example, EPO blocks cell injury in studies of diabetic retinal degeneration, maintains endothelial cell integrity during experimental models of DM, and regulates the detrimental effects of obesity in animal models. AMPK can have dual roles in cell survival. AMPK can limit oxidative stress that can lead to hypertension and reduce insulin resistance through autophagy that can have linked pathways to apoptosis. However, under other circumstances AMPK also can lead to neuroinflammation and cardiac dysfunction. Ultimately, a careful balance in the activities of autophagy and apoptosis is required through mTOR to foster cell survival during DM. mTOR: Mechanistic target of rapamycin; DM: Diabetes mellitus; AMPK: AMP activated protein kinase; EPO: Erythropoietin; p70S6K: p70 ribosomal S6 kinase; 4EBP1: 4E-binding protein 1; mTORC1: mTOR complex 1; TSC1/TSC2: Tuberous sclerosis 1/ tuberous sclerosis 2.

the complications of DM. These strategies include the recent focus upon sirtuins[24,47,52-56], protein tyrosine phosphatases[57,58], broad antioxidant therapies[3,7,17,31,34,38,59], forkhead transcription factors[56,60-63], and growth factors[32,64-68].

In reference to growth factors, the cytokine and growth factor erythropoietin (EPO) serves as a provocative model for potential treatments for the complications of DM (Figure 1). EPO blocks cell injury in studies of diabetic retinal degeneration[14], maintains endothelial cell integrity during experimental models of DM[27,28], facilitates wound healing during DM[65], reduces high glucose-induced oxidative stress in renal tubular cells[69], maintains cellular mitochondrial function and energy metabolism[32], and regulates the detrimental effects of obesity in animal models[33]. Although EPO affects multiple cellular signal transduction pathways in the body[70,71], of particular interest are the signal transduction pathways of the mechanistic target of rapamycin (mTOR) controlled by EPO that are intimately linked to cellular metabolism and DM[72-76]. mTOR can influence neuronal, glial, and cell to cell activity[77,78]. EPO uses mTOR to protect cells against oxygen-glucose deprivation[79,80], limit cell injury during β-amyloid exposure[81], control bone homeostasis[82], improve cognitive function in models of sepsis-associated encephalopathy[83], foster retinal progenitor cell survival during oxidant stress[84], and promote the neuronal phenotype of adult neuronal precursor cells[85].

mTOR, also known as the mammalian target of rapamycin and FK506-binding protein 12-rapamycin complex-associated protein 1, is a 289-ku serine/threonine protein kinase. mTOR is encoded by a single gene *FRAP1*[86-88] and is a component of the protein complexes mTOR complex 1 (mTORC1) and mTORC2 (Figure 1). Rapamycin, an agent that inhibits mTOR activity, blocks mTORC1 by preventing the phosphorylation of mTOR. In some cases with chronic administration, rapamycin also can inhibit mTORC2. mTORC1 is composed of raptor (regulatory-associated protein of mTOR), the proline rich Akt substrate 40 ku, deptor (DEP domain-containing mTOR interacting protein), and mLST8/G L (mammalian lethal with Sec13 protein 8, termed mLST8). Two important targets of mTORC1 through mLST8 that promote mTOR kinase activity are p70 ribosomal S6 kinase and the eukaryotic initiation factor 4E-binding protein 1[89,90]. mTORC2 is composed of rictor (rapamycin-insensitive companion of mTOR), deptor, mLST8, the mammalian stress-activated protein kinase interacting protein (mSIN1), and the protein observed with rictor-1 (Protor-1)[75,91].

In addition to phosphoinositide 3-kinase and protein kinase B (Akt)[6,92], mTOR signaling also is governed by AMP activated protein kinase (AMPK)[75,91]. AMPK can control the activity of the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2) complex that is an inhibitor of mTORC1. AMPK phosphorylates TSC2 as well as Raptor to block the activity of mTORC1 during energy stress[93]. AMPK also controls TSC1/2 activity through RTP801 (REDD1/ product of the *Ddit4* gene). AMPK activity can increase REDD1 expression, such as in the presence of hypoxic environments, to suppress mTORC1 activity by releasing TSC2 from its inhibitory binding to protein 14-3-3[94].

AMPK can have dual roles in cell survival (Figure 1). AMPK activation can suppress β-amyloid (Aβ) production[95], regulate tau phosphorylation[96], limit oxidative stress that can lead to hypertension[97], increase cell survival during hypoxia[98], and promote autophagy that may resolve memory impairment[99]. However, in other experimental models, AMPK activity has been suggested to influence neuroinflammation[100], lead to aberrant Aβ stress[96] and Aβ toxicity[101], result in cardiac dysfunction[102], and result in the hypertrophy of cardiac tissues[103]. In regards to cellular metabolism with AMPK[104], AMPK can reduce insulin resistance and diminish oxidative stress mediated through the programmed cell death pathway of autophagy[105], reduce myocardial ischemia in experimental models of diabetes[21], be necessary for proper metabolic function of cells[106], and block adipocyte differentiation, lipid accumulation, and obesity[107]. Loss of AMPK may lead to insulin resistance[108].

TARGETING APOPTOSIS AND AUTOPHAGY WITH mTOR FOR DM

For the development of new strategies against DM with mTOR, a careful balance in the activity of the programmed

cell death pathways of apoptosis and autophagy must be considered. Both apoptosis[4,7,17,32,38,109] and autophagy[6,74,110,111] can influence cell survival during oxidative stress[112]. In regards to cellular metabolic pathways, activation of mTOR that blocks apoptotic pathways may limit insulin resistance and vascular thrombosis in patients with metabolic syndrome[113]. Increased activity of mTOR also may prevent the development of atherosclerosis[114]. Furthermore, mTOR activation through glucagon-like peptide-1 agonists has recently been reported to protect pancreatic β-cells from cholesterol mediated apoptotic cell injury[115], promote pancreatic β-cell proliferation[116], and prevent neural apoptotic cell loss during DM through the epidermal growth factor receptor[117].

In other studies with DM, it is the induction of autophagy with requisite mTOR inhibition that is suggested to foster cellular protection. For example, metformin, an agent used to control hyperglycemia in DM, inhibits mTOR activity and promotes autophagy. Metformin can offer protection against endothelial cell senescence[24], limit androgen up-regulation during prostate cancer through mTOR inhibition[118], prevent cell loss during hypoxia through increased AMPK activity[98], and protect against neuronal cell apoptosis[119]. Metformin through pathways that activate AMPK also prevents cardiomyopathy in experimental models of DM[120], fosters cardiomyocyte cell survival[121], and reduces cortical infarction in stroke models[122].

Additional work suggests that autophagy irrespective of the contribution of mTOR may be protective during DM. Autophagy haploinsufficiency in murine animal models of obesity leads to increased insulin resistance with elevated lipids and inflammation[123], suggesting that loss of autophagy may foster the progression from obesity to DM. Autophagy also may be required to remove misfolded proteins and eliminate non-functioning mitochondria to prevent β-cell dysfunction and the onset of DM[124]. In addition, exercise in mice has been shown to initiate autophagy and regulate glucose homeostasis[125]. These results may be associated with observations that autophagy has been reported to improve insulin sensitivity during high fat diets in mice[105].

Yet, in other experimental models, autophagy may not be beneficial even though it can be less of a prominent modulator of cell survival than apoptosis in some experimental models[126]. Autophagy during high glucose exposure has been shown to impair endothelial progenitor cells, lead to mitochondrial oxidative stress, and prevent the formation of new blood vessels[127]. Increased autophagy also has been associated with significant loss of cardiac and liver tissue in diabetic rats during attempts to achieve glycemic control through diet modification[128]. During periods of elevated glucose that occur in DM, AGEs have been shown to lead to the induction of autophagy and vascular smooth muscle proliferation that can result in atherosclerosis[129] as well as cardiomyopathy[44].

FUTURE CONSIDERATIONS

DM is a significant and growing disorder throughout the world that leads to increased disability and death through multiple complications in the nervous, cardiac, and vascular systems. Current therapies for these complications are limited. As a result, novel therapeutic strategies are required to address the cellular mechanisms of oxidant stress and cell injury that can mediate complications of DM. Given the recent discovery that cytoprotective strategies against oxidative stress, *i.e.*, EPO, employ mTOR, the mTOR signaling pathways that include AMPK and TSC1/TSC2 have become increasingly recognized as a potential targets for the treatment of the complications of DM. However, future work will need to concentrate upon the complex relationship that the programmed cell death pathways of apoptosis and autophagy hold over cellular survival and longevity to attain both efficacy and safety for mTOR targeted strategies.

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REVIEW

Technology and diabetes self-management: An integrative review

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Abstract

Technology can be used to supplement healthcare provider diabetes care by providing both educational and motivational support. Education can be provided using technology allowing patients to learn new practices and routines related to diabetes management. Technology can support daily diabetes self-management activities including blood glucose monitoring, exercising, healthy eating, taking medication, monitoring for complications, and problem-solving. This article describes an integrative review conducted to evaluate the types of technology being used to facilitate diabetes self-management and the effect of that technology on self-management and diabetes outcomes for adults living with type 2 diabetes mellitus. A literature review was conducted by searching Medline, PubMed, and Psych INFO databases using the search terms: diabetes self-management, technology, type 2 diabetes, smartphones, cell phones, and diabetes mellitus covering the years from 2008-2013. Articles relying on secondary data (editorials, systematic reviews) and articles describing study protocol only were excluded. Fourteen studies including qualitative, quasi-experimental, and randomized controlled trial designs were identified and included in the review. The review found that technological interventions had positive impacts on diabetes outcomes including improvements in hemoglobin A1C levels, diabetes self-management behaviors, and diabetes self-efficacy. Results indicate that technological interventions can benefit people living with diabetes when used in conjunction with diabetes care delivered by healthcare providers.

Key words: Diabetes mellitus; Diabetes self-management; Type 2 diabetes mellitus; Technology; Integrative review

Core tip: Technology may be used to support diabetes self-management. Both mobile phone and internet-based technological interventions have been found to support self-management behaviors of people living with diabetes. Technology can extend the reach of diabetes self-management to patient's communities and homes, provide for individualized care, and provide just-in-time information.

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INTRODUCTION

Using technology to facilitate diabetes self-management is not a new idea, but as patients become more technologically savvy, devices become more available, and new technologies emerge, the variety of technological self-management strategies increases. Recent reports indicate that 90% of Americans have cellular phones and 58% of American adults have a smartphone. Among racial groups, Caucasians and African Americans have equal percentages of ownership (90%), while Hispanic Americans have a 92% ownership percentage[1].

Technology can be used to supplement healthcare provider diabetes care by providing both educational and motivational support. Technology can extend the reach of diabetes education and support when primary care resources are insufficient or patient resources and access to care are limited[2]. Patients may have difficulty scheduling and attending diabetes education classes or meeting regularly with a diabetes educator due to time, financial, or other constraints[3]. Education can be provided using technological resources so that patients learn new practices and routines related to diabetes management. Technology can support the daily diabetes self-management activities of blood glucose monitoring, exercising, healthy eating, taking medication, monitoring for complications, and problem-solving. Visual feedback of clinical information, including these self-management activities, improves patients' ability to see how diabetes is affected by their behaviors and promotes decision-making and problem-solving. Monitoring of self-management behaviors can be motivational and allows for more frequent contact between patients and healthcare providers. This can lead to necessary changes in self-management behaviors and treatment plans[4].

The purpose of this integrative review is to evaluate the types of technology being used to facilitate diabetes self-management and the effect of that technology on self-management and diabetes outcomes for adults living with type 2 diabetes mellitus. The paper identifies technological methods for self-management, outcomes from use of technology in self-management, and future recommendations for the development of technology in diabetes self-management.

RESEARCH

Articles were identified by searching Medline, PubMed, and Psych INFO databases using the search terms: diabetes self-management, technology, type 2 diabetes, smartphones, cell phones, and diabetes mellitus covering the years from 2008-2013. Articles relying on secondary data (editorials, systematic reviews) and articles describing study protocol only were excluded. Fourteen studies including qualitative, quasi-experimental, and randomized controlled trial designs were identified and included in the review. A summary of the reviewed articles is provided in Table 1.

MOBILE PHONES

Evidence suggests that mobile health applications may be used to deliver health services and self-management tools and overcome barriers to provider access[5]. Mobile phones can offer alternatives to in-person diabetes intervention delivery and support[6]. Mobile phones provide patients the ability to process and communicate data in real time. A meta-analysis of 22 intervention studies found that mobile phone interventions led to statistically significant improvements in glycemic control and self-management[7]. A Cochrane review of computer-based diabetes self-management interventions found a small beneficial effect on blood glucose control with a larger effect noted in mobile phone interventions. Reviewers concluded that mobile phone interventions may be more effective due to convenience, increased contact with the intervention, and cues and feedback provided through the phone[8].

Six of the studies examined in this review utilized a mobile phone intervention. A pilot study qualitatively evaluated a disease management program utilizing mobile phones and gaming systems for individuals living with type 2 diabetes[9]. Participants uploaded blood glucose readings using a smartphone, received charts with blood glucose daily, weekly, and monthly trends, emailed or text-messaged healthcare providers with questions, and received reminders and messages about self-management. Qualitatively, participants reported that connecting with a healthcare provider through email was beneficial, but an initial face-to-face meeting made the email communication more meaningful. Uploading data from glucose meters to visualize trends was also beneficial for most participants. They felt that the graphs enabled them to see how their exercise and eating patterns had affected blood glucose. Participants also felt that the intervention program promoted their own general health awareness. Most participants did indicate frustration with the smartphones due to difficulty using the phone. One participant recommended using phones with which patients are already familiar for future studies. Overall, the study results indicate that mobile phones can be effective in assisting people with diabetes self-management. Personal contact with healthcare providers should be included in technological interventions and participants should be included in the decision of which type of technology to be used[9].

A second study piloted an intervention to test the feasibility of an automated, two - way text messaging system to promote blood glucose monitoring in teenagers and young adults with diabetes[10]. Participants were randomized to receive messages *via* cell phone text messaging or email for a three month time period. Reminders were sent to check blood glucose and if no response occurred, a second reminder was sent. After the blood glucose value was submitted, a positive feedback message was sent. If the value was out of range, a warning to take action and recheck blood glucose was sent. Of the 40 participants who enrolled in

Table 1 Summary of reviewed articles

Ref.	Technology	Study purpose	Brief description of intervention	Major study results
Arsand *et al*[11]	Mobile phones	Qualitative evaluation of a mobile system for monitoring of blood glucose, nutrition habits, and physical activity as motivation for increasing and benefitting from these self-management behaviors in patients with type 2 diabetes	Participants assisted with development and testing of the mobile phone application. The application included blood glucose monitoring, a step counter that downloaded to the phone, and software for recording food habits and providing feedback on how users performed in relation to their own personal goals	Participant feedback demonstrated good usability of the system and several participants made adjustments in blood glucose, food habits, and/or physical activity based on the tracked self-management behaviors
Avdal *et al*[15]	Internet-based education program	Evaluation of the effect of providing internet-based diabetes education to individuals with type 2 diabetes	Participants in the intervention group viewed individualized diabetes education, asked questions of researchers, and monitored daily blood glucose levels using the internet-based system. Control group participants received education from a diabetes nurse in a clinic setting	After six months, HA1C levels in the intervention group significantly decreased and rates of health check attendance significantly increased. No differences in HA1C or health check attendance were noted for the control group
Fisher *et al*[16]	Internet-based diabetes self-management improvement program	Comparison of effectiveness of internet-based, CASM, CAPS, and computer-administered minimal support interventions	Participants in the CASM group received an internet-based diabetes self-management improvement program that included education, goal-setting, feedback from healthcare providers, and periodic phone calls to monitor progress. CAPS participants received the same plus a 60 min in person intervention to discuss problem-solving therapy related to diabetes distress. The minimal support intervention included computer-delivered health risk appraisal and diabetes information and phone calls from healthcare providers to answer questions about the information	Significant decreases were noted for diabetes distress and significant improvements in healthy eating, physical activity, and medication adherence in all three conditions, with no significant between-group differences
Glasgow *et al*[2]	Internet based DSMP	Comparison of an internet-based DSMP, internet-based DSMP with additional support, and enhanced usual care on healthy eating, physical activity, medication-taking, HA1C, body mass index, lipids, blood pressure, and psychosocial factors	Participants were randomized to one of the three groups. The internet-based DSMP participants used a website to select individual goals related to medication adherence, physical activity, and food choices, record progress, create action plans, identify barriers to self-management, and choose problem-solving strategies. Participants in the internet-based DSMP with additional support group received the above and two follow-up phone calls and three 120 min group sessions with other study participants	Internet conditions improved health behaviors significantly compared to usual care over the 12-mo period. No significant differences were noted between the two internet-based groups. All conditions improved moderately on biological and psychosocial outcomes
Hanauer *et al*[10]	Mobile phones	Pilot study for feasibility of a fully automated, two-way text messaging system to encourage increased blood glucose monitoring	Participants were randomized to receive electronic reminders to check blood glucose levels *via* mobile phone text messaging or email reminders. Participants determined the frequency and timing of reminders. Reminders were sent to check blood glucose. After entering the value, users received motivational feedback and, if the value was out of range, a warning to take appropriate action was sent. Participants could also receive daily diabetes facts to the mobile phone or email	Compared to the email group, participants in the mobile phone group received more reminders and responded with blood glucose results significantly more often. During the first month, mobile phone group participants submitted twice as many blood glucose values as email users
Lim *et al*[12]	Mobile phones	Improve glycemic control without hypoglycemia in elderly people living with type 2 diabetes using patient-specific messages and reminders delivered to mobile phones	All participants received diabetes education and then were randomly assigned to intervention, routine care, or SMBG groups. Participants in the intervention group received glucometers with a public switched telephone network-connected cradle that automatically transferred blood glucose results to a hospital-based server. Once the data was transferred to the server, an automated system generated and sent patient-specific messages by mobile phone. Routine care participants did not receive an intervention and were told to follow-up with their current medical care. SMBG participants were told to measure blood glucose at least eight times per week	After 6 mo of follow-up, HA1C was significantly decreased from 7.8 to 7.4 in the intervention group and from 7.9 to 7.7 in the SMBG group, compared with 7.9 to 7.8 in the control group. The proportion of patients with HA1C < 7% without hypoglycemia was 30.6% in the intervention group, 23.4% in the SMBG group, and 14.0% in the control group

Lorig *et al*[17]	Internet-based self-management program	Evaluation of effect of an internet-based DSMP on HA1C, diabetes symptoms, exercise, self-efficacy, and patient activation	Participants were randomized to the internet-based program, the internet-based program with e-mail reinforcement, or a usual care control group. The internet-based program consisted of six asynchronous educational sessions, weekly learning activities, discussion boards, and individualized action plans for self-management. Participants in the reinforcement group received the intervention followed by an online discussion group	At 6 mo, HA1C, patient activation, and self-efficacy were significantly improved for program participants compared with usual care control subjects. There were no changes in other health or behavioral indicators. The subgroup with initial HA1C > 7% demonstrated greater significant improvement in HA1C. The reinforcement intervention showed no additional improvements over the intervention alone
Lyles *et al*[9]	Mobile phones and gaming system	Qualitative evaluation of a disease management program utilizing mobile phones and gaming systems for individuals living with type 2 diabetes	Participants received a smartphone to upload blood glucose values and email or text message with a healthcare provider and a gaming system to gain access to a shared medical record that provided summaries of clinical information related to diabetes	Participants expressed frustration with using cell phones and gaming system, but liked collaborating with a healthcare provider on uploaded glucoses and receiving automatic feedback on blood glucose trends
Noh *et al*[18]	Internet-based information system for computers and mobile phones	Evaluation of the effect of a computer and mobile phone accessible internet-based system on blood glucose control	A web-based information system for mobile phone users and a website for Internet users provided diabetes education. Participants in this group were compared to a control group receiving conventional diabetes education	HA1C and postprandial glucose levels were significantly decreased in the intervention group, but not in the control group. There was a significant relationship between the change in HA1C and the frequency of web-based system access
Nundy *et al*[13]	Mobile phones	Qualitative exploration of mechanisms by which a text-message based diabetes program affected self-management among African-Americans living with type 2 diabetes	Participants completed a 4-wk text messaged based diabetes program in which they received text message reminders about diabetes self-management	Themes that emerged from the study included self-awareness and control of diabetes, reinforcement of success in managing diabetes, acceptance and awareness of seriousness of diabetes, and caring and support
Pacaud *et al*[19]	Internet-based system to provide education for newly diagnosed people with type 2 diabetes	Comparison of three varied media educational systems on diabetes knowledge, self-efficacy, and diabetes self-management activities	Participants were randomly assigned to either the web interactive group who received electronic education and virtual appointments using both synchronous and asynchronous communication, the web static group who received electronic education and virtual appointments using asynchronous communication, or the control group who received face-to-face education and synchronous and asynchronous communication	All three groups had similar improvements in diabetes knowledge, self-efficacy, and diabetes self-care activities. Independent of which group subjects were randomized to, findings were significant when examining correlation between website usage and outcomes: a higher total use was significantly associated with a higher diabetes knowledge score, a higher total diabetes self-efficacy score, and lower HA1C by final study visit
Quinn *et al*[14]	Mobile phones	Evaluation of a diabetes coaching system that used mobile phones and patient-provider portals for individualized treatment and communication	Participants were randomly assigned to one of three treatment conditions or a usual care control group. Participants utilized a patient-coaching system consisting of a mobile diabetes management software application that allowed them to enter diabetes self-care data including blood glucose values, carbohydrate intake, and medications into mobile phones and receive automated, real-time educational, behavioral, and motivational messages related to entered data. The intervention also included a web portal consisting of a secure messaging center for patient and provider communication, personal health record, a learning library, and logbook to review entered data	The mobile phone-based intervention significantly improved HA1C compared to the usual care group. No differences were observed between groups for diabetes distress, depression, diabetes symptoms, blood pressure, or lipid levels
Song *et al*[3]	Internet-based DSMP	Evaluation of the efficacy of an internet-based diabetes self-management education program for newly diagnosed patients with type 2 diabetes as an alternative to group lectures	Participants in the intervention group participated in an internet-based diabetes self-management program and control group participants attended three hours of group lectures provided by healthcare professionals specializing in diabetes care	HA1C and diabetes care knowledge improved significantly in the intervention group at six weeks and diabetes care behaviors improved significantly at six weeks and three months. Diabetes

				care knowledge and diabetes care behaviors improved significantly in the control group at six weeks, but HA1C did not significantly change at six weeks or three months
Tang *et al*[20]	Internet-based system	Evaluation of an online disease management system supporting patients with uncontrolled type 2 diabetes	Multicomponent intervention that included: wirelessly uploaded home glucometer readings with graphical feedback; patient-specific diabetes summary status report; nutrition and exercise logs; insulin record; online messaging with healthcare providers; self-management advice and medication management; and personalized text and video educational messages	Participants in the intervention group had significantly reduced HA1C levels at 6 mo compared to a usual care group. At 12 mo, the differences were not significant

SMBG: Self-monitored blood glucose; CASM: Computer-assisted self-management; CAPS: Computer-assisted self-management plus diabetes distress specific problem-solving; DSMP: Diabetes self-management program; HA1C: Hemoglobin A1C.

the study, 22 were randomized to the cell phone group and 18 to the email group; however, only 18 of the cell phone group and 11 in the email group actually used the system. Participants in the cell phone group requested more reminders and submitted more blood glucose values than those in the email group, but over time both groups significantly decreased the number of requested reminders and submitted blood glucose values[10].

In another study, researchers developed a mobile phone application in conjunction with 12 people living with type 2 diabetes to assist with self-management[11]. Participants participated in focus groups and feasibility testing during development of the application. Qualitative interviews were conducted at study conclusion. The application included blood glucose monitoring, step counter, software for recording food habits, and feedback based on personal goals established prior to application use. Results of the study were positive. The majority of participants utilized the blood glucose sensor system one or more times per day and had a slight decrease in average blood glucose over the study period. As a group, participants had a reduced intake of carbohydrate-rich foods by the end of the study compared with the beginning of the study. Some participants found daily entry of consumed foods to be a tedious task. The step counter automatically transferred number of steps to the mobile phone once per day. Overall, participants increased their number of daily steps from study beginning to end. Participants especially liked the tips and feedback related to personal goals[11].

A study designed to improve glycemic control without hypoglycemia in elderly people living with type 2 diabetes utilized mobile phones for the intervention[12]. Participants received a glucometer that downloaded to a hospital-based server and based on the data, patient-specific messages were generated and sent to their mobile phone. Text messages included instructions about changes to medications based on blood glucose values and reminders to check blood glucose as instructed. The intervention group had significantly lower hemoglobin A1C (HA1C) values compared to two control groups at six months follow-up. Participants in the intervention group did have higher rates of hypoglycemia than participants in the two control conditions, but the difference was not statistically significant[12].

Support for diabetes management has been provided through text messaging using mobile phones. A sample of 18 African American people living with type 2 diabetes completed a 4 wk text message diabetes program[13]. Participants were required to receive a daily medication reminder, question about medication adherence, question about foot care, and appointment reminders for diabetes-related visits. Participants could also receive additional diabetes management text reminders if desired. A certified diabetes educator (CDE) phoned participants weekly to obtain feedback on the experience and make adjustments to the personalized text message. The CDE did not provide any education, counselling, or clinical support for participants. Qualitative interviews revealed that the text message program reinforced the importance of self-management, increased awareness of diabetes, and improved feelings of control over diabetes[13].

A mobile diabetes intervention study examined the effect of mobile phones and patient and provider portals for individualized patient treatment and communication on HA1C levels[14]. Participants with type 2 diabetes enrolled in the one year study and utilized a patient-coaching system consisting of a mobile diabetes management software application that allowed them to enter diabetes self-care data including blood glucose values, carbohydrate intake, and medications. After entering this data into mobile phones, participants received automated, real-time educational, behavioral, and motivational messages related to entered data. The intervention also included a web portal consisting of a secure messaging center for patient and provider communication, personal health record, a learning library, and logbook to review entered data. Researchers found a statistically significant improvement in HA1C levels in the intervention group compared to a usual care control group. The study did not evaluate how the mobile intervention affected behavior leading to

blood glucose changes. How the intervention affected medication adherence, physical activity, quantity and quality of patient-provider communication, and treatment intensification are important variables which should be considered in future studies[14].

Of the six mobile phone intervention studies, three were qualitative and three were randomized controlled trials. Participants in the qualitative studies generally reported positive outcomes from using the mobile phone intervention. Participants appreciate the personalized feedback and education received from the intervention[11]. Participants in randomized controlled trials using a mobile phone intervention noted improvements in HA1C levels[12,14]. Overall, mobile phone interventions had small sample sizes making generalization of study findings difficult.

INTERNET-BASED

Internet diabetes interventions provide opportunities to offer diabetes education, support, and motivation for self-management behaviors[2]. Web-based learning provides easy access without time or location restrictions and allows users to work at their own pace[3].

A randomized controlled trial evaluated the effects of web-based diabetes education on HA1C levels and health check attendance[15]. Participants in both the experimental and control groups had completed basic diabetes education prior to this study. After six months of individualized patient education delivered over the web, the experimental group had significant decreases in HA1C and significantly higher health check attendance rates compared to the control group who received education from a diabetes nurse in a polyclinic setting[15]. No information was provided regarding amount of time participants spent accessing diabetes education either over the web or in the polyclinic.

A second randomized controlled trial aimed at reducing distress and enhancing effective management of type 2 diabetes compared three interventions to reduce diabetes distress and improve self-management[16]. The study enrolled 392 participants who were randomly assigned to computer-assisted self-management, computer-assisted self-management plus diabetes distress-specific problem-solving, or a computer-administrated minimal support intervention. Computer-assisted self-management included a web-based diabetes self-management improvement program that allows patients to select goals for medication adherence, diet, or exercise and monitor those goals. Participants in this group also had access to a forum to ask questions of diabetes experts and received phone calls from an interventionist to monitor progress and problems. The second group received this same computer-assisted self-management plus problem-solving therapy specifically for diabetes distress. The third intervention group received a computer-delivered health risk appraisal and diabetes information regarding healthy living, diet, and physical activity. Significant decreases in diabetes distress, emotional burden, and regimen distress occurred in all three groups with no significant between-group differences. The study did not include a usual care control group so the effect of attention alone could not be measured[16].

Similarly, a three-arm randomized controlled trial compared computer-assisted diabetes self-management, computer-assisted diabetes self-management plus human support, and enhanced usual care[2]. Participants in the computer-assisted diabetes self-management program (DSMP) selected achievable goals in the areas of medication adherence, physical activity, and food choices. They were able to view displays of their biophysical data, record progress toward goals, participate in a moderated forum, and view diabetes self-management information. Participants in this group also received periodic motivational calls. The computer-assisted plus human support group received the same computer intervention and received follow-up calls from an interventionist and opportunities to attend group educational sessions. The internet interventions significantly improved health behaviors including eating habits and adherence to medications compared to usual care over the 12 mo study period. All three conditions moderately improved self-efficacy, problem-solving, and HA1C[2].

A six month randomized trial evaluated the effect of an internet-based DSMP on HA1C, diabetes symptoms, exercise, self-efficacy, and patient activation[17]. Participants were randomized to the intervention, intervention plus email reinforcement, or usual care. The internet-based DSMP consisted of six weekly sessions that participants could view asynchronously anytime during the week. The site also contained a learning center where participants could respond to a posed question and develop an action plan for dealing with diabetes-related problems. A discussion center included interactive threaded discussion boards viewable by all participants where comments, questions, and discussions could be posted. Lastly, a help section was included that allowed participants to email program administrators. The study had a large sample size with 732 completing the six months study and 645 completing the six months completion questionnaire. Following the 18 mo reinforcement period, 528 participants completed questionnaires. At six months, HA1C, self-efficacy, and patient activation were significantly improved for intervention group participants compared to usual care participants. The subgroup with a pre-study HA1C greater than seven demonstrated stronger improvement in HA1C. No significant changes were noted for diabetes symptoms and exercise. Reinforcement did not affect study outcomes. Those in the intervention plus email reinforcement had no significant improvements compared to the intervention group. Researchers recommend follow-up to determine if the type of reinforcement was not beneficial or if it was not properly utilized[17].

A randomized controlled trial was conducted to

evaluate the effect of a web-based comprehensive information system on blood glucose control[18]. The system was available using a computer or cellular phone. The system provided real-time information about diet, dining out, hypoglycemia, sick day management, stress management, and diabetes management. HA1C and postprandial blood glucose were levels were significantly decreased in the intervention group, but not the control group after six months. There was a significant relationship between the HA1C change and frequency of website access with greater decreases in HA1C associated with higher website usage. The most frequently accessed information using cellular phones was the dining out section which may have contributed to improved postprandial glucose levels. Participants accessed the website more often using the cellular phone than the computer[18].

A randomized controlled trial found that electronic presentation of diabetes education was as effective as traditional face-to-face education in newly diagnosed patients with type 2 diabetes[19]. A total of 68 participants were randomly assigned to one of three educational models. The control group received structured diabetes education in a traditional classroom setting. This group also had direct verbal communication with health care providers. The second group had access to electronic educational materials and tools and used asynchronous communication through email for interactions with providers. The third group had access to electronic education materials and tools, used asynchronous and synchronous communication with providers and patients, and used an electronic blood glucose journal and other functions. All three groups had similar improvements in diabetes knowledge, self-efficacy, diabetes self-care activities, and HA1C with no significant between-group differences[19].

A quasi-experimental design study was conducted to compare a web-based diabetes education program with a traditional classroom diabetes education program for newly diagnosed adults with type 2 diabetes[3]. The web-based program included six education modules covering diabetes basics, dietary management, exercise, medications, stress management, and foot care. The website also included a password-protected space where participants could enter glucose levels and see a display of those levels, calculate caloric content of meals consumed, record activities, and measure daily stress levels. Participants in the control group attended one hour lectures every week for three consecutive weeks in a group setting consisting of 30 to 40 participants that were taught by a diabetes care specialist nurse, dietician, and physician. Diabetes knowledge, care behaviors, and glycemic control were compared for the intervention and control groups at baseline, six weeks, and three months. Diabetes care knowledge significantly increased in both intervention and control groups from baseline to six weeks, but not from six weeks to three months. Diabetes care behaviors significantly increased in both groups from baseline to six weeks and also significantly increased from six weeks to three months in the intervention group. HA1C levels for the intervention group significantly decreased from baseline to six weeks, but not from six weeks to three months. No differences in HA1C were found in the control group. Limitations of this study include the small sample size (31 participants) and the lack of random group assignment. Participants in the intervention group were required to have the ability to use the internet which prevented random assignment. The improvement in diabetes care behaviors and HA1C in the intervention group offers promise for using web-based diabetes education as a substitute for group education[3].

A randomized controlled trial evaluated an online diabetes management system for patients with uncontrolled type 2 diabetes[20]. A usual care control group was compared to an intervention group that utilized an online disease management program that included wireless uploading of glucose readings, individualized diabetes summary status reports, nutrition and exercise logs, insulin records, online messaging with the health care team, advice and medication management from a nurse care manager and dietician, and personalized educational information. Participants in the intervention group had significantly lower HA1C levels at 6 mo compared to the control group, but at 12 mo, the difference was no longer significant. As in other studies, participants who utilized the online system more often achieved greater benefits[20].

Internet interventions include education, goal-setting, tracking of behaviors, patient feedback and support. Of the eight internet studies reviewed, seven were randomized controlled trials and the remaining study had a quasi-experimental design. All studies that measured changes in HA1C levels noted improvements and all improvements were significant with one exception[2]. In two of the studies, short-term improvements were noted in HA1C, but not at the second, long-term follow-up[3,20]. Several studies noted improvements in outcomes in both intervention and control groups[2,3,16,19].

CONCLUSION

Previous reviews found mixed results with some noting significant improvements in HA1C and self-management behaviors[8]. This review found mainly positive results though some interventions had no effect or only short term improvements. It is important to note that greater usage of technological interventions, both mobile and internet-based, was associated with greater improvements in outcomes[18,20]. One of the reviewed studies included a web-based intervention that could be accessed using the computer or mobile phone. Researchers found that participants in the mobile phone group accessed the site more often than those using the computer[18]. Mobile phone interventions are an important source of diabetes self-management

to pursue as their convenience may increase access of information and support for people living with diabetes.

Due to time constraints of both patients and healthcare providers, web-based education and monitoring may be beneficial and can be used to complement healthcare provider visits[15]. Increased access, whether in-person or electronic, to diabetes education and healthcare providers can improve diabetes knowledge and self-efficacy[19]. The increased use of diabetes-related mobile applications indicates that people living with diabetes are interested in using these methods to improve self-management and diabetes outcomes. The use of applications to provide education and real-time feedback needs to be developed[5].

RECOMMENDATIONS

While technology can be effective for promoting diabetes education, support, and self-management, patients report a need for personal contact with health care providers in addition to technology[9,13]. In the study by Nundy *et al*[13], automated text messages were sent, but participants stated they preferred to think of them as coming from the certified diabetes educator (CDE) who enrolled them in the study. They also appreciated the weekly calls from the CDE to obtain feedback on the experience and make adjustments to text messaging as needed. Some participants felt the text messaging intervention would not be effective for them without a person to monitor and provide clinical support[13]. A website that provides diabetes education, monitoring, and support through communication with a healthcare provider may be most effective[15]. Web-based interventions can be used in conjunction with healthcare provider education and support and as a follow-up to healthcare provider interventions[21].

Researchers and healthcare providers should include participants in the development of technological interventions and in the decision of which technology to use[9]. Patient needs must be explored to determine the best method for individual needs realizing that not all patients will be amenable to technological interventions[21]. A previous review of mobile diabetes applications found that current applications are lacking personalized education and decision support features are not being included. Additionally, inclusion of peer support features through mobile applications are largely underused and could be beneficial for people living with diabetes[22].

IMPLICATIONS FOR PRACTICE

Healthcare providers should actively select and adapt technological self-management methods to extend the reach of diabetes self-management to patient's communities and homes, provide for individualized care, and provide just-in-time information. People living with diabetes who have limited access to care due to lack of transportation, physical restrictions, or other limitations could benefit from technological interventions that bring care to them[21]. Additionally, with limited primary care resources, technology can provide cost-effective ongoing diabetes self-management education and support[2].

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REVIEW

Type 2 diabetes after gestational diabetes: The influence of changing diagnostic criteria

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Abstract

A previous diagnosis of gestational diabetes (GDM) carries a lifetime risk of progression to type 2 diabetes of up to 60%. Identification of those women at higher risk of progression to diabetes allows the timely introduction of measures to delay or prevent diabetes onset. However, there is a large degree of variability in the literature with regard to the proportion of women with a history of GDM who go on to develop diabetes. Heterogeneity between cohorts with regard to diagnostic criteria used, duration of follow-up, and the characteristics of the study population limit the ability to make meaningful comparisons across studies. As the new International Association for Diabetes in Pregnancy Study Group criteria are increasingly adopted worldwide, the prevalence of GDM is set to increase by two-to three-fold. Here, we review the literature to examine the evolution of diagnostic criteria for GDM, the implications of changing criteria on the proportion of women with previous GDM progressing to diabetes, and how the use of different diagnostic criteria may influence the development of appropriate follow-up strategies.

Key words: Gestational diabetes; Pregnancy; Type 2 diabetes; Impaired glucose tolerance; Diagnostic oral glucose tolerance test criteria

Core tip: Gestational diabetes (GDM) is associated with a greatly increased future risk of type 2 diabetes, but there are many different GDM diagnostic criteria in clinical use. Criteria with lower glucose thresholds increase GDM prevalence, and therefore the number of women requiring follow-up to detect progression to diabetes. However, lower diagnostic thresholds are also likely to decrease the proportion that progress to diabetes. Heterogeneity across studies with regard to diagnostic criteria, demographics, and duration of follow-up, limit direct comparison. As the International Association of Diabetes in Pregnancy Study Groups criteria enter widespread use, follow-up of these women will be an important issue.

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INTRODUCTION

Background

Gestational diabetes (GDM) has long been recognised clinically. First described in pregnancy in 1824 in Germany[1], Joslin[2] described in 1916 a case of diabetes which presented in pregnancy, resolved with delivery, and recurred later in life. In the 1940s and 1950s, Hoet *et al*[3] recognised the association of this type of diabetes with adverse perinatal outcome, and characterised the relationship between glucose tolerance during pregnancy, and in the post-partum period. However, despite the long-recognised association, no standardised criteria for diagnosis were devised until 1964. In Boston City Hospital, O'Sullivan *et al*[4] carried out 3-h 100 g oral glucose tolerance tests on 752 patients at different stages of pregnancy. Women with 2 out of 4 values that were greater than 2 standard deviations (rounded to the nearest 5 mg/dL) above the mean glucose levels determined in this cohort were classified as having GDM. These criteria (with some modification) have continued in clinical use over the following four decades.

Evolution of diagnostic criteria for GDM

The major feature of these criteria was that they defined a cohort of women with a greatly increased future risk of progression to type 2 diabetes, demonstrating a lifetime risk of up to 60%[5]. The National Diabetes Data Group (NDDG) criteria, proposed in 1979[6] (Table 1), converted the O'Sullivan/Mahan criteria from whole blood to plasma values (see Figure 1 for timeline). The Carpenter-Coustan criteria[7], proposed in 1982, also converted the O'Sullivan/Mahan criteria to plasma values, but in addition, took a change in enzymatic methods into account. They soon entered widespread clinical use, and were subsequently validated for prediction of adverse perinatal outcome[8-12]. Essentially, therefore, all 3 sets of criteria were intended to define a similar population.

Studies directly comparing the prevalence of GDM by either NDDG or Carpenter-Coustan criteria show, however, significant differences, with an approximately 50% relative increase in GDM prevalence if the Carpenter-Coustan criteria are used[9,11-13]. In addition, in 2001, the American Diabetes Association (ADA), having previously endorsed the Carpenter-Coustan criteria, also allowed for the use of a 75 g, 2-h oral glucose tolerance test (OGTT) to make a diagnosis of GDM, using the same one- and two-hour cut-offs as the three-hour 100 g OGTT. The post-load glucose levels are estimated as being 0.9 mmol/L lower at one hour, and 0.5 mmol/L lower at two hours with the lower glucose load[14], therefore these criteria will identify a different group of women. Indeed, only weak diagnostic agreement has been noted between the two glucose loads[15] (Cohen kappa index 0.18; although some this difference may also be attributable to day-to-day glycaemic variability).

The World Health Organisation (WHO) also recommended alternative criteria for the diagnosis of gestational diabetes beginning in 1980 (the 1965 WHO report did not comment on this issue). These thresholds were the same as those for non-pregnant adults. Initially, the WHO recommended a fasting glucose threshold of 8 mmol/L (see Table 1). These recommendations were revised again in 1985[16] (fasting glucose threshold lowered to 7.8 mmol/L, recommendation to treat impaired glucose tolerance added) and 1999[17] (fasting glucose threshold reduced to 7.0 mmol/L) (see Table 1). Although these thresholds were not chosen on the basis of predicting adverse pregnancy outcome, a subsequent large (n = 4998) prospective cohort study did show that these thresholds predicted increased risk for macrosomia (RR = 1.45, 95%CI: 1.06-1.95) and preeclampsia (1.94, 95%CI: 1.22-3.03), even when women with values diagnostic of diabetes in the nonpregnant adult[18].

The European Association for the Study of Diabetes also proposed new GDM criteria in 1996[19], using a fasting value of 6.0 mmol/L and a two-hour post 75 g glucose load value of 9.0 mmol/L, based on the distribution of glucose values on 75 g OGTTs on over 1000 European women. A subsequent retrospective cohort study supported this 2-h value in prediction of adverse perinatal outcome[20]. However, subsequent analysis of women in this cohort, with 2-h values below the 2-h threshold of 9.0 mmol/L (not treated for GDM), demonstrated a linear relationship between 2-h glucose and pregnancy outcome, with no clear threshold value[21].

In addition to these major criteria, multiple different diagnostic criteria are in use worldwide, some related to older criteria, some derived on the basis of local data. Therefore, the situation still exists where different centres in the same country, or even the same region, may employ different criteria for GDM diagnosis.

GDM criteria to predict adverse perinatal outcome

However, none of the available criteria had been designed specifically to predict adverse pregnancy outcome. To look at this issue, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) convened a consensus conference in 2008 to review the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study findings (published and unpublished), along with other relevant studies. This consensus conference had two major outcomes[22]. Firstly, women meeting the cut-off values for diagnosis of diabetes in the non-pregnant adult (Table 1) would now fall into the new category of "overt diabetes" rather than GDM. The rationale for this was that

Table 1 Comparison of thresholds for criteria for gestational diabetes diagnosis

Criteria	Glucose load	Fasting glucose mmol/L (mg/dL)	1-h glucose mmol/L (mg/dL)	2-h glucose mmol/L (mg/dL)	3-h glucose mmol/L (mg/dL)	No. of criteria required
O'Sullivan *et al*[4]	100 g	5 (90)	9.2 (165)	8.1 (145)	6.9 (125)	⩾ 2
NDDG	100 g	5.8 (105)	10.6 (190)	9.2 (165)	8.1 (145)	⩾ 2
WHO 1980	75 g	8 (144)	N/A	8 (144)	N/A	⩾ 1
Carpenter and Coustan	100 g	5.3 (95)	10 (180)	8.6 (155)	7.8 (140)	⩾ 2
ADA	75 g or 100 g	5.3 (95)	10 (180)	8.6 (155)	7.8 (140)	⩾ 2
WHO 1985	75 g	7.8 (140)	N/A	7.8 (140)	N/A	⩾ 1
EASD	75 g	6 (108)	N/A	9 (162)	N/A	⩾ 1
WHO 1999	75 g	7 (126)	N/A	7.8 (140)	N/A	⩾ 1
IADPSG GDM	75 g	5.1 (92)	10 (180)	8.5 (153)	N/A	⩾ 1
IADPSG overt diabetes	[a]None/75 g	7 (126)	N/A	11.1 (200)	N/A	⩾ 1

[a]This diagnosis can also be made on a random glucose sample, a fasting glucose sample, or on an HbA1c value [if 6.5% (48 mmol/mol or over)]. NDDG: National Diabetes Data Group; WHO: World Health Organisation; EASD: European Association for the Study of Diabetes; ADA: American Diabetes Association; IADPSG: International Association of Diabetes and Pregnancy Study Groups; GDM: Gestational diabetes; N/A: Not applicable.

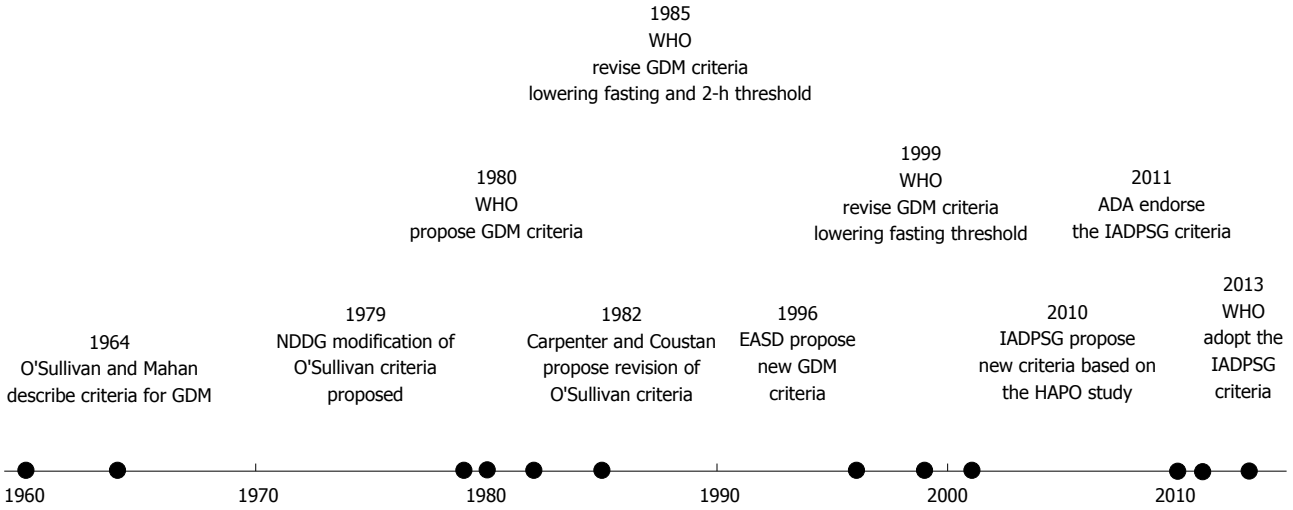

Figure 1 Timeline of evolution of criteria used to diagnose gestational diabetes from 1964-present. NDDG: National Diabetes Data Group; WHO: World Health Organisation; EASD: European Association for the Study of Diabetes; ADA: American Diabetes Association; IADPSG: International Association of Diabetes and Pregnancy Study Groups; GDM: Gestational diabetes; HAPO: Hyperglycemia and pregnancy outcomes study.

this group were felt to be distinct clinically and biochemically from women with milder degrees of hyperglycaemia. Secondly, the data from the 2008 HAPO study[23] was reviewed. This large (over 25000 participants screened), multicentre study showed that glucose levels at all time points on the 2-h 75 g OGTT were associated with adverse pregnancy outcomes (large for gestational age, macrosomia, cord c-peptide concentration greater than the 90th centile). In the absence of a clear threshold effect, and having considered various cutpoints, the IADPSG consensus committee ultimately decided to set new values for GDM diagnosis at the mean glucose values for which the odds ratio for adverse pregnancy outcome was 1.75. This lowered the fasting and 1-h values compared to previous values, while raising the 2-h value slightly. However, the major change was allowing a diagnosis to be made on just a single abnormal value, a change likely to greatly increase the prevalence of gestational diabetes. On applying these criteria retrospectively to the HAPO cohort, 17.8% (range 9.3%-25.5%) met the criteria for diagnosis[24].

These consensus criteria were published in March 2010, and began to enter clinical use shortly afterwards. At the time of writing, in addition to the IADPSG endorsing the criteria, the ADA[25] and WHO[26] have also endorsed the criteria. However, the American College of Obstetricians and Gynaecologists (ACOG) have not adopted the new criteria, and still recommend a 100 g OGTT using the Carpenter-Coustan criteria, for diagnosis, a position endorsed by a National Institute of Health Consensus Conference in March 2013[27].

With this in mind, we will review the impact of changing criteria for GDM diagnosis with regard to the prevalence/cumulative incidence of abnormal glucose tolerance/diabetes post GDM, risk factors for progression to diabetes, and follow-up of women with previous GDM. This is a clinically relevant problem for 2 major reasons - firstly, prevention or delay of type 2 diabetes in women with previous GDM is a possibility,

as demonstrated by a subgroup analysis of the diabetes prevention program[28], and the Troglitazone In the Prevention Of Diabetes[29] and Pioglitazone In the Prevention Of Diabetes[30] studies. Secondly, undetected type 2 diabetes developing prior to a subsequent pregnancy carries the risk of congenital malformation and an increased risk of pregnancy complications.

HETEROGENEITY OF STUDIED COHORTS

Many studies have assessed the risk of progression to type 2 diabetes post gestational diabetes.

A major issue with all studies in this area however, is their marked heterogeneity. This is seen in several ways: (1) As discussed, the diagnostic criteria in clinical use for GDM diagnosis over the last four decades are numerous. This leads to the identification of cohorts who may not be directly comparable in terms of the severity of glucose intolerance; (2) Both the criteria and method used to diagnose diabetes and/or abnormal glucose tolerance in women who have previously had GDM varies significantly; (3) The ethnic mix of the cohorts is extremely heterogeneous with some composed entirely of a single ethnicity, and others showing very mixed composition; and (4) Duration of follow-up varies between studies, from 6 wk to almost 30 years[31].

In summary, meaningful comparison of the actual cumulative incidence or prevalence across studies is not possible. It is clear, however, that regardless of the criteria used, GDM signifies a high risk of future progression to type 2 diabetes.

RISK FACTORS FOR FUTURE PROGRESSION

Despite the heterogeneity of the cohorts, many studies identify similar factors predicting progression to diabetes/abnormal glucose tolerance. We will consider the most commonly associated risk factors here.

Pre-pregnancy factors

Given that most studies identify women with GDM at the time of diagnosis, most studies assess pre-pregnancy risk factors retrospectively. Therefore, information on this is limited. The exception to retrospective recall of pre-pregnancy factors is the large long-term longitudinal cohort population-based studies, such as the Nurse Health Study[32], which have detailed information preceding the index pregnancy. However, these also use self-reported GDM as an outcome measure. Although the diagnosis has been validated in a subset by medical record review, the precise criteria used by the healthcare provider are uncertain, and therefore lie outside the scope of this review. Of pre-pregnancy variables assessed, weight or BMI is the most common measure, and is commonly associated with increased risk of progression to abnormal glucose tolerance or diabetes[33-38], although the relationship is not particularly strong. Polycystic ovary syndrome has also been reported in a one retrospective study to be associated with later progression to abnormal glucose tolerance[39] on multivariable analysis, although this study used two different sets of criteria to diagnose GDM.

Index pregnancy-related factors

Pregnancy glucose values: Higher glucose values during pregnancy, as reflected by the index pregnancy OGTT, are consistently associated with increased later progression to diabetes. This is measured in various ways (number of abnormal values, area under the curve), but most commonly the values for plasma glucose at fasting, one hour, two hours (and three hours if applicable) are used. Fasting glucose shows the strongest association, being the most commonly identified risk factor associated with later abnormal glucose tolerance and diabetes[31,40-45]. Studies that have not identified fasting glucose as a factor associated with later progression to abnormal glucose tolerance tend to have either not measured it[46], not included it in the statistical models[47], or have excluded women with the highest fasting glucose levels from follow-up[48,49]. One large Australian study found fasting glucose was not associated with later abnormal glucose tolerance and diabetes despite its inclusion in the model[50]. One-hour[48,50,51] and two-hour glucose levels[37,40,51,52] are also associated with later glucose abnormalities, although less consistently, and to varying degrees. Also, higher haemoglobin (HbA1c) during pregnancy, although much less frequently studied, has been found to be associated with future risk of progression to diabetes[52,53].

More detailed characterisation of glycaemic response to a glucose load such as measures of insulin secretion[43], when undertaken, are also associated with later progression to abnormal glucose tolerance and diabetes. These measures, of course, are generally not available routinely clinically. Insulin use during pregnancy has also frequently been shown to be associated with increased risk of future progression to diabetes/abnormal glucose tolerance[36,46,54-56], presumably as a marker of higher glucose levels in pregnancy, even taking into account likely differences in prescribing practice between centres.

Body weight/body mass index: Body weight [or body mass index (BMI)] during the index pregnancy is commonly reported in studies of GDM cohorts, occasionally with waist circumference or body fat measurements. Studies are inconsistent as to whether weight or BMI persist as a risk factor when adjusted for other risk factors using multivariate analysis. Studies that have not found an association between pregnancy weight and BMI tend to examine women who have progressed to abnormal glucose tolerance in the early post-partum period. BMI during pregnancy may be

associated with abnormal glucose tolerance at this stage, but is not independently associated when antepartum glucose levels (indicating severity of hyperglycaemia) are included in the model[41]. Most studies that do show an association between pregnancy BMI and later abnormal glucose tolerance, independent of antepartum glucose measurements, involve longer-term follow-up post delivery[43,57,58], although this is not a universal finding[59].

Gestational age at diagnosis: Gestational age at diagnosis is another commonly reported association[37,38,41,42,44,60,61]. However, many of the studies also specify a screening protocol that involves screening higher-risk women in early pregnancy, causing a significant bias. Women diagnosed with GDM in early pregnancy, before insulin resistance begins to rise[62,63], are likely to have a greater degree of hyperglycaemia, and therefore an increased likelihood of progression to abnormal glucose tolerance/diabetes. However, gestational age at diagnosis remains a risk factor, even when measures of glycaemia from the index pregnancy are included in the model, in many of these studies[41,42,44,60,61].

Ethnicity: There are few studies specifically examining the effects of ethnicity, although these that do have generally found an increased prevalence among those women of ethnicity other than white European origin[47,64-68]. Other studies have found no association[40,69]. The reasons for this are unclear. However, many studies have examined ethnically homogenous cohorts, who are often already at high risk of GDM. The prevalence of GDM is higher among ethnic groups who are not of white European origin, while the prevalence of GDM increases at a lower BMI[70] in the Asian populations studied. In addition, adoption of the IADPSG criteria may cause a disproportionate rise in GDM prevalence among Asian populations[71], which will be of relevance when determining the future risk of abnormal glucose tolerance or diabetes in these populations. In addition to the studies outlined above examining this question, comparison between studies does suggest a higher proportion of women of non-white European ethnicity progress to abnormal glucose tolerance[68]. However, meaningful comparison between studies is generally not possible due to the heterogeneity of the studies on the points listed above.

Family history of diabetes: This is uncommonly associated with progression to abnormal glucose tolerance/diabetes among women with GDM after measures of glycaemia are taken into account. Several studies examining family history have found no effect[49,72,73]. Although some studies have shown an independent effect[39,47,59,74], it appears to be small, and the association is often not seen when analysed as part of a multivariate model[38,58,75,76]. Therefore, family history does not appear to play a major independent role in predicting future risk of diabetes or abnormal glucose tolerance.

Other factors: Age at diagnosis of GDM[44,52,54,76] has been associated with future abnormal glucose tolerance or diabetes also, but is inconsistent, with other studies showing no association[57,77,78], and again, is rarely significant[54] when other variables are taken into account. Parity, most commonly classified as a binary variable (multiparous or nulliparous) has been identified[53,55,79] as potentially associated with higher risk of progression later, but this finding is inconsistent[41,78]. Potential gene associations have also been identified, but currently appear to add little to clinically assessing individual risk[80]. Autoantibody testing also been examined[81], and appears to be associated with risk of progression to type 1 rather than type 2 diabetes.

Risk factors post-pregnancy

Breastfeeding: Breastfeeding among women with GDM is associated with improved glycaemic indices in the early post-partum period[47,82]. Its role in prevention of later progression to abnormal glucose tolerance is at present unclear, although long-term follow-up of the Study of Women, Infant Feeding and type 2 diabetes mellitus after GDM (SWIFT) pregnancy cohort will address this issue.

Body weight/BMI: Weight (or associated measures) after the index pregnancy has been shown to be correlated in a number of studies[33,56,59,83-86] with progression to diabetes or abnormal glucose tolerance. This correlation appears more robust than that seen with pregnancy weight/BMI, which often loses significance in multivariable models (see above). Also, weight gain since the index pregnancy has been associated with metabolic deterioration[43]. Studies not demonstrating BMI as a predictive factor may take high-risk cohorts, for example, entirely composed of participants with postpartum impaired glucose tolerance[87], or are carried out in the early post-partum period[41,69,88]. Interestingly, Wang *et al*[84] showed that both waist circumference and body fat performed better than BMI in predicting type 2 diabetes in a Chinese cohort, while Jang demonstrated that waist circumference showed a stronger association than BMI in a Korean cohort[37]. This may help to explain why some Asian cohorts[38,87] have not demonstrated an association between BMI and future abnormal glucose tolerance or diabetes, despite longer-term follow-up.

Others: The type of contraceptive - specifically the progesterone-only oral contraceptive - is thought to confer a higher risk[89]. Subsequent GDM is also associated with greater risk of progression to diabetes/abnormal glucose tolerance[83]. Age at follow-up is commonly reported. Although an association with later abnormal glucose tolerance has been noted[40,44,90-92], and despite the increasing prevalence of type 2 diabetes with advancing age in the general population, this is not a universal finding[38,93], particularly in multivariate analysis[94]. This may be due to the relatively small difference in ages within the cohorts of women involved in these studies, compared to the population as a whole.

Despite the heterogeneity of the studies for the reasons above, including diagnostic criteria used, there is consistency among most studies in the factors associated with a greater risk of diabetes after the index pregnancy in women with GDM. As can be seen, measures of glycaemia during the index pregnancy are not only the strongest predictor, but also frequently attenuate or remove the predictive ability of other traditional risk factors for type 2 diabetes. Thus, the most important risk factor for future abnormal glucose tolerance or diabetes in these women is simply a previous diagnosis of GDM, taking into account the degree of hyperglycaemia at diagnosis.

PREVALENCE OF DIABETES POST-GDM

The prevalence of progression from GDM to abnormal glucose or type 2 diabetes varies greatly. The lifetime cumulative incidence of diabetes among women with GDM is frequently cited at up to 60%, but this summary figure does not illustrate the many underlying different factors (*e.g.*, time since delivery, cohort demographics, and criteria for diagnosis of GDM and postpartum diabetes).

Duration of follow-up

With regard to timing, many studies have documented short-term follow-up only (*i.e.*, to the first post-partum test). Prevalence rates for diabetes at this time point differ, and are generally less than 10%, but depending on the cohort studied, and criteria used, may be significantly higher - Metzger *et al*[40] showed a prevalence of 38% up to one year post-partum in women meeting NDDG criteria[40]. These women are likely to be different from those developing diabetes at a later post-partum interval, and are more likely to have had pre-existing type 2 diabetes. It is therefore unlikely that any of the criteria in use for GDM diagnosis would fail to detect these women.

Beyond the post-partum period, prevalence or cumulative incidence figures continue to show great variation. Figures may be as low as 3% (up to 3 years post-partum from a Swedish cohort, using area under the glucose curve measures from the OGTT for diagnosis[85]), and as high as 62% (at up to 6.5 years in a cohort from Trinidad meeting the 1980 WHO criteria[64]). Follow-up of O'Sullivan's original cohort at 16 years showed a cumulative incidence by life-table analysis of 60%[85]. A systematic review from 2002[31] attempted to control for the marked heterogeneity in time among studies, by plotting actuarial projections of cumulative incidence of cohorts at up to 28 years follow-up, and concluded that most cohorts progressed to diabetes at a similar rate in the first 5 years post index pregnancy, and then levelled off by 10 years with few cases after this (however, this calculation included NDDG-diagnosed women only).

Cohort features

Cohort selection also plays a vital role in determining later progression to abnormal glucose tolerance/diabetes, and makes comparison difficult. Selection of women who are known to have normal glucose tolerance in the early post-partum period[48], or restricting follow-up to those who did not require insulin for glycaemic control in pregnancy[34], would be expected to reduce the proportion progressing to abnormal glucose tolerance or diabetes, removing those women with the highest glucose levels during pregnancy. Ethnicity, as outlined above, appears also to be a risk factor for progression, with non-white populations demonstrating increased risk, although comparison across studies is difficult.

Criteria used

There is little evidence to directly compare future progression to diabetes or abnormal glucose tolerance among the different criteria in use. Studies directly comparing progression in women meeting the NDDG *vs* Carpenter-Coustan criteria[78] showed little difference in prevalence of diabetes at a median of 6 years post-partum (25.5% *vs* 25.3%) or at 3 mo post-partum (4.0% *vs* 3.2%)[95].

However, the WHO criteria (Table 1) would be expected to show a smaller proportion of women progressing to diabetes/abnormal glucose tolerance, given the increased number of women identified with GDM compared to the NDDG and Carpenter-Coustan criteria. However, again, direct comparison across studies is difficult. In any given population, therefore, lower diagnostic thresholds will lead to a greater prevalence of GDM. Conversely, criteria using higher thresholds to define GDM will identify fewer women with GDM, but these women will, on average, have higher glucose levels. Therefore, the proportion progressing to abnormal glucose tolerance/diabetes will be higher, despite the lower GDM prevalence.

Also, the criteria used to diagnose type 2 diabetes and abnormal glucose tolerance postpartum may differ - older cohorts in particular, using the NDDG or older WHO criteria would be expected to show a lower prevalence at follow-up due to higher thresholds for diagnosis of diabetes in the nonpregnant adult.

RELEVANCE OF IADPSG GDM CRITERIA

The new IADPSG criteria pose an important clinical question with regard to intensity of follow-up. With potentially up to one in four pregnancies in some centres meeting the new criteria for GDM diagnosis[24], lifelong follow-up of these women will have important clinical and resource implications. However, the optimal mode and timing of a follow-up strategy remains unclear. More women with milder degrees of hyperglycaemia are now classified as GDM. Accordingly, the proportion progressing to abnormal glucose tolerance should decrease. There are as of yet no prospective figures

on progression to type 2 diabetes or abnormal glucose tolerance post-partum in women with IADPSG-defined GDM. The ATLANTIC-DIP study retrospectively classified women using IADPSG criteria after a universal screening programme, and found that 19% had abnormal glucose tolerance at early post-partum follow-up[47]. Capula *et al*[39] looked at a mixed (approximately 60% diagnosed by IADPSG criteria) cohort, and found 4% had diabetes, and a further 32% abnormal glucose tolerance at 6-12 wk post-partum, although conclusions on the relative contribution of each set of criteria are not possible. Overall, it appears certain that more women will need to be tested to identify those women progressing to abnormal glucose tolerance and diabetes.

Some clues as to how women diagnosed with GDM by IADPSG criteria may behave on follow-up may be seen in several papers which follow women meeting just a single abnormal value on the pregnancy OGTT using the older criteria. Retnakaran *et al*[95,96], using NDDG criteria for GDM diagnosis, examined early post-partum outcomes among women along the spectrum of glucose tolerance: from normal glucose tolerance, to abnormal glucose challenge test (GCT) with normal OGTT, a single abnormal value on OGTT, and GDM. This demonstrated a graded relationship in abnormal glucose tolerance; from 3.2% in the normal glucose tolerance (NGT) group, 10.2% in the GCT abnormal, OGTT normal group, 16.5% in the GCT abnormal, single abnormal value on OGTT group, to 32.8% in the GDM group. Indeed, detailed characterisation of these groups[97] demonstrates the similarity between women with a single abnormal value at 1-h post glucose load (as opposed to later abnormal values) and women with GDM, as measured by AUC curve on OGTT and beta-cell dysfunction at 3 mo postpartum.

Thus we can see that a cohort of women with a single abnormal value only, albeit using higher cut-offs than the new IADPSG values, still have a clinically important increased risk of abnormal glucose tolerance. Other prospective studies examining similar cohorts, although at a longer follow-up interval, have drawn similar conclusions; Stuebe *et al*[98], using the stricter Carpenter-Coustan criteria, showed a higher HbA1c in women with a single abnormal value at 3-year follow-up, *vs* both women with GDM, and those with NGT in pregnancy. Vambergue *et al*[76] (using Carpenter-Coustan criteria) showed a similar graded relationship for progression to type 2 diabetes at almost 7 years follow-up, with 6% of women with a single abnormal value progressing to diabetes, as compared with 18% in the those meeting GDM criteria (less than 1% of those with no abnormal values had progressed to diabetes). Carr *et al*[99] (using Carpenter-Coustan criteria), in a large retrospective cohort study, found a HR of 2.0 for diabetes diagnosis among women with a single abnormal value on OGTT *vs* those who did not.

Therefore, all degrees of glucose abnormalities in pregnancy, even those not meeting older GDM criteria, are associated with an increased risk of later glucose abnormalities. This may have important implications for those women with lesser degrees of hyperglycaemia who will now be classified as having GDM by IADPSG criteria.

RELEVANCE OF OVERT DIABETES

Women meeting criteria for diabetes diagnosis in the non-pregnant adult are now classified as separate category by the IADPSG criteria and represent the highest-risk GDM cohort, having an increased risk of congenital abnormalities and diabetes complications, and are likely to have had undiagnosed type 2 diabetes preceding the index pregnancy[22]. The future risk of these women is unclear at present. A retrospective audit of 254 women meeting criteria for overt diabetes demonstrated that 41% had normal glucose tolerance at 6-8 wk postpartum (although testing was carried out at 24-28 wk rather than at the booking visit, and diagnoses based on a 2-h value of $\geqslant$ 11.1 mmol/L were not confirmed with HbA1c or FPG measurements)[100]. Further prospective follow-up comparing women meeting both sets of IADPSG criteria will therefore be useful in further refining risk in this population.

POST-PARTUM FOLLOW-UP STRATEGIES

Current recommendations for follow-up of women with gestational diabetes vary from region to region. The ADA recommend an early post-partum OGTT (in line with ACOG guidelines) and follow-up with HbA1c, fasting plasma glucose (FPG) or 75 g OGTT thereafter, on a 1-3 yearly basis[101]. The International Diabetes Federation[102] recommend an early post-partum OGTT, and thereafter vary recommendations on whether a further pregnancy is planned, (OGTT prior to conception) and whether the woman is high-risk (annual OGTT) or low-risk (FPG every 2-3 years), the criteria for which are not defined. The British National Institute for Health and Care Excellence guidelines[103] recommend FPG alone in the early post-partum period, and an OGTT at follow-up only if a further pregnancy is planned. Several studies have examined the use of HbA1c and FPG[104-107] for both early and medium term follow-up in women with previous GDM, in order to avoid the inconvenience associated with the OGTT. Sensitivity for the detection of abnormal glucose tolerance after delivery varies widely according to the thresholds chosen, ranging from 23%-65% (specificity 68%-96%) for HbA1c values, increasing to a sensitivity of 82%-93% (specificity 84%-92%)when combined with FPG values. Further prospective study will be needed to examine the potential use of these approaches. This will be particularly important if IADPSG criteria are used, as the optimum frequency and mode of testing for such a large cohort of women with previous GDM is unknown.

CONCLUSION

Marked heterogeneity across studies of women with previous GDM with regard to the diagnostic criteria used, duration of follow-up, and cohort demographics limits the ability to compare findings across studies. However, regardless of which criteria are used, a history of GDM confers a large excess risk of progression to type 2 diabetes in later life, and the risk factors predicting progression remain similar across cohorts. The new IADPSG criteria increase the prevalence of GDM by 2-3 fold, and lifelong follow-up of these women has significant clinical and resource implications. Therefore, further prospective studies are necessary to determine the longer-term risk of progression to diabetes in those diagnosed using the new criteria, and also to determine the optimal method and frequency follow-up needed.

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REVIEW

Diabetic cardiac autonomic neuropathy: Do we have any treatment perspectives?

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Abstract

Cardiac autonomic neuropathy (CAN) is a serious and common complication of diabetes mellitus (DM). Despite its relationship to an increased risk of cardiovascular mortality and its association with multiple symptoms and impairments, the significance of CAN has not been fully appreciated. CAN among DM patients is characterized review the latest evidence and own data regarding the treatment and the treatment perspectives for diabetic CAN. Lifestyle modification, intensive glycemic control might prevent development or progression of CAN. Pathogenetic treatment of CAN includes: balanced diet and physical activity; optimization of glycemic control; treatment of dyslipoproteinemia; correction of metabolic abnormalities in myocardium; prevention and treatment of thrombosis; use of aldose reductase inhibitors; dihomo-γ-linolenic acid (DGLA), acetyl-L-carnitine, antioxidants, first of all α-lipoic acid (α-LA), use of long-chain ω-3 and ω-6 polyunsaturated fatty acids (ω-3 and ω-6 PUFAs), vasodilators, fat-soluble vitamin B_1, aminoguanidine; substitutive therapy of growth factors, in severe cases-treatment of orthostatic hypotension. The promising methods include research and use of tools that increase blood flow through the vasa vasorum, including prostacyclin analogues, thromboxane A_2 blockers and drugs that contribute into strengthening and/or normalization of Na^+, K^+-ATPase (phosphodiesterase inhibitor), α-LA, DGLA, ω-3 PUFAs, and the simultaneous prescription of α-LA, ω-3 PUFA and DGLA.

Key words: Diabetes mellitus; Cardiac autonomic neuropathy; Postural hypotension; treatment

Core tip: Cardiac autonomic neuropathy (CAN) is a serious complication of diabetes mellitus, that is strongly associated with increased risk of cardiovascular mortality. CAN manifests in a spectrum of things, ranging from resting tachycardia and fixed heard rate to development of "silent" myocardial infarction. Although it is common complication, the significance of CAN has not been fully appreciated and there are no unified treatment algorithms for today. In this review we have analyzed the effectiveness of lifestyle modification, prescription of α-lipoic acid, aldose reductase inhibitors; γ-linoleic acid, acetyl-L-carnitine, antioxidants, long-chain ω-3 polyunsaturated fatty acids, vasodilators, vitamin B_1 and some other substances.

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INTRODUCTION

Diabetes mellitus (DM) is a global epidemic affecting at least 8.3% of the population and 371 million people worldwide with a significant proportion (50%) remaining undiagnosed. It is estimated that almost one of six people are currently at risk of developing diabetes-related complications[1,2].

The majority of patients with long-term course of DM [mainly type 2 diabetes (T2DM)] are diagnosed with coronary heart disease (CHD) due to coronary vessels arterial sclerotic disease. Often the course of CHD is complicated by combination of hypertension, specific kidney arterial involvement, eyes and lower limbs affection. Metabolic alterations in the myocardium are combined with early coronary atherosclerosis. All these changes in heart occur out of prolonged duration of DM among middle age and elderly patients [coronary vessels affection, myocardium changes, diabetic cardiac autonomic neuropathy (CAN) and arterial sclerotic disease] are associated with the term "diabetic heart or diabetic cardiomyopathy". Conditionally, there are two main forms of heart disease in case of DM: diabetic cardiomyopathy (non-coronary genesis); ischemic heart disease. There is a metabolic stage (actual cardiomyopathy); metabolic-ischemic stage-ischemic heart disease; myocardial infarction (MI); dystrophic coronary cardiosclerosis; CAN[3-5].

Cardiac autonomic neuropathy among T2DM patients, is characterized by lesion of nerve fibers in the sympathetic and parasympathetic divisions of the autonomic nervous system, is diagnosed unsatisfactorily and may be accompanied by severe postural hypotension, decreased tolerance to the physical loadings, and cause the cardiac arrhythmias, ischemia of coronary vessels, "silent" MI, sudden death syndrome[6-9]. The aim of this study is to review the latest evidence and own data about the treatment perspectives of patients with DM and CAN.

THERAPEUTIC APPROACHES FOR CAN

Based on the CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy[10], CAN is defined as the impairment of cardiovascular autonomic control among patients with established DM following the exclusion of other causes.

CAN in T2DM, which is characterized by lesion of nerve fibers in parasympathetic and sympathetic nervous systems, is one of the leading causes of heart arrhythmias and an independent risk factor for cardiovascular mortality among these patients[11,12]. CAN, especially at the early stages, can be subclinical and thus as the disease progresses, it becomes clinically evident.

Therefore, the problem of effective treatment of CAN is particularly relevant. Pathogenetic treatment of CAN includes: balanced diet and physical activity; optimization of glycemic control; treatment of dyslipoproteinemia (DLP); correction of metabolic abnormalities in myocardium; prevention and treatment of thrombosis; use of aldose reductase inhibitors (ARI); γ-linolenic acid, acetyl-L-carnitine, antioxidants, first of all α-lipoic acid (α-LA), use of long-chain ω-3 and ω-6 polyunsaturated fatty acids (ω-3 and ω-6 PUFAs), vasodilators, fat-soluble vitamin B_1, aminoguanidine; substitutive therapy of growth factors and others[13-17].

It is obvious that the foreground should be therapy aimed at reducing insulin resistance (IR), correction of hyperglycemia, prevention and treatment of cardiomyopathy, symptomatic treatment of concomitant diseases and syndromes (hypertension, coronary artery disease, heart failure and arrhythmias)[18,19]. In this regard it is necessary to perform the following preventive and remedical therapy.

Lifestyle modification

Nutrition and physical activity. Correction of obesity. Limit salt intake to 2-4 g/d. Limit smoking, alcohol, foods that contain caffeine. It has been established that compliance with recommended lifestyle modifications (exercise, weight loss, *etc*.) help improve insulin sensitivity level. Sedentary lifestyle (less than 1000 kcal/wk) is accompanied by the risk of mortality three times higher than when living an active lifestyle. Dosed physical activity reduces hyperinsulinemia and encourages the tendency to normalize lipid metabolism in addition to body weight decrease. Physical activity is associated with higher heart rate variability (HRV) and lower heart rate, therefore may be a predictor of positive changes in HRV indices[20]. Obtaining the necessary amount of energy combined with physiologic food ration forms the dietary principles. The traditional Mediterranean diet (Greece and Southern Italy) is associated with longevity and/or low mortality due to cardiovascular disease (CVD) complications, decrease the incidence of T2DM, low frequency of wide range of chronic diseases, including rheumatoid arthritis, Parkinson's disease and others[21-23].

Intensive glycemic control

Compensation state of T2DM is recognized as a primary goal in the prevention of development and/or progression of CVD[2]. IR is a defining feature in most cases of T2DM and plays a key role in the pathogenesis of myocardial alternations. Obviously, pharmacological agents that are used in the treatment of diabetes should have positive qualities for correction of functional and structural disorders of the cardiovascular system[3,11,12].

Theoretically, pharmacological agents that improve insulin sensitivity [metformin, thiazolidinediones (TZD)]

appear to be the most appropriate in this regard. It is established that metformin has a positive effect on glucose metabolism; Ca^{2+} concentration in cardiomyocytes, but metformin, unlike TZD, does not show any positive effect on optimization of glucose metabolism in the myocardium[4,24]. TZD stimulate receptor transcription factors, activated by peroxisome proliferator activated receptor-γ (PPAR-γ), which improves insulin sensitivity and reduces the level of circulating free fatty acids (FFA). It is likely that TZD, despite the absence of the myocardium PPAR-γ type receptors, improve the functional state of the myocardium by reducing the content of FFA. However, the use of TZD among patients with CVD is limited due to the possibility of fluid retention and/or development of edema[25,26].

Insulin and/or insulin secretagogues: Theoretically, their use may improve glucose metabolism in the myocardium and reduce the content of FFA, however, the assignment of these pharmacological agents is not conducive to the prevention of CVD in the experiment[4]. Inhibition of PPAR-α expression, which stimulates glucose metabolism and inhibit the metabolism of FFA's, prevents the development of CVD in the experiment, and activation causes the formation of severe cardiomyopathy. Reduction of fat contents in nutrition among animals with increased expression of PPAR-α is accompanied by myocardium lesions warning, confirming the pathophysiological significance of activation of FFA metabolism. Similarly, the use of PPAR-γ agonist medications encourages the activation of glucose metabolism, inhibition of FFA metabolism, prevention of CVD[4].

Glucagon-like peptide-1 medication: Glucagon-like peptide-1 is one of the two leading "incretins" in the body-hormones that stimulate postprandial secretion and improve insulin sensitivity. The experiment established that the use of glucagon-like peptide-1 (GLP-1) improves the functional state of left ventricular (LV) hemodynamic parameters[27]. However, GLP-1 medication can not be used in pharmacological therapy of CVD as under influence of dipeptidyl peptidase-4 (DPP-4), GLP-1 is rapidly destroyed (effective half-life is only 1-2 min). Exenatide is 53% GLP-1 homologous and functions as a partial GLP-1 agonist receptor. Alternative to GLP-1 is the use of antagonists of DPP-4 (sitagliptin). However, the exenatide effectiveness as well as antagonists and DPP-4 in suspension/prevention of CVD in T2DM is not clear[27].

Treatment of dyslipoproteinemia

For DLP pharmacotherapy using statins, fibrates, bile acid sequestrants, nicotinic acid and its derivatives, products of long-chain ω-3 and ω-6 PUFA, or as an alternative-their combination with cholesterol absorption inhibitors[28].

Statins: Statins (along with lifestyle changes) should be prescribed to patients with T2DM aged over 40 where there is at least one of the risk factors for CVD (regardless of basic lipid levels); prescription of statins among patients with T2DM aged under 40 years without diagnosed CVD should be considered when low density lipoprotein (LDL) cholesterol level exceeds 2.6 mmol/L[29,30]. Achievment of LDL level in the blood < 1.8 mmol/L or reduction by 30%-40% compared with initial level (in case of failure to achieve value targets in the course of the prescription of the maximum tolerable dose statin) is suitable for patients at high risk of CVD, particularly patients with T2DM. However, statins are often ineffective when used for treatment of atherogenic DLP as pharmacological agents to achieve reduction in triglycerides (TG) and increase high density lipoprotein (HDL) cholesterol; statin use (even at high doses) only partially solves the problem of the risk of CVD[31-33].

Fibrates: Fibrates limit the availability of substrates for the synthesis of TG in the liver, encourage lipoprotein lipase effects, increase LDL receptor/ligand interaction, stimulate cholesterol secretion with bile; stimulate reverse cholesterol transport, that is accompanied by reduction of TG and very LDL (VLDL) cholesterol levels, and improve insulin sensitivity. Possible mechanisms that help fibrates improve insulin sensitivity are: fibrate binding to receptors that activate PPAR-β enhances fatty acids oxidation in the liver and, consequently, causes increase of insulin sensitivity; fibrates are involved in the regulation of adipokine expression [adiponectin, leptin, tumor necrosis-α (TNF-α), resistin, *etc.*], accompanied by the increase of insulin sensitivity[34].

Bile acid sequestrants: Bile acid sequestrants are safe lipid-lowering medicaments, however often causing gastrointestinal adverse reactions. The second generation bile acid sequestrants, including colesevelam binds bile acids with higher affinity and better tolerance. It is used as a supplement to diet therapy and physical activity to reduce the concentration of LDL cholesterol among patients with primary DLP, during monotherapy and/or in combination therapy with statins and to improve glycemic control among patients with T2DM. In addition, it is important that the bile acid sequestrants reduce the concentration of glucose and HbA_{1c} in the blood (approximately 0.9%)[35] and thus may be useful in the treatment of hypercholesterolemia among patients with T2DM.

Niacin: Niacin is the most efficient pharmacological agent for raising HDL cholesterol level and, to a lesser extent, to reduce the concentration of TG and LDL cholesterol. It is reported that the therapeutic effect of prolonged forms of niacin on lipid profile occurs with the medicament intake in the dose range 0.5-2.0 g. A common reason for not using niacin, which significantly affects patien's susception and accurate application is the problem of "flushing". Current approach to this issue

is the use of combined prolonged form of niacin with laropiprant, an inhibitor of prostaglandin D_2[36,37].

Long-chain ω-3 PUFAs: The use of long-chain ω-3 PUFAs due to their effects on glucose homeostasis and IR (IR reduction in muscle > adipose tissue >> liver; presumably inhibit insulin secretion and delay the development of T2DM); influence on the state of lipid metabolism (decrease TG concentrations, presumably increase the concentration of HDL cholesterol, improve lipid profile among patients with T2DM and DLP); moderately reduce blood pressure (BP); improve endothelial function; reduce the inflammation and improve antioxidant protection[38-41].

Ezetimibe: Ezetimibe is used as a nutrition and exercise supplement to reduce the concentration of LDL cholesterol, total cholesterol (TC), and treatment of homozygous familial hypercholesterolemia. Despite some reservations, ezetimibe remains the medicine of first choice among other pharmacological agents in the absence of target specific level of LDL cholesterol using statin monotherapy[42].

Combined treatment: Therapy of first choice for T2DM in case of lipid profile correction is usage of statins to achieve specific target of LDL cholesterol level < 2.6 mmol/L for primary prevention and < 1.8 mmol/L for secondary prevention of CVD. Failure to get this target is the indication to combine statins with other lipid-lowering agents of other pharmacological groups. A number of international guidelines as a compulsory component of CVD risk monitoring recommend to control apolipoprotein B level on the first-priority basis. However, no results in multicentred, randomized, double-blind, placebo-controlled clinical trials makes it a therapeutic dilemma, since it is uncelar whether the intensification of statin therapy or combination of statins with fibrates and/or nicotinic acid will give the desired results[42,43].

Correction of metabolic abnormalities in the myocardium

Correction of metabolic abnormalities in the myocardium is the basis of pharmacotherapy that aims at optimization of the energy metabolism of the myocardium. Pharmacological impact system includes the following main aspects: use of metabolism regulators; energy-saving solutions; activators of endogenic high-energy compounds and O_2 transportation; inhibitors of metabolic acidosis; membran protection: inhibition of lipid peroxidation membranes of cardiomyocytes; stabilization of lysosomal membranes, neutralization of membranotropic action of humoral agents of lysosomal proteases and others. Medicaments that enhance cell energy state (means of potential energy supply survival of ischemic myocardium). Deterioration of intracellular reserves of carbohydrates needs to be replenished by use of glycolysis activation measures. The use of macroergic phosphates (ATP, *etc*.) as a direct energy source is problematic, as the therapeutic effect of ATP in case of ischaemia, probably has less to do with disposing of its macroergic bonds but more with involving products of catabolism of ATP into energy metabolism of cardiomyocytes[4,44,45].

Modulators of metabolism: Insulin resistance affects myocardial function by reducing glucose transportation and oxidation of carbohydrates; enhancing the use of FFA; inhibition of Ca^{2+} transportation in the sarcolemma; violation of the structure and function of regulatory contractile proteins of myofibrils. In case of DM the reduction of myocardial energy formation leads to inhibition of glucose oxidation and preferential oxidation of fatty acids in the myocardium and skeletal muscle, which increases sensitivity to myocardial ischemia and leads to significant disturbances of Ca^{2+} homeostasis, deterioration of diastolic and systolic myocardial function. The presence of coronary artery disease (CAD) among patients with diabetes worsens the disease and significantly increases cardiovascular mortality. It is considered that even the initial stages of glycemic profile violations may influence the myocardial metabolism and contribute to the development of cardiomyopathy[4,44,45]. It is important that myocardial dysfunction is a supposititive stage of chronic hyperglycemia elaboration. Thus, dysfunction of cells metabolism, rather than systemic hyperglycemia is the reason for the elaboration of cardiac malfunction[4,46,47].

Metabolic medicaments: Optimization of myocardial energy metabolism is based on increased myocardial glucose oxidation, which enhances cardiac function and protects myocardial fibers from ischemic and reperfusion injuries. Myocardial use of glucose in case of chronic disease may be improved due to intake of the medicines, that can improve fatty acids metabolism and inhibit their oxidation. New therapeutic approach has been implemented after advent of trimetazidine- the first representative of a new class of metabolic agents- inhibitors of 3-ketoacyl coenzyme A thiolase. Trimetazidine reduces oxidation of fatty acids; stimulates glucose intake; restores the link between glycolysis and carbohydrate oxidation, which leads to the formation of ATP, reducing O_2 consumption; redirects fatty acids towards phospholipids; increases cell tolerance to ischemic and reperfusion injuries; increases the oxidation of glucose, the activity of Na^+, K^+-ATPase and Ca^{2+}-pomps in the sarcoplasmic reticulum. Antiischemic properties of trimetazidine do not depend on changes in hemodynamics and are associated with a distinct recovery of mechanical function after ischemia, which makes it recognized as cardyo-cytoprotective agent. Trimetazidine prescription improves glucose metabolism; reduces endothelin-1 among patients with diabetic cardiomyopathy, that is taken to have effect on the vascular endothelium; accompanied by a significant

positive changes in ejection fraction (EF) parameters among patients with heart failure; improves quality of life parameters and NYHA functional class[48,49]. Another pharmacological agent that facilitates the inhibition of metabolism of fatty acids is perhexiline. Perhexiline prescription to patients with heard failure significantly contributes to the improvement of EF, VO_2max and quality of life. Unfortunately, the clinical use of this medicament is limited because of the risk of hepatotoxicity and peripheral neuropathy[50]. Ranolazine is the third antianginal pharmacological agent with a potential of metabolism modificator. However, the following factors do not allow to implement its use: the degree of inhibition of fatty acids metabolism is limited by physiological indicators; ranolazine prescription associates with the possibility of corrected QT interval prolongation[51].

Limitation of extracellular Ca^{2+} into the cell: Blockers of Ca^{2+}-channels show a protective effect on myocard in case of ischemia. In terms of correction of cell power the most pathogenetically efficient option is the use of Ca^{2+} blockers, however they only eliminate secondary dysfunction links of oxidative phosphorylation in mitochondria. Prescription of β-adrenergic receptor blockers for T2DM with CAD and CAN has significant pathogenetic grounds as high sympathetic activity that is followed by CAN, accelerate the development of CVD and significantly affects prognosis. In addition, several studies demonstrated the ability of β-blockers to reduce the incidence of "silent" myocardial ischemia episodes and improve prognosis among these patients. However, adrenergic receptors β-blockers negatively affect the performance of glycemic profile, increase the risk of hypoglycemia, showing a negative effect on blood lipid profile and can provoke acute heart failure. The above described events occur with prescription of non-selective β-blockers. Selective β-adrenergic receptor blockers, including metoprolol, are free of side effects, including the effectiveness of metoprolol in the treatment of CVD demonstrated in numerous controlled studies. Metoprolol has cardioprotective properties; improves prognosis among patients with CAD; has a fair tolerance in case of prolonged use. Cardioselective β-blockers can also balance the effects of autonomic dysfunction in particular by resisting sympathetic stimulation they can restore parasympathetic-sympathetic balance. However, traditional antianginal agents that affect hemodynamic parameters (β-blockers, Ca^{2+} antagonists, *etc*.), have lower tolerance among elderly due to the high risk of the interaction of pharmacological agents with a significant incidence of side effects[3,4,45,46].

Medicaments that contain micro- and macro-elements, primarily Mg^{2+}: One of the risk factors that can decrease insulin sensitivity is hypomagnesaemia. It is suggested that Mg^{2+} deficiency plays a significant role in increasing the risk of diabetic macro- and microvascular complications and, especially, risk of CAD[4,16,17].

Thrombosis prevention and treatment

Platelets obtained from patients with T2DM and tested *in vitro* are characterized by a real ability to aggregate under the influence of ADP, adrenaline, collagen, arachidonic acid, and thrombin. Aggregation of platelets is significantly increased in the second, irreversible phase, which depends on the transformation of arachidonic acid into labile prostacyclin and thromboxane. Thus, the possibility of ADP receptors of platelet membranes blocking is a pathogenetically justified measure. Prescription of antiplatelet agents, namely acetylsalicylic acid (ASA), clopidogrel and others can help prevent blood clots, stenocardia and development of MI. The active clopidogrel metabolite irreversibly binds to ADP receptor on the platelet membrane, which leads to inhibition of adenylate cyclase; inhibition of ADP-dependent secretion of platelet granules; inhibition of ADP-dependent process of binding fibrinogen receptor to the platelet membrane; does not affect the expression of receptors directly; blocks myointymal proliferation in case of vascular damage; unlike ASA does not affect the activity of cyclooxygenase. Effect of clopidogrel and ASA synergy is demonstrated in the study of platelet *ex vivo*. However, clopidogrel is more effective pharmacological agent within the frames of the combined risk of MI, stroke, and the syndrome of "sudden death" reduction[52-55].

ARI

ARI inhibit the glucose polyol way metabolism, prevent the reduction of the redox potentials. Analysis of the double-blind, placebo-controlled study established that tolrestat contributes to the improvement of independent tests results and vibration sensitivity among patients with symmetric diabetic peripheral neuropathy (DPN). Zenarestat prescription for 12 mo was accompanied by a dose-dependent changes in the spissitude of nerve tissue, increased the velocity of nerve impulses, improved myocard systolic function. Zoporestat, ranirestat-medicaments of a new generation of ARI group showed sufficient efficacy in experimental studies[56-59].

Replacement therapy with help of myoinositol

Several individual clinical trials were conducted for the study of myoinositol efficacy in the treatment of diabetic neuropathy. The results are quite positive, but the future clinical double-blind, placebo-controlled trials are needed[60-62].

Aminoguanidine

Aminoguanidine improves capacity of nerve velocity, increases blood flow, inhibits the formation of advanced glycation endproducts, delays the emergence and development of albuminuria. Analysis of controlled trials confirmed quite aminoguanidine high efficiency among patients with diabetic neuropathy, but the development of a number of side effects terminated their application.

The use of aminoguanidine derivatives is accompanied by clinical efficacy and lack of adverse side effects[6,8,11]. The results are promising, but need further clinical double-blind, placebo-controlled studies.

Neurotrophic therapy

Inhibition of nerve growth factor (NGF) expression and its receptors suppresses NGF axonal retrograding transport and reduces the activity of small demyelinamted neurons and their neuropeptides, including substance P and gene-linked calcitonin peptide. The use of recombinant human NGF normalizes neuropeptide concentration and prevents the development of sensory neuropathy in the experiment. However, the results of clinical placebo-controlled studies deny the positive impact of recombinant human NGF among patients with diabetic neuropathy[6,8].

Antineural autoimmunity human immunoglobulin for intravenous use

Intravenous human immunoglobulin prescription is recommended for patients with DPN, which have signs of antineural autoimmunity symptomes. The side effects include headache, and the main danger could be the development of an anaphylactic reaction, however, it affects mainly patients with deficiency of immunoglobulin A[6,8].

Endoneural perfusion inhibition with the development of hypoxia

Experimental and clinical studies have shown benefit in the efficiency of vasodilators when used for improvement of nerve flow velocity, but there is not enough information about the impact of vasodilators on the course of DPN during clinical double-blind placebo-controlled studies. The research results of characteristics that impact the angiotensin-converting-enzyme inhibitors on heart rate variability parameters among diabetic patients with CAN appeared to show diametrically opposed results. In particular, prescription of quinapril for 3 mo was accompanied by statistically significant increased parasympathetic activity, and the use of trandolapril for 12 mo did not affect the performance of autonomic myocardial function. However, most of these pharmacological agents have no proven clinical and electrophysiological positive effects and have certain limitations and contraindications[4-6,11].

Activation of free radical stress

Considering that one of the major pathogenetic mechanisms of neuropathy is oxidative stress (OS), the need for antioxidants prescription is obvious. Great therapeutic potential is observed in α-LA and creates pathogenic evidence for the use of this pharmacological agent[63-65]. Mechanism of α-LA action is not fully developed, but specific attention should be paid to two hypotheses. Firstly, α-LA phenomenon causes dose-dependent proliferation of neuroblastoma cultured cells. Changes in the membrane fluidity that are mediated through sulfhydryl groups α-LA are considered to cause this effect. This is confirmed by the following results of several studies, including experimental neuropathy induced by acrylamide, followed by a significant inhibition of proliferation of the above phenomenon; overlay and/or progression of experimental distal neuropathy, mainly caused by a decrease of content of substances in axons containing sulfhydryl groups (*e.g.*, glutathione); α-LA *in vivo* and *in vitro* enhances spontaneous processes of expansion and improvement of the structural and functional nerve terminals membranes state; prescription of α-LA stimulates the regeneration of nerve terminals in case of the partial denervation, as well as experimental hexacarbon neuropathy. Secondly, and the most probable mechanism is the ability of α-LA to function as a radical binder ("cleaner")[66-69].

Vitamins with antioxidant properties [a liposoluble vitamin B_1 (benfotiamin)], combined medications

There is enough experimental and clinical results of studies that suggest that the hyperinsulinemia, IR, and chronic hyperglycemia in T2DM have a negative impact on the metabolism of thiamine particularly due to the inhibition of the functional state of the thiamine transporter-1 and thiamine transporter-2, responsible for the reabsorption of vitamin in the proximal tubules of the kidneys; transketolase activity, which can lead to the congestion of intermediates in the initial stages of glycolysis [glyceraldehyde-3-phosphate (GA3P), fructose-6-phosphate (F6P) and dihydroxyacetone-phosphate]. Congestion of intermediates in case of chronic hyperglycemia increases the production of free radicals in the mitochondria, followed by inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Increased concentrations of GA3P, F6P and GAPDH can initiate induced hyperglycemia, metabolic fates that favor the overlay of vascular injury, including activation of proteinkinase-C, accumulation of advanced glycation end products (AGEs) hexosamine biosynthetic fates activation, dicarbonyl compounds. Activation with dicarbonyl compounds is followed by further stimulation of the AGEs formation, which is also associated with functional impaired and structural state of cardiomyocytes[70-72].

It is clear that the correction of thiamin deficiency must be performed using exogenous vitamin B_1, or benfotiamine (monophosphate S-benzoyl-thiamine, high-bioavailable liposoluble vitamin B_1 derivatives). Results of experimental and clinical studies suggest a positive effect of benfotiamine prescription on prevention of diabetic vascular disease progression. Benfotiamine broad therapeutic potential has a good efficiency on medications containing soluble thiamine derivatives for the purpose of regulating the activity of free radical processes; correction of endothelial dysfunction in case of CVD, stabilization of clinical and antioxidant effects. Benfotiamine favoring the transketolase (TK)

activity prevents the activation of pathophysiological mechanisms by reorientation towards of F6P and GAPDH metabolism[73-75]. Benfotiamine can promote neuronal and vascular deficiency correction through participation of nitrogen oxide processes, which have a significant therapeutic potential for the treatment of CVD. The use of thiamine and α-LA combination has a great significance in the treatment of diabetic angio-neuropathy. In particular, it demonstrated that prescription of benfotiamine and α-LA to patients with T1DM was followed by normalization of hyperglycemia and for 4 wk it promoted the normalization of prost-acyclin synthase suppressed by diabetes; increase of TK activity in monocytes in 2-3 times[76-80].

Fatty acids metabolism disorders (γ-linolenic acid, acetyl L-carnitine)

Vasoactive prostanoids, metabolites and dihomo-γ-linolenic acid (DGLA), including prostaglandins and other eicosanoids are necessary for the physiological behavior of nerve conductivity and blood flow. The results of double-blind, placebo-controlled studies showed that prescription of DGLA to patients with DPN is followed by positive dynamics in clinical course, as well as increase in the speed of nerve conductivity. L-carnitine's main function is to strengthen the metabolism of fatty acids, but there are experimental evidence of L-carnitine's ability to activate glucose metabolism. It is believed that T2DM is characterized by malfunction of L-carnitine exchange in the mitochondria. The results of several studies showed that prescription of L-carnitine helps to improve energy supplies and LV function. It is established that propionyl-L-carnitine improves the functional status, used as glucose energy oxidation in the rat's affected myocardium (despite the increased level of fatty acids). Nutrition of diabetic mice with obesity with L-carnitine addition increases the level of acyl-carnitine in the blood, muscle, liver and adipose tissue; increases levels of pyruvate dehydrogenase activity in the muscles; prescription of zinc-carnitine mixture reduces hyperglycemia and improves glucose tolerance. L-carnitine infusion with the help of hyperinsulinemic-euglycemic clamp improves glucose profile control, reduces the concentration of circulating lipids. L-carnitine prescription for 3 or 6 mo for newly diagnosed patients with T2DM with lipid metabolism disorders is followed by a statistically significant decrease in lipoprotein(a) [Lp(a)] levels. The results of double-blind, placebo-controlled studies among patients with verified hyperLP(a) established that L-carnitine (2 g/d) encouraged a significant decrease in the concentration of Lp(a) levels; L-carnitine incorporation into nutrition of patients with newly diagnosed T2DM is followed by similar changes; combined L-carnitine with simvastatin (20 mg/d) treatment is much more efficient in decreasing the concentration of lipids, including TG and Lp(a) than statin monotherapy. Thus, L-carnitine can be used as one of the components for lipid-modifying therapy among patients with T2DM[81,82].

ω-3 and ω-6 PUFAs medications

A fundamentally new approach to assessing the biological role of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) is associated with long-term epidemiological studies results among Inuits, which established a small percentage of CVD. The Greenlandic Inuits were observed to have an increased bleeding duration, lower levels of TC, TG, VLDL-cholesterol and a significant increase in TC lipid membranes of EPA and DHA contents, arachidonic acid concentration reduction and linoleic acid. For the first time these results allowed to express a reasonable assumption about the protective effect of DHA and especially EPA from the damaging effects on the internal vessel wall cause capable of inducing experiment CAD-a phenomenon of TC activation and high blood viscosity, enhanced the cyclic endoperoxide synthase, including prostaglandin H_2, TXA_2 activation of endothelial cell proliferation, hypercholesterolemia and hypertriglyceridemia. Prescription of EPA and DHA is followed by a decrease in the "rigidity" of red blood cells, which is obviously associated with labilization of erythrocyte plasmolemma based on rapid and intensive incorporation of long-chain ω-3 PUFA phospholipids into membrane and decreased synthesis of vasoconstrictor active ingredients. The ability of exogenous EPA and DHA to incorporate phospholipid blood cell membranes and membrane phospholipids of endothelial cells blood vessels affects the fundamental plasmolemma properties and receptors function for the perception and processing of extracellular information. Accumulating long-chain polyenes acids, labilize plasmolemma, changing the microviscosity of its lipid matrix, which causes the transformation of the basic plasmolemma properties-permeability, generation of biopotentials, ions transit. Changes in the lipid environment of receptor structures affects their functional activity and enzyme systems control in the cell, which primarily relates to the corpuscular adenylate cyclase, whose function is related to the metabolism of phospholipids[83-85].

Analysis of experimental and clinical studies proves that ω-3 PUFA inhibit the absorption of cholesterol in the intestine and its synthesis in the liver, lead to increased clearance of lipoproteins in the blood, prevent the development of IR in experimental diabetes, decrease level of BP, dose-dependently prevent the development of diabetes, improve the sensitivity of platelets to ADP and collagen, contribute to positive changes in the parameters of coagulation, endothelial cells migration, inhibits the proliferation of smooth muscle cells. However, the studies aimed to investigate the features of ω-3 PUFA in T2DM are numerically small and obtained results do not always testify to their effectiveness[86-93]. In particular, the results of the ORIGIN trial demonstrated, that administration of 1 g ω-3 PUFA did not reduce the rate of death caused by cardiovascular reasons or their outcomes during a period of 6 years among patients with dysglycemia and additional cardiovascular risk factors. In this trial the dose of ω-3 PUFA was not chosen

Table 1 N-terminal fragment of the prohormone brain natriuretic peptide level and lipid metabolism parameters after 3-mo of omega-3 polyunsaturated fatty acid therapy

Parameter	Patients with T2DM and CAN (n = 36)	
	Control (n = 15)	ω-3 PUFA (n = 21)
	Group 1	Group 2
NT-proBNP	-3.0 ± 1.1	-6.8 ± 1.1[a]
LDL cholesterol	-8.3 ± 1.4	-12.8 ± 1.9
HDL cholesterol	4.1 ± 1.0	7.1 ± 0.5[a]
TG	-8.3 ± 1.2	-35.4 ± 2.6[c]
TC	-6.7 ± 1.0	-8.2 ± 1.1

The results are presented as % change from baseline, (Δ%, Mean ± SEM); [a]P < 0.05, [c]P < 0.001. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; ω-3 PUFA: Omega-3 polyunsaturated fatty acid; NT-proBNP: N-terminal fragment of the prohormone brain natriuretic peptide; LDL cholesterol: Low density lipoprotein cholesterol; HDL cholesterol: High density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol.

on the basis of any estimate of its effect on TG levels, nevertheless, a significant reduction in the TG level was shown. However, this study did not apply to treatment of CAN and it was decided to continue the study for a few more years[94]. In the same time, American Diabetes Association (ADA, 2005) recommend the prescription of α-LA and ω-3 PUFA in algorithms of DPN treatment[95] and in ADA recommendations (2014) and results of some trials-prescription of ω-3 PUFA in DLP treatment among patients with T2DM and cardiovascular diseases[2,90-92].

To explore the effectiveness of some above-mentioned compounds we examined 81 patients with T2DM and CAN, patients were aged between 50-59 years with disease duration 1-6 years and median HbA_{1c} 7.1% ± 0.4%. CAN was diagnosed according to previously proposed criteria[8,10,12]. The work was done according to the principles of the Declaration of Helsinki (2004) and all subjects signed an informed consent prior their inclusion in the study. Patients were allocated to five treatment groups: first group received traditional antihyperglycemic therapy (n = 15, control group); patients in group 2 (n = 21), received in addition to standard treatment 1 capsule/d of the ω-3 PUFA; patients in 3rd group (n = 12) - benfotiamine 300 mg/d; patients in 4th group (n = 18) -600 mg of α-LA, patients in 5th (n = 15) -1 capsule/d of the ω-3 PUFA, benfotiamine 300 mg/d and 600 mg of α-LA. Each one gram capsule of the ω-3 PUFA contains approximately 465 mg of EPA and 375 mg of DHA. The duration of the treatment was three months.

The concentration of glucose in the blood was determined by the glucose oxidase method while HbA_{1c} was assessed by using a highly sensitive method of ion-exchange liquid chromatography with D-10 analyzer and BIO-RAD reagents (United States). Determination of immunoreactive insulin (IRI) was performed using commercial kits from immunotech insulin immunoradiometric assay reagents (Czech Republic); leptin level-from Immunotech Leptin (Czech Republic) test kits; TNF-α-from Vector-Best (Russia); high-sensitivity C-reactive protein (hsCRP)-from diagnosis-related group (United States); N-terminal fragment of the prohormone brain natriuretic peptide (NT-proBNP)-from Biomedica (Austria) kits and an enzyme-linked immunosorbent assay analysis technique. Lipid metabolism was assessed by the concentration of TC, LDL-, HDL-, VLDL-cholesterol measurements. The lipid fractions were determined by using HUMAN reagents (Germany) for the analyzer HUMANLAYZER 2000.

We found out that the HbA_{1c} of patients with T2DM and CAN was not statistically significant influenced by the treatment (P > 0.05). Treatment with the drug containing ω-3 PUFA among patients with T2DM and CAN (group 2) led to a significant increase of the HDL cholesterol level [7.1% ± 0.5%, (P < 0.05)] and reduction of TG [-35.4% ± 2.6%, (P < 0.05)]. The treatment also lead to a significant decrease of the NT-proBNP level [-6.8% ± 1.1%, (P < 0.05)] compared to the control group. Changes of NT-proBNP and lipid metabolism parameters among patients with T2DM and CAN after 3-mo of ω-3 PUFA therapy are given in Table 1.

Benfotiamine prescription to patients with T2DM and CAN did not cause any significant changes in lipid profile and leptin levels (P > 0.05), while it probably helped reduce the IRI concentration [-12.7% ± 1.4%, (P < 0.05)]. The use of benfotiamine in the comprehensive treatment of T2DM helped reducing hsCRP [-13.3% ± 2.1%, (P < 0.05)] and TNF-α [-10.2% ± 1.6%, (P < 0.05)] concentrations, but the prescription of α-LA was followed by a significant decrease in these parameters [-15.2% ± 1.9%, (P < 0.01) and -14.7% ± 1.8%, (P < 0.001), accordintly] and facilitated visible LDL cholesterol [-14.2% ± 1.8%, (P < 0.05)], IRI [-15.9% ± 1.6%, (P < 0.01)] and leptin [-16.3% ± 1.2%, (P < 0.001)] reduction, also increased HDL cholesterol level [7.8% ± 0.7%, (P < 0.01)]. Combined ω-3 PUFA, benfotiamine and α-LA prescription was followed by the more pronounced decrease of IRI, leptin and some inflammation factors (Table 2).

Obtained results of this study could prove that prescription of ω-3 PUFA is accompanied by more significant decrease of TG and increase of HDL cholesterol levels compared to patients in control group. The complex therapy with α-LA contributes to more evident antiatherogenic effect, in particular decrease of LDL and TC, increase of HDL cholesterol level (compared to patients of 1st, 2nd and 3rd groups). Combined prescription of ω-3 PUFA, benfotiamine and α-LA is followed by more statistically significant positive changes of lipid profile (Table 3).

In order to evaluate the artery stiffness parameters during active and passive periods of the day the 24-h blood pressure profile, aorta (AIxao) and brachial augmentation index (AIxbr), pulse wave velocity (PWV) and ambulatory arterial stiffness index (AASI) were

Table 2 Changes of the immunoreactive insulin, leptin, high reactive C-reactive protein and tumor necrosis factol alpha levels after 3-mo of treatment

Parameter	Patients with T2DM and CAN (n = 81)				
	1st group (n = 15)	2nd group (n = 21)	3rd group (n = 12)	4th group (n = 18)	5th group (n = 15)
IRI	-6.8 ± 2.0	-10.3 ± 1.1	-12.7 ± 1.4[a]	-15.9 ± 1.6[b,e]	-20.9 ± 0.9[c,f,i,j]
Leptin	-7.1 ± 1.8	-15.8 ± 1.7[b]	-6.4 ± 1.4[f]	-16.3 ± 1.2[c,i]	-18.4 ± 1.4[c,i]
hsCRP	-7.2 ± 1.6	-14.8 ± 2.4[a]	-13.3 ± 2.1[a]	-15.2 ± 1.9[b]	-22.6 ± 1.6[c,e,h,k]
TNF-α	-6.1 ± 1.0	-14.1 ± 2.1[b]	-10.2 ± 1.6[a]	-14.7 ± 1.8[c]	-19.8 ± 1.6[c,d,i,l]

The results are presented as % change from baseline, (Δ%, Mean ± SEM); [a]P < 0.05, [b]P < 0.01, [c]P < 0.001 - compared to 1st group; [d]P < 0.05, [e]P < 0.01, [f]P < 0.001 - compared to 2nd group; [h]P < 0.01, [i]P < 0.001 - compared to 3rd group; [j]P < 0.05, [k]P < 0.01, [l]P < 0.001 - compared to 4th group. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; IRI: Immunoreactive insulin; hsCRP: High reactive C-reactive protein; TNF-α: Tumor necrosis factol alpha.

Table 3 Changes of the lipid metabolism parameters after 3-mo of treatment

Parameter	Patients with T2DM and CAN (n = 81)				
	1st group (n = 15)	2nd group (n = 21)	3rd group (n = 12)	4th group (n = 18)	5th group (n = 15)
LDL cholesterol	-8.3 ± 1.4	-12.8 ± 1.9	-7.6 ± 1.0[d]	-14.2 ± 1.8[a,h]	-33.1 ± 2.4[c,f,i,l]
HDL cholesterol	4.1 ± 1.0	7.1 ± 0.5[a]	5.7 ± 0.6	7.8 ± 0.7[b,g]	13.9 ± 1.3[c,f,i,l]
TG	-8.3 ± 1.2	-35.4 ± 2.6[c]	-13.3 ± 3.4[f]	-9.3 ± 1.1[f]	-27.9 ± 3.9[c,h,l]
TC	-6.7 ± 1.0	-8.2 ± 1.1	-7.1 ± 1.2	-10.7 ± 1.3[a,g]	-27.2 ± 1.9[c,f,i,l]

The results are presented as % change from baseline, (Δ%, Mean ± SEM); [a]P < 0.05, [b]P < 0.01, [c]P < 0.001 - compared to 1st group; [d]P < 0.05, [f]P < 0.001 - compared to 2nd group; [g]P < 0.05, [h]P < 0.01, [i]P < 0.001 - compared to 3rd group; [l]P < 0.001 - compared to 4th group. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; LDL cholesterol: Low density lipoprotein cholesterol; HDL cholesterol: High density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol.

assessed by TensioMed™ Arteriograph 24 (Hungary). The program orders the values of the AIxbr and PWV into ranges as follows: optimal values: AIxbr > -30%, PWV < 7 m/s; normal values: -30% < AIxbr < -10%, 7 m/s < PWV < 10 m/s; elevated values: -10% < AIxbr < 9.8%, 9.8 m/s < PWV < 12 m/s; pathological values: AIxbr > 10%, PWV > 12 m/s[96]. The study involved 51 patients with T2DM, among them 12 patients without CVD and CAN, 39 patients with moderate CAN. Patients with diagnosed CAN were allocated to two groups: control group (n = 18) received traditional antihyperglycemic therapy and treatment group (n = 21) received in addition to standard treatment 1 capsule/d of the ω-3 PUFA. Control-12 healthy volunteers. Artery stiffness parameters among patients with T2DM without CAN were within normal limits, but this group has a tendency toward increase of vascular wall stiffness parameters. The arterial stiffness parameters among patients with moderate CAN exceed the physiological values, in particular AIxao 26.2% (P < 0.01), AIxbr 66.2% (P < 0.001), PWV 24.7% (P < 0.001), AASI 30.6% (P < 0.01) compared to patients with T2DM without CAN and were considered as high (Table 4). After 1.5 mo of treatment we found out that there was a decrease of AIxbr (-10.0% ± 2.62%, P < 0.05) and PWV (9.8 ± 0.42 m/s, P < 0.01) values in treatment group. Prescription of ω-3 PUFA for three months was followed by more significant decrease of AIxao (27.8% ± 1.13%, P < 0.05), PWV (9.3 ± 0.42 m/s, P < 0.01) during the 24 h; decrease of AIxao (16.2% ± 3.12%, P < 0.01), PWV (-11.6% ± 2.09%, P < 0.05) during the day and decrease of AIxao (-11.2% ± 4.2%, P < 0.05), AIxbr (-98.0% ± 18.1%, P < 0.05), PWV (-18.9% ± 3.9%, P < 0.01) during the night. At the same time there wasn't significant influence on the AIxbr during the active period of day (Tables 5 and 6). Therefore, the administration of ω-3 PUFA to patients with T2DM for three months promotes arterial stiffness parameters improvement.

We previously reported that the use of ω-3 PUFA, which contains in one capsule approximately 90% ω-3 PUFA, mainly EPA and DHA, in the treatment of patients with T2DM and CAN improved the general condition of the patients. Thus, prescription of ω-3 PUFA contributed to significant decrease of mean diastolic blood pressure (DBP), time index of diastolic hypertension, diastolic hypertension area index and variability of DBP during the day and night hours and was followed by a tendency to a low pulse pressure[97-101]. The influence of ω-3 PUFA on the dynamics of metabolism is probably caused by their effects on IR, glucose homeostasis and lipid metabolism (improvement of the lipid profile in patients with T2DM and DLP). In addition, ω-3 PUFA moderately reduce BP, improve endothelial function, reduce proinflammatory status and improve antioxidant protection. The combination of the positive influences of ω-3 PUFA on NT-proBNP, lipid profile and their moderate hypotensive effects suggests the feasibility of their use in the complex treatment of patients with T2DM and CAN. Further investigations aimed to establish the influence of ω-3 PUFA on dynamics of independent cardiovascular tests, daily monitoring of electrocardiography, daily monitoring of BP, arterial wall stiffness parameters among patients with T2DM and CAN are necessary[102-104].

Orthostatic hypotension treatment

Postural hypotension syndrome is manifested by dizziness and possibility of consciousness loss. Hypovolemia and sympathoadrenal disorders are the most characteristic features among patients with T2DM and orthostatic hypotension. Postural hypotension among most diabetic patients progresses asymptomatically and, therefore, does not require correction. However, in severe cases-it is key traumatic factor. Treatment of symptomatic postural hypotension among patients with CAN is very complicated because of the need to achieve a balance between changes in BP in the vertical and horizontal position. The increase of peripheral venous inflow is achieved through

Table 4 Arterial stiffness parameters in patients with type 2 diabetes mellitus and cardiac autonomic neuropathy

Parameter	Control (n = 12)	Patients with T2DM without CVD and CAN (n = 12)	Patients with T2DM and CAN (n = 21)
	1st group	2nd group	3rd group
AIxao (%)	20.6 ± 1.71	26.7 ± 1.84[a]	33.7 ± 1.24[c,e]
AIxbr (%)	-33.7 ± 2.86	-23.4 ± 1.91[b]	-7.9 ± 2.67[c,f]
PWV (m/s)	7.2 ± 0.31	8.9 ± 0.25[c]	11.1 ± 0.39[c,f]
AASI	0.3 ± 0.02	0.36 ± 0.02[a]	0.47 ± 0.03[c,e]

Δ%, Mean ± SEM; [a]P < 0.05, [b]P < 0.01, [c]P < 0.001 - compared to 1st group; [e]P < 0.01, [f]P < 0.001 - compared to 2nd group. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; CVD: Cardiovascular diseases; AIxao: Aortha augmentation index; AIxbr: Brachial augmentation index; PWV: Pulse wave velocity; AASI: Ambulatory arterial stiffness index.

Table 5 Changes of day arterial stiffness parameters after 3 mo omega-3 polyunsaturated fatty acid therapy

	Patients with T2DM and CAN (n = 39)			
	Groups	Baseline	After treatment	% change from baseline
AIxao (%)	Control group	30.4 ± 1.97	28.4 ± 1.68	-4.3% ± 4.76%
	Treatment group	32.0 ± 1.32	26. 4 ± 1.12[b]	-16.2% ± 3.12%
AIxbr (%)	Control group	-10.6 ± 3.37	-12.0 ± 3.11	-19.3% ± 12.14%
	Treatment group	-9.8 ± 2.76	-14.3 ± 2.84	-42.8% ± 9.0%
PWV (m/s)	Control group	10.2 ± 0.4	9.6 ± 0.4	-6.0% ± 2.21%
	Treatment group	11.0 ± 0.35	9.7 ± 0.39[a]	-11.6% ± 2.09%

The results are given as absolute values and as % change from baseline, (Δ%, Mean ± SEM); [a]P < 0.05, [b]P < 0.01, - compared to baseline. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; AIxao: Aortha augmentation index; AIxbr: Brachial augmentation index; PWV: Pulse wave velocity.

Table 6 Changes of night arterial stiffness parameters after 3 mo omega-3 polyunsaturated fatty acid therapy

	Patients with T2DM and CAN (n = 39)			
	Groups	Baseline	After treatment	% change from baseline
AIxao (%)	Control group	33.2 ± 1.98	30.1 ± 1.27	-6.6% ± 4.15%
	Treatment group	36.6 ± 1.65	31.7 ± 1.23[a]	-11.2% ± 4.2%
AIxbr (%)	Control group	-4.2 ± 2.8	-5.9 ± 2.48	-10.0% ± 17.23%
	Treatment group	-1.6 ± 2.79	-10.4 ± 3.23[a]	-98.0% ± 18.1%
PWV (m/s)	Control group	10.9 ± 0.4	10.3 ± 0.36	-4.93% ± 1.41%
	Treatment group	11.3 ± 0.48	9.0 ± 0.44[b]	-18.9% ± 3.9%

The results are given as absolute values and as % change from baseline, (Δ%, Mean ± SEM); [a]P < 0.05, [b]P < 0.01, - compared to baseline. T2DM: Type 2 diabetes mellitus; CAN: Cardiac autonomic neuropathy; AIxao: Aortha augmentation index; AIxbr: Brachial augmentation index; PWV: Pulse wave velocity.

the use of elastic tightening body linen. It is inappropriate to prescribe psychotropic and diuretic drugs, and eliminate the possibility of electrolyte disorders and/or reduce the fluid volume. Prescription of glucocorticoids is efficient among some patients with postural hypotension, but may be followed by the development of edema, risk of arterial hypertention. Metoclopramide is effecient among patients with excessive dopaminergic activity, or increased sensitivity to dopaminergic stimulation. The ineffectiveness of the above remedial measures requires the prescription of α_1-adrenergic agonists (midodrine) or dihydroergotamine combined with caffeine. Exceptional refractory to the treatment, often postprandial orthostatic hypotension forms determine the necessity of octreotidum prescription[105,106].

PROSPECTIVE DIRECTIONS OF CAN TREATMENT

The revival of interest in vascular hypothesis of CAN, OS index, neurotrophic hypothesis and importance of autoimmune disorders opens up new areas of treatment. The promising methods include research and use of tools that increase blood flow through the vasa vasorum, including butaprost (prostacyclin analogue), TXA_2 blockers and drugs that contribute into strengthening and/or normalization of Na^+, K^+-ATPase (cilostazol-a potential phosphodiesterase inhibitor), α-LA, DGLA, ω-3 PUFAs, and the simultaneous prescription of α-LA, ω-3 PUFA and DGLA[107-112]. In addition, the combination of α-LA, ω-3 PUFAs, DGLA and ARI is the

most rational pathogenetically justified use.

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REVIEW

Short and long term neuro-behavioral alterations in type 1 diabetes mellitus pediatric population

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Abstract

Type 1 diabetes mellitus (T1DM) is one of the most prevalent chronic conditions affecting individuals under the age of 18 years, with increasing incidence worldwide, especially among very young age groups, younger than 5. There is still no cure for the disease, and therapeutic goals and guidelines are a challenge. Currently, despite T1DM intensive management and technological interventions in therapy, the majority of pediatric patients do not achieve glycemic control goals. This leads to a potential prognosis of long term diabetic complications, nephrological, cardiac, ophthalmological and neurological. Unfortunately, the neurological manifestations, including neurocognitive and behavioral complications, may present soon after disease onset, during childhood and adolescence. These manifestations may be prominent, but at times subtle, thus they are often not reported by patients or physicians as related to the diabetes. Furthermore, the metabolic mechanism for such manifestations has been inconsistent and difficult to interpret in practical clinical care, as reported in several reviews on the topic of brain and T1DM. However, new technological methods for brain assessment, as well as the introduction of continuous glucose monitoring, provide new insights and information regarding brain related manifestations and glycemic variability and control parameters, which may impact the clinical care of children and youth with T1DM. This paper provides a comprehensive review of the most recently reported behavioral, cognitive domains, sleep related, electrophysiological, and structural alterations in children and adolescences from a novel point of view. The review focuses on reported impairments based on duration of T1DM, its timeline, and modifiable disease related risk parameters. These findings are not without controversy, and limitations of data are presented in addition to recommendations for future research direction.

Key words: Type 1 diabetes mellitus; Cognitive; Behavioral; Brain; Alterations; Children; Adolescences

Core tip: Neurocognitive and behavioral functions vary with age and depend on interactions between multiple endogenous (brain structure, integrity) and exogenous (metabolic) factors. Understanding of brain development with age is an emerging field of research, and delineation of type 1 diabetes mellitus (T1DM) impact on those processes is even more challenging and unclear. We review the most recent information in a novel format, relevant for clinicians practicing pediatric medicine and diabetologists taking part in clinical oriented research, in order to clarify: what is known, what is its association to modifiable diabetes related aspects, what should clinicians pay attention to, and what is needed in future research. The available studies presented hereby already indicate the need for a change in the care of pediatric population with T1DM. Periodic psychological and neurological ongoing evaluation of children and youth with T1DM, including cognition specific questionnaires and direct testing, should be performed as part of clinical care, especially while taking into account patients' daily and nocturnal glucose variability.

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INTRODUCTION

The notion that diabetes mellitus (DM) impacts brain function and structure is not new. The theory arose for the first time in 1922[1], and since then the idea has intrigued many investigators, especially in regard to its effect on quality of life in young children and adolescents.

Although it is well-known that type 1 DM (T1DM) is associated with neuro-cognitive impairments, there are still some open debates regarding which abilities are impaired, their appearance according to disease acquisition, and their underling mechanisms. Understanding the full impact of T1DM on the brain, and glycemic control in particular, is critical[2], especially in children and adolescents, a key period for development of brain matter as well as cognitive functions[3,4]. One should remember that this is the most challenging period of T1DM management due to the demanding and exhausting therapeutic self-care guidelines of this chronic disorder, especially in this pediatric and youth age group[5].

The current review aims to explore the reported neuro-behavioral alterations in children and adolescences from a novel point of view. The review focuses on cognitive and behavioral impairments according to duration of T1DM and its timeline, and according to modifiable disease related risk parameters. We do not present data regarding the physiological and tissue or cell related pathogenic processes, which are available elsewhere. Our aim is to summarize for clinicians the historical and novel data available, as compared to normal cognitive and behavioral age related development, in order to direct, modify and individualize the specific clinical goals in order to encourage both better outcomes in day-to-day life, as well as to increase long-term outcome, with a focus on age and developmental stage.

We wish to clarify: what is known about the disorder, what is its association to modifiable diabetes related metabolic aspects, what clinicians should pay attention to and prevent by clinical care and what is needed in future research.

BEHAVIORAL ALTERATIONS

Adolescence is a crucial time for the formation of healthy and responsible habits, yet it is an age characterized by an increasing tendency for risk-taking behavior. This is especially notable for adolescents with T1DM, who may require long-term healthcare support in creating healthy and responsible habits[3].

Studies show that mastery over one's environment, an important component of resiliency, is related to life satisfaction, quality of life and improved daily functioning, including school functioning[6,7]. Better glycemic control is related to resiliency and quality of life, which are both associated with better school functioning in youth with T1DM[6].

Importantly, children and adolescents with T1DM show lower levels of life satisfaction than control populations[8]. Life satisfaction is often discussed in the context of quality of life, which in children and adolescents with T1DM is associated with better glycemic control parameters, assessed as lower HbA1c (Glycated hemoglobin) levels[9].

Unfortunately, poor health behaviors, such as drinking alcohol and smoking, are equally high among youth with T1DM as compared to their healthy peer counterparts, despite the increased risk associated with such behaviors in T1DM[8].

Self-care

Older studies found that young adults with T1DM have a higher tendency to live at home and study at local colleges[10,11]; however, more recent research no longer shows such differences between those with T1DM and the typical population[12]. This lack of a difference in more recent research may be explained by the progress in T1DM education, self-care empowerment and technological progress in T1DM management and glycemic control. These advances enable youth with T1DM to more flexible self-care and better glycemic control outcomes[13].

School

Teachers often have limited knowledge about diabetes, leading to confusion in many areas associated with the disorder, particularly in regard to physical education. This lack of knowledge may have great implications on the child and their disorder. Teachers may wrongly exclude diabetic children from activities in which they are actually able to participate, and likewise children may get away with using their illness to avoid participation in activities that are in fact not harmful, and are possibly even favorable to their health. This may lead to skipping physical activity, which can in turn cause isolation among children with T1DM[14]. Further, increased absences from school or missing class time in order to check glucose levels[9,13,15] can contribute to lower academic achievement among students with T1DM[7,13,15,16]. Importantly, overall it was found that children with fair to good glycemic control earn better grades in school as compared to children with poor glycemic control[17]. The mechanism involved is not yet discerned. Both direct effects of glycemic control on the ability to learn may operate here, in addition to indirect paths, such as by affecting sleep-wake rhythms. School grades and school absences of youth with T1DM were found to be related to sleep duration and those, in turn, were found to be related to increased burden associated with diabetes management[7]. Hence, a special focus on sleep quality is warranted for more optimal care of T1MD.

Physical activity

Results are inconsistent in regard to physical activity levels in those with and without T1DM. Few studies found that both groups showed similar levels of self-reported physical activity, while others found lower physical activity among youth with T1DM as compared to controls[18], and one research reported higher activity among diabetic youth[19]. It should be emphasized that physical activity has many health benefits for T1DM patients, in that it is known to improve their physical fitness, strength and overall well-being[18,20-22], while decreasing long-term health deficiencies, such as vascular complications, for which T1DM patients are particularly vulnerable[23].

Sleep

There are several reports regarding associations between sleep patterns and T1DM. Restricted sleep may contribute to reduced peripheral insulin sensitivity in T1DM[24,25]. Children with T1DM experience ventilatory dysfunction during sleep, which is related to diabetes duration and to glycemic control during sleep[26]. Adolescences with T1DM have more awakenings due to glucose fluctuations[27] and they do not sleep as deeply as healthy population[7,28]. Further, there may be increased sleep disturbances among adolescent males with T1DM as compared to females and healthy peers[8]. In addition, children with T1DM are known to experience longer and more frequent apnea (sleep disordered breathing) events, increased awakening from night sleep, more daytime sleepiness, decrease in total sleep time, decreased sleep efficiency and increased sleep latency, as compared to healthy children[27]. Those with T1DM spend slightly less time in slow-wave sleep during the first half of the night and report less restorative sleep than their healthy peers as well[25]. Based on the reported influence that T1DM may have on sleep, glycemic variation and poor glycemic control were associated with sleep-wake cycles in T1DM patients[29], and associations between sleep loss and high-caloric intake as seen in Western lifestyles may reinforce this circle given that glycemic alterations during the night may affect the waking phase of the sleep-wake cycle[29]. Youth with T1DM who spend less time in slow-wave sleep have higher average daily glucose values, higher HbA1c levels and more frequent hyperglycemia occurrences. More time in fast-wave sleep was associated with parental reporting of sleepiness, depressive moods, emotional and behavioral difficulties, lower grades in school and lower overall quality of life[28].

Healthier life styles, which include proper quality and duration of sleep, may improve metabolic control, which in turn will further improve sleep quality. This suggests that proper diagnosis and treatment for sleep disorders among the T1DM population is paramount to improving outcome for the disorder.

Mood

During adolescents, particularly in patients who are challenged by school's academic and social demands symptoms of depression arise more frequently, these symptoms may lead to yet poorer glycemic control, and thus increase complications associated with diabetes[30]. Youth with T1DM report more mental health symptoms as compared to healthy peers[6]. This includes increased depression and more anxiety as compared to controls, although the differences seem to be smaller in more recent studies[31]. Hanna *et al*[32] reported that depressive symptoms among adolescents with T1DM are associated with diabetes related weight control behavior. Depressive symptoms were found to be a predictor for poorer diabetic management and glycemic control among adolescences with T1DM, along with increased age, longer durations of diabetes, insulin *via* injections and diabetic specific family conflict[33]. Further research found that depression and anxiety are associated with poorer glycemic control and more long term complications, which can apparently be explained by the negative impact these symptoms have on the capacity to follow diabetes treatment routines[31,34,35].

Importantly, better parental relationships are associated with fewer depressive symptoms in emerging adulthood in this population[36], and increased family support among youth with diabetes is related to better glycemic control[37]. Mood and parental relationships then play an important role in good glycemic control, which can predict less diabetes related long term

damages. This underscores the importance of addressing family relations early during T1DM diagnosis and in management education.

Disturbed eating behavior

In considering family relations for glycemic control in T1DM, familial eating habits and familial management of gender specific maturational issues should be addressed. Importantly in this regard are the findings that adolescent females with T1DM tend to develop eating disorders twice as often as those without the disorder, and eating disorders are associated with poorer glycemic control[38]. Therefore, disturbed eating behavior is another relevant aspect that has strong implications for diabetes related complications in T1DM[39].

COGNITIVE ALTERATIONS

Taking together the physiological, psychological and academic issues reviewed above in T1DM, it is important to explore the cognitive alterations that may account for some of these symptoms. Many studies have reported cognitive impairments in people with T1DM, including children and adolescents. In adults, deficits in cognitive abilities are seen mostly in the domains of general intelligence, psychomotor speed and mental flexibility. In children, lower overall IQ scores were found in those with T1DM as compared to controls, and more noticeable difference were found in children with early onset diabetes (usually under 7 years old)[40]. In addition, multiple studies have reported that children with T1DM show difficulties in response time, abstract reasoning, cognitive flexibility and verbal memory, as compared to controls; however, the differences reported are fairly small and inconsistent with borderline significance[4,13,41-50].

The progression of these deficits when no other microvascular complications are present is generally present yet slow[51]. Cognitive impairments may be seen within two years of diagnosis[52], while after six years after diabetes onset, measures of intelligence, attention, processing speed, long term memory, executive function and self-monitoring were found to be lower than those of healthy peers, with stronger effects in attention, processing speed and executive function in children who were younger than 4 at disease onset[53].

Twelve years after diagnosis, youth with T1DM showed impaired performance on working memory, compared to healthy controls. Early diabetes onset was associated with poorer attention, learning and mental efficiency. This has been thought to be related to both hypoglycemic and hyperglycemic events: Hypoglycemic events were associated with impaired learning and memory and slower processing speed, and hyperglycemia was associated with impaired working memory[54]. Yet results are not consistent: In another study on preschoolers, T1DM patients showed no difference in neurocognitive performance as compared to healthy controls, however, poor glycemic control was associated with lower cognitive abilities, slower fine motor speed and lower receptive language scores[55]. Poor glycemic control in children 4-10 years old was associated with lower verbal comprehension scores, and a history of hypoglycemic seizures was associated with lower processing speed, lower full scale IQ score, impaired working memory and perceptual reasoning[56]. A recent study on T1DM children from the same age group assessed the influence of hypoglycemic as well as hyperglycemic excursions on cognitive functioning, demonstrating a tendency for lower general IQ scores and executive functions compared to healthy peers[57]. It may be the case that the presence of several risk factors rather than one or none is associated with poorer cognitive performance that may compromise learning and memory and executive functions in T1DM[54].

Learning and memory

Diabetes effect on memory and learning was first raised in 1922 by Miles *et al*[1], who reported that people with diabetes complained of memory loss and attention difficulties. Later research found that early onset of T1DM was associated with verbal and visual learning and memory skills[46], verbal and nonverbal intelligence, attention and psychomotor skills[58]. In a recent study chronic hyperglycemia was associated with learning and memory in children[57]. Further, one research found that in boys with T1DM, lower verbal intelligence was associated with poor glycemic control; however, most studies found no gender differences in neurocognitive abilities[59].

Memory, especially working memory, is influenced by multiple risk factors[60,61] including glycemic excursions, but by itself this was not proven to cause long term damage in T1DM, yet early diabetes onset may be a relevant additional risk factor in this respect[45,62].

Executive functions

Early onset T1DM is associated with deficits in executive functioning[46,58], which include the ability to initiate, plan, consolidate and sustain problem solving in working memory, to control emotions and behavior and to modify a cognitive set through proper inhibition control[63]. The integrity of executive functions may be of particular importance for management of T1DM: People with T1DM have many daily tasks that must be organized, including timely insulin administration, blood glucose monitoring and regulating dietary intake[64]. This suggests that executive functions are critically important in adolescents with T1DM during a time in which diabetes self-management must be learned and maintained. In fact, a relation was found between executive functioning and adherence to a self-management schedule in adolescents with T1DM[64,65]. A mild trend was recently noticed in young children with T1DM, such that lower executive function was associated with hyperglycemia, a deficit that becomes more apparent and more debilitating

in older children or in those with longer durations of the disorder[57]. Further, impaired decision making was found to be associated with T1DM in several studies, and was associated with comorbid depression, cognitive deficits and hypoglycemia unawareness[59,66,67]. Impaired decision making may be related to white-matter microstructural deficits that were reported on neuroimaging studies in youth with T1DM[66]. This cognitive disadvantage may cause poorer glycemic control, and thus elicit more brain alterations.

BRAIN STRUCTURE ALTERATIONS

Neuroplasticity is the ability of the brain to change its structure and function due to environmental changes. Deficiencies in brain trajectories involved in neuroplasticity were reported among T1DM, including hyperglycemia and hyper insulinemia[68]. Yet, the findings in this regard do not yet offer a coherent framework.

Consistent findings in neuroimaging research show structural changes, especially in cortical grey matter[69]; however, very few studies have investigated participants younger than 20 years old[70]. The few that exist show that brain volume alterations are detectable already in childhood[70-72] and that these alterations have long term influences into adulthood[51]. On the other hand, there is a consensus that some reported atrophic changes are short term and may be related to glucose excursion[70]. For example, early results from a magnetic resonance imaging (MRI) study found that higher rates of ventricular atrophy and hippocampal white matter lesions were correlated with early diabetes onset[62], and larger hippocampal volumes were associated with recurrent severe hypoglycemia[73]. Future studies may explore a potential vicious circle between poor glycemic control in this population, learning and memory deficits and these brain structural changes. In this regard, it is important to consider both grey and white matter volume changes.

Grey matter volume

Smaller grey matter volume in the left superior temporal region and in the thalamus were reported among youth with T1DM, as well as among children ages 4-10, as compared to healthy controls[56,74,75]. Those findings in both reports were associated with a history of severe hypoglycemic events. Smaller grey matter volume in the right cuneus and precuneus was also reported in T1DM patients, but associated with greater exposure to hyperglycemia[74]. The decreased grey matter in those areas was also associated with high rest activity and dramatic decrease in brain activity during goal-oriented tasks[70]. No other findings dealing with children and adolescents with T1DM support these associations to grey matter volume, however studies in adults showed similar findings[70]. Smaller grey matter volume in bilateral temporal-occipital and cerebellar regions, and larger grey matter volume in left inferior prefrontal, insula and temporal pole regions, associated with hyperglycemia, were seen in young children (mean age 7 years old) with early onset of T1DM[71]. Similarly, higher HbA1c was associated overall decrease in grey matter volume[76].

White matter volume

Perantie *et al*[74] reported that smaller white matter volume in the right posterior parietal region is associated with greater exposure to hyperglycemia in T1DM patients, and that distinct decrease in white mater volume, especially in the occipito-parietal cortex, is associated with sever hypoglycemia[76]. Compared to healthy controls, young children between 4-10 years of age with T1DM who experienced hypoglycemic seizures showed significantly altered age-related white matter development (amygdala and hippocampus as well) and smaller white matter volumes[56].

It is obvious that findings regarding T1DM, glycemic control and glucose excursions and their association to white and grey brain matter regions, are inconsistent and rely on few reports. In order to clear up these inconsistencies, further studies are required regarding the clinical and neurocognitive impact of such findings, and their cause. Whether there is any long term effect of severe hypoglycemia or of poor glycemic control on the developing brain, and whether the effect is subtle, as suggested by Arbelaez *et al*[70], should be clarified with further research. These different findings raise new questions regarding the possible explanations of increase or decrease in brain volume, the processes they may reflect as a function of age, gender, and severity, and the impact they may have on the lives and well-being of young patients with diabetes, in order to improve management at the various age groups[77]. Some of these disparities concerning structural changes at different ages and glycemic control may be resolved in the future by adding brain properties into the model.

BRAIN PROPERTIES

White matter integrity

Barnea-Goraly *et al*[78] recently suggested that differences in fibers integrity and radial diffusivity are negatively associated with age of T1DM onset. Yet little is still known with regard to young patients.

Middle-aged adults with T1DM showed a decrease in white matter integrity of posterior parietal region[51]. In children with T1DM, microstructural white matter integrity changes were seen in the thalamus, hippocampus and superior parietal regions[72]. White matter microstructural changes were more apparent in children with higher HbA1c. White matter microstructural changes manifested especially in the frontal and temporal regions, including lower axial diffusivity values in diabetic children compared to controls, and in higher radial diffusivity among those who had higher HbA1c[79]. These finding support the association between poor glycemic control

and demyelination and gliosis in frontal and temporal lobes[80]. Lower axial diffusivities involving many white matter trajectories in all cortical lobes were observed in T1DM children as compared to controls. These white matter integrity alterations were associated with hyperglycemia and with cognitive impairments. White matter integrity alterations in the superior parietal and particularly in the precuneus and cuneus region, and decreased density in the hippocampus were associated with hyperglycemia[72].

Connectivity

Alterations in neural networks were correlated with cognitive functions, such as with attention and memory[81] and with mental illness, both of which are known to be associated with T1DM[68]. In adults, abnormalities in functional magnetic fields and in the brain neural connectivity were detected in individuals with T1DM using magnetic encephalography (MEG)[69]. Several studies employing various modalities and methodologies [electroencephalography (EEG), functional MRI (fMRI), MEG] agree on the overall influence that T1DM has on the brain's functional connectivity[68,82-84]. These researches support the hypothesis that brain connectivity is altered due to diabetes, but with respect to affected brain regions, the results vary substantially[51]. Additionally, these findings have not yet been replicated on children[70]. The influence of hypoglycemia, glucose excursions or overall glycemic control on those findings requires further research.

BRAIN ACTIVITY ALTERATIONS

EEG differences have been seen between individuals with T1DM and healthy controls, including loss of high frequency activity in temporal, frontal and occipital regions and lower frequencies overall. These changes were attributed to the metabolic disturbances caused by a history of prolonged diabetes and past severe hypoglycemic events[85-89]. However, most studies did not assess simultaneous glucose concentration, thus relation to hyperglycemic excursions could not be delineated, as well as duration of the disorder. Transient changes in electrical activity in various brain regions were reported during hyperglycemia during wakefulness and sleep among youth with T1DM. Glucose excursions above 280 mg/dL during wakefulness was associated with a decrease in the power of high frequency bands (α, β, and γ), and increase in the power of low frequency bands (δ and θ) in the EEG from central and occipital regions. Glucose concentration > 200 mg/dL during sleep was associated with increased power in high frequency bands in the EEG in frontal and central areas and more low frequencies generally. However, the clinical neurocognitive relevance of those findings is still not clear[90].

T1DM RELATED RISK FACTORS

Glycemic control

Daily hypoglycemic and hyperglycemic excursions are frequent among T1DM patients despite efforts to keep glucose concentration within a narrow range, especially in the pediatric and adolescent population. Despite efforts to maintain strict intensive management, including dietary restriction, multiple daily glucose measurements and insulin injections in order to prevent glucose excursions in daily life, glucose concentration in children and young adolescents is more frequently in the hyperglycemic range[91]. Possible effects of those excursions and their length on cognitive functions, brain activity and brain structure have mostly been examined in adults; yet, their effects on young children and adolescent brains may be critical. This age is a common period for marked developmental changes in the brain and in the maturation of cognitive functions, making the brain more susceptible to an array of pathogeneses, including metabolic instability[3,4].

Hypoglycemic events

The anxiety associated with severe hypoglycemia is a major barrier in optimizing glycemic control, yet, a recent ten-year longitudinal study testing 1770 participants found that poor glycemic control was not associated with an increased risk for severe hypoglycemia[92]. This is an important factor, since frequent mild hypoglycemic events are related to impaired cognitive functions, including abstract reasoning, motor responses, processing speeds, selective attention and behavioral inhibition[40] all critical for problem solving, academic and social achievements and to well-being. Early studies reported that severe hypoglycemia is a primary cause of neurocognitive impairments[42,93,94], but this was not supported by two meta-analyses that investigated poor glycemic control as a potential risk factor[16,46]. Unfortunately, hyperglycemic and hypoglycemic events may be convolutedly intertwined, which makes it complicated to rule out severe hypoglycemia's role in cognitive impairments[74].

Hyperglycemic excursions

Poor glycemic control, defined by high values of HbA1c, is often referred to as chronic hyperglycemia[70]. Chronic hyperglycemia is associated with negative effects on memory[95-97], and with lower estimated verbal intelligence[49] in children with T1DM. The effects of chronic hyperglycemia on young children include lower cognitive abilities, slower fine motor speed and lower receptive language scores[55]. Hence, hyperglycemia is a major risk factor for cognitive decrements.

Age of diabetes onset

A diagnosis of T1DM in the first 4-7 years of life appears to be a major risk factor for significant clinical neurocognitive deficiencies[62]. Both severe hypoglycemia and

chronic hyperglycemia may impair cognitive abilities at this age range[98].

Specifically, early onset diabetes, particularly before the age of 5, is associated with a significant reduction in IQ scores, slower motor speed, visuospatial processing deficits, selective attention, verbal memory deficits and executive function deficits. Not all domains of functioning were impaired in children with early onset diabetes, and different studies showed contradicting outcomes regarding the impact of early diabetes onset[49,94]. From a neuro-structural perspective early onset (5-9 years old) was associated with bilateral grey matter decrease in the cerebellum and occipital regions and with grey matter increase in left insula, inferior frontal and temporal poles[71]. The meaning of these findings has yet to be investigated in particularly with regard to implications on cognitive function and on management.

DISCUSSION

Neuro-behavioral alterations and cognitive deficits associated with T1DM may be found in children and in adolescence soon after diabetes onset, as well as after long standing disease duration. However, its reported frequency and severity differ with age, T1DM duration and reported timeline according to medical therapeutic development. These clinical alterations seem to be correlated with brain changes (*i.e.*, structural and white matter integrity findings), and were found to be associated with diabetes metabolic consequences. By reviewing the recent literature we can emphasize some ideas and insights to help us understand the known findings that may be associated with T1DM, and to suggest future research goals.

Looking at the history of the disorder, less pervasive and behavioral findings are reported in children and adolescents with T1DM in more recent research. This is highly promising given the rise in affected children, and may be a result of better care, better education, and better health technologies that gradually became available, together with improved glycemic control management plans.

Overall, findings point to the notion that T1DM is associated with interconnected behavioral alterations and glycemic control pathologies, a timeline summarizing core finding is presented in Figure 1.

In the cognitive realm T1DM seems to be associated with impaired memory and learning, slow cognitive processing, reduced general intelligence and impaired attention and executive functions.

As for the psychological - emotional domain, T1DM seems to be tied to depression, anxiety and low life satisfaction, which may contribute to poorer glycemic control, but may also be exacerbated by it. Glucose concentration excursions may impair the length and efficiency of night sleep, affecting school achievements and physical activity, which are also in turn associated with yet poor glycemic control. Poor glycemic control

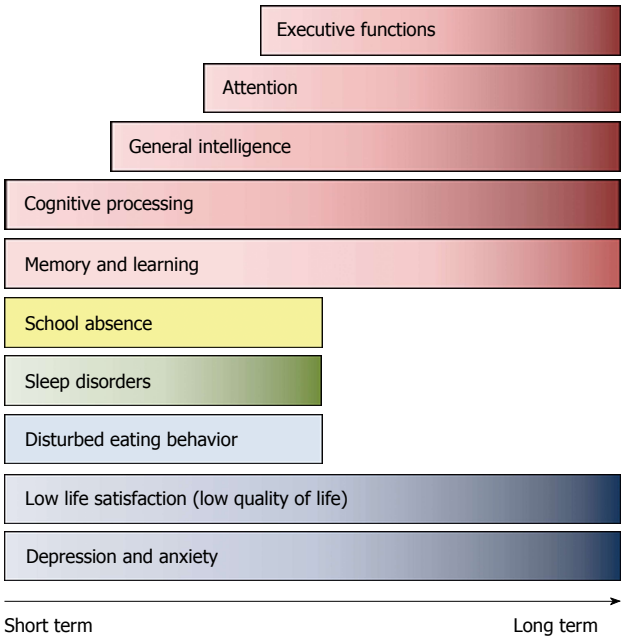

Figure 1 Short and long term behavioral and cognitive reported alterations following type 1 diabetes mellitus onset in children and adolescents according to type 1 diabetes mellitus duration. Figure is general and based on data retrieved from published reports as detailed in text.

may also affect mood, sleep, physical activity and grades in school, which once again circularly lead to poorer glycemic control, more depression and anxiety, cognitive impairments and eventually diabetes complications. The good news is that good glycemic control may balance self-control, mood and physical shape and moderate this escalating deterioration to some degree.

In recent studies, only mild cognitive deficits were observed as compared to healthy controls, which may become a marked deficit in the presences of additional risk factors. Combinations of risk factors are known to increase the probability of significant impairments in T1DM. Among these, we reviewed early onset diabetes from the ages 4-7 years of age, severe hypoglycemic seizures, hypoglycemia, hyperglycemia and poor glycemic control. Early diabetes onset, together with severe hypoglycemic seizures, may cause more significant impairments in some cognitive domains. Figure 2 summarizes the associations between cognitive domains and diabetes risk factor.

Overall, it seems that early achievement of glycemic control (*i.e.*, age dependent goal of HbA1c), may reduce the risk of cognitive impairments. Fear of hypoglycemia is considered to be a barrier to reaching good glycemic control, but recent studies support the understanding that though recurrent episodes of moderate hypoglycemia may compromise memory and executive function, in manners that are corroborated by alterations in the hippocampus, in the short term; implications for affects on long term cognitive abilities have not been proven. On the other hand, poor glycemic control and recurrent hyperglycemia seems to have long term effects on

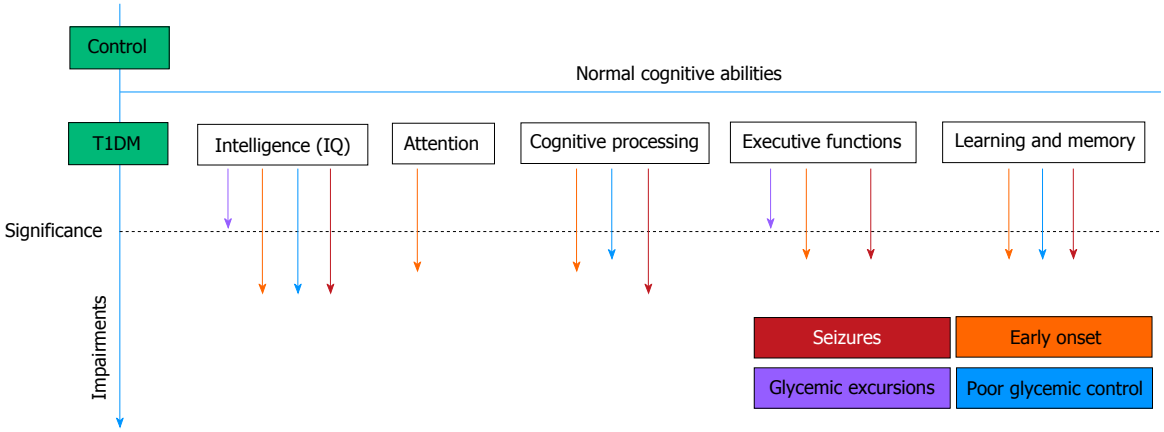

Figure 2 Impaired cognitive domains in children and adolescents with type 1 diabetes mellitus. Mild impairments were found compared to healthy controls, but several risk factors (marked by arrows) increase potential deficits. This figure is generalized, qualitative and not quantitative, based on reported studies. T1DM: Type 1 diabetes mellitus.

cognitive abilities.

Poor glycemic control is also associated with changes in brain structure, brain integrity and brain activity. Studies focusing only on the adult population demonstrated differences in connectivity underscoring the importance of extending this research line to younger patients in whom, plasticity is expected to be marked both as a function of poor glycemic control and by affecting cognitive competence.

PERSPECTIVES

Despite a wide range of previous research dealing with adults, this line of study in the pediatric population is still in its beginning phases for several reasons. First, glycemic control is accompanied with glycemic excursions, transient hypoglycemia and transient hyperglycemia, and their impact on brain functionality and on cognitive and behavioral domains has yet to be proven. Second, new studies with novel brain imaging data analysis methods have uncovered associations and impacts that diabetes has on brain properties and on brain function. Third, technological development introduced new modalities of brain study as well as ability to assess glucose excursions longitudinally and glycemic control parameters more accurately. Importantly, due to a lack of research dealing with developing age groups whose neurobehavior performances are essential to understanding the impact that T1DM, the brain the review revealed open questions regarding sensitivity and specificity of brain-behavior relations in typical and in atypical development in children and adolescents. Some reported findings with T1DM patients actually be part of normal variability of brain development, others may be a general response to stress that is not necessarily specific to T1DM.

Further research is required in a longitudinal prospective manner, beginning with T1DM diagnosis and continuing throughout the years of management. This research should include simultaneous objective assessments of neurocognitive function, behavioral environmental objective questionnaire based assessment and brain function analysis. Brain function analysis may include novel modalities of fMRI, infusibility and connectivity assessment, and electroencephalographic assessment. Analysis should be performed in an attempt to assess simultaneous glucose excursions in a wide range of glucose concentrations, including the hypoglycemic and hyperglycemic ranges.

Studies should be performed in large populations, but within specific age groups to enable comparison with normal brain development and to avoid statements of possible abnormalities that may actually be part of normal brain function and development variability and not related to T1DM. On the other hand, this will elucidate the modifiable parameters of disease management.

In summary, review of neurobehavioral findings with pediatric populations with T1DM indicates that neurocognitive and behavioral function varies with age and depends on multiple endogenous and exogenous factors that are relevant to care methodologies. These functions are dependent on brain structure, integrity, connectivity, metabolic immediate and long term changes and vary constantly. Since understanding of brain maturation with age is an emerging field of research, delineation of T1DM impact on these processes is challenging but possible with current advances.

Importantly the available studies presented hereby already indicate the need for a change in the care of pediatric population with T1DM. Periodic psychological and neurological ongoing evaluation of children and youth with T1DM, including cognition specific questionnaires and direct testing, should be performed as part of clinical care, especially while taking into account patients' daily and nocturnal glucose variability. Clear goals of glycemic control criteria should be stated to families, including explanation of possible immediate cognitive impact in order to improve compliance. Gaining a deeper understanding of the effects of T1DM on cognitive functions, brain activity and brain connectivity may

deepen the understanding of the aftermaths of diabetes and eventually lead to better individual titration of management of young patients with T1DM in order to prevent long term and short term neurocognitive complications.

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REVIEW

Role of phytoestrogens in prevention and management of type 2 diabetes

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Abstract

Type 2 diabetes (T2D) has become a major public health threat across the globe. It has been widely acknowledged that diet plays an important role in the development and management of T2D. Phytoestrogens are polyphenols that are structurally similar to endogenous estrogen and have weak estrogenic properties. Emerging evidence from pre-clinical models has suggested that phytoestrogens may have anti-diabetic function *via* both estrogen-dependent and estrogen-independent pathways. In the current review, we have summarized the evidence linking two major types of phytoestrogens, isoflavones and lignans, and T2D from epidemiological studies and clinical trials. The cross-sectional and prospective cohort studies have reported inconsistent results, which may due to the large variations in different populations and measurement errors in dietary intakes. Long-term intervention studies using isoflavone supplements have reported potential beneficial effects on glycemic parameters in postmenopausal women, while results from short-term small-size clinical trials are conflicting. Taken together, the current evidence from different study designs is complex and inconsistent. Although the widespread use of phytoestrogens could not be recommended yet, habitual consumption of phytoestrogens, particularly their intact food sources like soy and whole flaxseed, could be considered as a component of overall healthy dietary pattern for prevention and management of T2D.

Key words: Type 2 diabetes; Phytoestrogen; Isoflavone; Lignan; Epidemiological study; Clinical trial

Core tip: Phytoestrogens are a group of polyphenols that are structurally similar to endogenous estrogen. Animal experiments and pre-clinical models have provided strong evidence that phytoestrogens may have anti-diabetic function *via* both estrogen-dependent and estrogen-independent pathways. A number of epidemiological studies and clinical trials have thus been conducted in different populations linking two major types of phytoestrogens, isoflavones and lignans, to the prevention and management of diabetes. Although the current evidence is complex and inconsistent, habitual consumption of phytoestrogens, particularly their intact

food sources, could be considered as a component of overall healthy dietary pattern for prevention and management of diabetes.

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INTRODUCTION

Diabetes has become a global public health crisis, and the International Diabetes Federation estimated that 382 million adults were affected by diabetes in 2013, and 5.1 million deaths due to diabetes occurred annually. More than 90% of the diabetes cases are type 2 diabetes (T2D). The global prevalence of T2D has doubled in the last 30 years and is predicted to continue to rise at an alarming rate, and the number is projected to reach 592 million by 2035. The health and economic burden from diabetes is enormous[1]. T2D is a constellation of disorders precipitated by complex and poorly understood interactions between environmental and genetic factors, leading to diminished insulin sensitivity and pancreatic β cell failure. However, diabetes is largely preventable by the adoption of a healthier lifestyle, including normal body weight, not smoking, regular exercise, and a balanced and healthy diet.

It has been widely acknowledged that diet plays an essential role in the development of T2D. Historically, the prevalence of T2D was very low in the traditional Asian society. One hypothesis speculates that the traditional Asian diet, characterized with high intakes of whole grains, large amount of vegetables and fruits, but small portions of meat products, contains many protective components against the development of T2D. Among the many food groups, soybean and soy products as the unique element of traditional Asian diet have aroused much interest because of considerable difference in its intake levels comparing with Western diet[2]. Although there are several potential beneficial compounds (soy protein, dietary fiber, monounsaturated and polyunsaturated fat, vitamins and minerals) in soybean and soy products, one group of polyphenols concentrated in soy products, isoflavones, have been suggested to be beneficial for diabetes prevention and management[2,3].

Isoflavones belong to a group of phytochemicals called phytoestrogens[4,5]. Phytoestrogens are plant-derived compounds that are structurally similar to endogenous estrogen and also have weak estrogenic properties[4,5]. There are two major types of phytoestrogens: isoflavones and lignans[4,5]. The former is concentrated in beans and soy products, and the latter is concentrated in flaxseed, sesames, whole grain and other plant-based foods[4,5]. The other types of phytoestrogens, like prenylated flavonoids and coumestans, are not commonly consumed in daily diet and are not discussed in this article.

In this review, we aimed to examine the current evidence linking phytoestrogens and T2D from epidemiological studies and clinical trials, to explore the potential underlying mechanisms of phytoestrogens' effect on glucose metabolism from animal and experimental studies, and to propose research priorities for future investigations in this field.

PHYTOESTROGENS

Isoflavones are primarily found in members of leguminosae family and occur in varying amounts in legumes consumed by humans, but soy exceptionally contains the highest isoflavone content[6]. Isoflavone contents of soy food ranges from approximately 0.1 to 5 mg/g of soy protein[7,8]. Asians generally consume very high amount of soy products, and studies have reported that the daily mean intake level of soy protein ranged from 2.0 g in Thailand to 9.6 g in North Korea[9]. Other studies have reported similar results: 5-9 g in Japanese[10] and Chinese[11-13]. The mean isoflavone intake was reported to be from 6 to 75 mg/d in these countries[9], while it was approximately 0.4 mg/d in Spain[14] and Dutch[15] populations, and approximately 0.3 mg/d in the United States population[16].

There are three main soy isoflavones, genistin, daidzin, and glycitin, in which the first two are the major ones available as sugars conjugated form (glycosides) in soybeans and most soy foods in Asian cuisines[4,5]. These biologically inactive forms are hydrolyzed in intestinal wall by the bacterial β-glucosidases and converted into the corresponding bioactive aglycones, daidzein and genistein, which then could be absorbed by intestine[7]. After initial hydrolysis of the glucoside moiety in colon, daidzein can be further metabolized to equol by colonic bacteria. In addition to the conversion by intestinal microflora, genistin and daidzin can also be converted into bioactive forms by *in vitro* fermentation that is common in traditional Asian methods of preparing soy foods[8]. The blood isoflavone concentration would be in the nanomolar range (< 40 nmol/L) in people who do not eat soy food, and can be increased to micromolar range by acute ingestion of dietary soy. Isoflavones and their metabolites are rapidly excreted in urine with a half-life of about 9 h for daidzein and 7 h for genistein[7].

The other type of phytoestrogens, plant lignans, are more ubiquitous than isoflavones, and the common food sources include oilseeds (flaxseed, sesame, soy, rapeseed), whole-grain cereals (wheat, oats, rye), and various vegetables[17,18]. Cereal fiber and wholegrain foods are among few food groups with established preventive effect for T2D[19], and lignans may be partially responsible for protective effects of dietary fiber complex[20]. Studies have suggested the use of urinary lignan excretion as a marker for fiber and whole

grain intake[21,22]. Plant lignans (secoisolariciresinol and matairesinol) are converted to mammalian lignans, enterolactone and enterodiol, by mammalian gut microflora, and enterodiol can also be further oxidized to enterolactone[4,5]. Like isoflavones, the main factors influencing circulating concentration of enterolactone are the food contents of lignans and microflora function[20].

Because of the lack of complete databases of dietary phytoestrogens, large variations of phytoestrogen contents of foods, and comprehensive metabolism pathways influencing circulating concentrations of phytoestrogens, studies have started to use objectively measured blood or urinary phytoestrogen concentrations as a good indicator of dietary intake[22-24]. In Asians with high variations of soy intakes, studies have reported a reasonably well correlation between urinary concentrations of isoflavone metabolites and dietary soy intakes (mostly assessed by food-frequency questionnaires), using morning spot urine[25], or overnight urine samples[26]. This was consistently observed in Japanese[27] and other populations as well[24]. Some studies in United States populations also confirmed that urinary concentrations of isoflavone metabolites are reasonable options for assessing isoflavone intake in epidemiologic studies[28-30]. Studies also suggested that urine samples performed better than serum samples for correlating with dietary intakes[31].

For lignans, studies in Western populations indicated that enterolactone concentrations in overnight urine samples moderately correlated with fruit and vegetables intake[32], concentrations of enterodiol and enterolactone in spot urines were significantly correlated with dietary intakes of fiber[32,33], vegetables and rye products[34]. Few studies have been performed to estimate the urinary lignan metabolites in Asian populations. A small study in 19 Japanese adults found that concentration of urinary lignan metabolites was about one third of isoflavone metabolites, and was correlated with intakes of green and yellow vegetables, pulses and beans[27]. A study in 75 Korean postmenopausal women found that the concentration of lignan metabolites in 24-h urine samples was about half of isoflavone metabolites[35], similar results were found in a study among 68 Chinese T2D patients using first morning urine samples[36]. In another large cross-sectional study of 2165 middle-aged and elderly Chinese women, despite that the concentrations of lignan metabolites were substantially lower compared to isoflavone metabolites in spot urines, the urinary enterodiol was higher than and enterolactone was similar to that among United States women of comparable age[12]. This was observed in another study that collected urine samples from several Asian countries (Japan, Vietnam, India, and Cambodia) and United States[37]: high concentrations of isoflavone metabolites were detected in urine samples from Japan and Vietnam, while the concentrations in urine samples from Cambodia and India were much lower and comparable to that found in United States samples; the differences between urinary lignan metabolites were relatively small among samples from the five countries.

RECENT HUMAN STUDIES LINKING PHYTOESTROGENS TO DIABETES AND GLUCOSE HOMEOSTASIS

The epidemiological studies on the relation between phytoestrogens and risk of T2D or diabetes biomarkers are shown in Table 1. We have described the findings by study designs as below.

Cross-sectional study evidence

Several cross-sectional studies have assessed the association between soy protein and isoflavone intakes and diabetes related markers. In the Shanghai Women's Health study of 39385 women aged 40-70 years, it was observed that soy protein intake was inversely associated with glycosuria, an important indicator of diabetes, but only in normal weight postmenopausal women[38]. However, in another study among 2811 Chinese adults, soy protein intake was significantly associated with increased odds of hyperglycemia in men, but null association in postmenopausal women[11]. The median soy protein intake was around 8 g/d in both studies[11,38]. The increased odds of hyperglycemia in men could be a chance finding, and residual confounding and reverse causation are possible in the cross-sectional studies. The sex-specific effects may also linked to the estrogen-like activity of isoflavones[4,5], but the underlying mechanisms are complex and unclear[11]. In a study of 208 American postmenopausal women who ate much lower levels of soy foods, genistein intake was significantly associated with 2-h post challenge insulin concentrations, but not fasting or 2-h glucose concentrations[39]. This suggested that isoflavones may have direct effect on β-cell function and insulin secretion, which is supported by experimental studies[40]. Among 299 pregnant women who participated in the United States NHANES 2001-2008 surveys, Shi *et al*[41] found that urinary concentrations of total isoflavone metabolites were inversely associated with fasting glucose, insulin and homeostatic model assessment of insulin resistance (HOMA-IR).

Consumption of soy products is generally low in Western diet leading to modest effect of isoflavones on metabolic markers, while lignans may be the major form of phytoestrogens and exert a stronger effect. Dietary lignan intake was inversely associated with fasting insulin and C-peptide in 468 United States men, but the association was not found for isoflavones[42]. In the Framingham Offspring Study with 939 postmenopausal women in United States, high intake of phytoestrogens was associated with a favorable metabolic cardiovascular risk profile (waist-to-hip ratio, triglyceride and overall metabolic score), with stronger association for lignans

Table 1 Epidemiological studies on the relation between phytoestrogens (lignans or isoflavones) and risk of diabetes or diabetes biomarkers

Ref.	Ethnicity	Population	Sample size, total (outcome)	Mean follow-up years	Main exposures	Outcome	Exposure level (mean or median)	Maximum effect (highest *vs* ref.)
Cross-sectional study								
Goodman-Gruen *et al*[39]	Mix[1]	Postmenopausal women, aged 45-74 yr	208	-	Dietary isoflavones	Diabetes biomarkers	4.4 mg/d genistein (mean)	Inverse with 2-h insulin (β = -0.2); not significant for FG and insulin
Yang *et al*[38]	Chinese	Women aged 40-70 yr	39385 (323)	-	Tofu and other soy products	Glycosuria	9 g/d soy protein	Inverse association in postmenopausal women
van der Schouw *et al*[42]	Caucasian	Men aged 47-83 yr	468	-	Dietary lignans and isoflavones	Diabetes biomarkers	Approximately 1 mg/d total phytoestrogens	Inverse association of lignans with fasting insulin and C-peptide; no significant association with isoflavones
Pan *et al*[11]	Chinese	Men and women aged 50-70 yr	2811	-	Dietary soy protein	Hyperglycemia (FG ⩾ 5.6 mmol/L)	7.8 g/d soy protein	Increased odds in men, but not in women
Shi *et al*[41]	Mix[1]	Pregnant women aged 28 yr	299	-	Urinary isoflavones	Diabetes biomarkers	502 mg/g creatinine	Inverse association with FG, insulin, and HOMA-IR
Longitudinal study								
Villegas *et al*[44]	Chinese	Women aged 40-70 yr	64191 (896)	4.6	Soy protein, soybeans, soy products	T2D	7.7 g/d soy protein	Inverse association with soybeans; inverse but not significant relation with soy protein or other products
Nanri *et al*[46]	Japanese	Men and women aged 45-75 yr	59791 (1114)	5	Soy products, daidzein, genistein	T2D	Approximately 73 g/d soy products, approximately 23 mg/d genistein, and 14.5 mg/d daidzein	No significant association
Morimoto *et al*[47]	Mix[2]	Men and women aged 45-75 yr	75344 (8564)	14	Soy products	T2D	Approximately 14.5 g/d in Japanese, approximately 8 g/d in Hawaiians, and 0 g/d in Caucasians	A modest increased risk in men and women
Mueller *et al*[45]	Chinese	Men and women aged 45-74 yr	43176 (2252)	5.7	Isoflavones, unsweetened and sweetened soy products	T2D	Approximately 5.2 g/d for soy protein, 15.8 mg/d for soy isoflavones	Inverse association for soy isoflavones and unsweetened soy products, while increased risk for sweetened soybean drinks
Zamora-Ros *et al*[48]	European whites	Men and women with mean age 52.4 yr	11559 cases and 15258 subcohort, case-cohort design	Approximately 12	Dietary isoflavones and lignans	T2D	0.9 mg/d isoflavones, 1.4 mg/d lignans	No significant association for isoflavones and lignans
Sun *et al*[49]	Caucasian	Women aged 65.6 yr from NHS and 45.4 from NHS Ⅱ	1107 cases and 1107 controls, nested case-control design	Approximately 6	Urinary lignin metabolites (enterodiol and enterolactone)	T2D	2.2 μmol/g creatinine for NHS women, and 1.9 μmol/g creatinine for NHS Ⅱ women	Inverse association and odds ratio 0.64 (95%CI: 0.45-0.91) comparing extreme quartiles

[1]Mostly non-Hispanic whites; [2]Caucasian, Japanese American, and Native Hawaiian. FG: Fasting glucose level; NHS: Nurses' Health Study; T2D: Type 2 diabetes; HOMA-IR: Homeostatic model assessment of insulin resistance.

compared to isoflavones[43]. No study has been conducted so far to investigate the cross-sectional relation between lignans and diabetes risk markers in Asian populations.

Prospective study evidence

A few larger prospective cohort studies have been conducted to investigate the relation between soy food consumption and risk of incident T2D in different populations. In a study with an average 4.6 years of follow-up among Chinese women from the Shanghai Women's Health Study, Villegas *et al*[44] reported that soybean and soymilk intakes were significantly associated with a lower risk of incident T2D, while soy protein and other soy products were related to a trend of reduced risk, although not statistically significant. In another large prospective study in Chinese population, the Singapore Chinese Health Study, Mueller *et al*[45] pointed out that consumption of unsweetened soy products was inversely associated with T2D risk in a graded fashion (*P* for trend = 0.02), while consuming sweetened soybean drink was positively associated with T2D risk. The findings underline the importance of food context and preparation method. Furthermore, after full adjustment including sweetened soy items, the authors observed a marginally significant inverse association between intake of isoflavones and T2D (relative risk comparing extreme quintile: 0.76; 95%CI: 0.58-1.00; *P* for trend = 0.08). In a large-scale study in middle-aged and elderly Japanese from the Japan Public Health Center-Based Prospective Study, Nanri *et al*[46] found no significant association between soy products and isoflavones with incident T2D in either men or women. The suggestive protective association in overweight women disappeared when energy-adjusted intake was considered[46]. In the Multiethnic Cohort study in Hawaii with three ethnicities (Caucasian, Japanese American, and Native Hawaiian), Morimoto *et al*[47] reported a moderately elevated risk of T2D with soy food consumption and risk of T2D during 14 years of follow-up in men and women, particularly in overweight adults. However, the consumption level of soy products was substantially lower compared to that in the Asian populations. In the European populations, the recent EPIC-InterAct case-cohort study in 12403 incident T2D cases and a subcohort of 16154 participants found no significant association between isoflavones and risk of T2D, while a suggestive trend with lignans (the hazard ratio comparing extreme quintiles 0.88; 95%CI: 0.72-1.07; *P* for trend = 0.12)[48].

Therefore, the current evidence from large longitudinal studies regarding the relation between phytoestrogen and related food sources and incident T2D is still inconsistent. One methodology challenge could be the measurement error of dietary assessment by questionnaire data. This may be due to the incomplete inclusion of phytoestrogen-enriched food items in the questionnaire and lack of comprehensive food composition databases of phytoestrogens. Furthermore, phytoestrogen metabolism and circulating concentrations in human body can be influenced by many other factors in addition to dietary intake. Thus, studies have started to measure blood or urinary phytoestrogens and evaluate the relation with disease outcomes. Recently, a nested cases-control was conducted among 1107 T2D cases and 1107 control subjects from the Nurses' Health Study (NHS) and NHS Ⅱ[49]. Urinary concentrations of the lignan metabolites were assayed by liquid chromatography-mass spectrometry. After multivariate adjustment for lifestyle and dietary risk factors of T2D, the odds ratio for T2D was 0.70 (95%CI: 0.53-0.92) for each SD increment of urinary concentrations of total lignan metabolites. The association was seen in both enterolactone [odds ratio comparing the extreme quartiles 0.62 (95%CI: 0.44-0.88), *P* for trend = 0.003] and enterodiol [odds ratio comparing the extreme quartiles 0.67 (95%CI: 0.48-0.96), *P* for trend = 0.08]. Thus far, this is the only prospective study using objectively measured phytoestrogen biomarkers to link with diabetes risk. More studies are needed to examine the relation of urinary phytoestrogen excretion and risk of developing T2D in different studies and populations with varying intake levels.

Clinical trial evidence in participants without T2D

A meta-analysis of 24 intervention studies (*n* = 1518 in total) on soy intake and glycemic control was done including trials published before March 2010[50]. While no significant effect on fasting glucose and insulin was generally observed for soy intake, the authors found 3.85 mg/dL (95%CI: 2.41-5.28) reduction in fasting glucose concentrations in a subgroup analysis of 9 studies that used whole soy foods or soy diets as the intervention regime. No statistically significant association was identified in 8 studies with isoflavone extract (ranged 40 to 132 mg/d isoflavones) or 6 trials with isolated soy protein containing isoflavones as the main intervention. This suggests that other components of soy like soy protein and fiber, polysaccharides, phytosterol, and unsaturated fatty acid or their interactions may play roles in glycemic control in addition to isoflavones. However, the majority of the studies in this meta-analysis had small sample size (ranged from 14 to 203) and short intervention period (ranged 4 to 52 wk). One of the largest studies so far was a 1-year double-blind, randomized, placebo-controlled trial in 203 Chinese postmenopausal women aged 48 to 62 years[51]. They were randomly assigned to receive daily doses of 0 mg (placebo, *n* = 67), 40 mg (*n* = 68), and 80 mg (*n* = 68) isoflavone supplements along with 500 mg calcium in all groups. The mean differences in the changes of fasting glucose between the intervention and placebo groups were -5.2 mg/dL (95%CI: -9.4 to -1.0) and -3.3 mg/dL (95%CI: -7.5 to 0.9), respectively, for the mid-dose and high-dose groups, and the effect was much more significant in women with higher baseline glucose levels[51].

Another meta-analysis of 12 clinical trials conducted before October 2010 focused on the effects of isoflavone supplementation on blood glucose and insulin in non-Asian postmenopausal women[52]. Zhang *et al*[52] found that isoflavone supplementation significantly reduced fasting glucose by 0.19 mmol/L (95%CI: 0.03-0.34), and this effect was limited to the studies with more than 6-mo period of intervention. The meta-analysis also reported a significant reduction in fasting insulin by 0.94 μU/mL (95%CI: 0.16-1.72). One of the largest and longest studies so far was done in Italian postmenopausal women with osteopenia[53]. Participants were randomly assigned to receive genistein (54 mg/d; *n* = 198) or placebo (*n* = 191) for 2 years. Both groups received 500 mg/d calcium carbonate and 400 IU/d vitamin D. Compared with placebo, genistein significantly reduced fasting glucose and insulin as well as HOMA-IR after both 12 and 24 mo of treatment[53].

Since 2010, a few more trials have been published on the effects of isoflavone supplementation on glucose homeostasis. Two long-term (24 mo) clinical trials by the same research group found that daily intake of 40 mg of soy isoflavones together with lifestyle modification (Mediterranean diet and exercise) reduced HOMA-IR compared to lifestyle modification alone among 116 Spanish postmenopausal women with insulin resistance[54], this was confirmed using same study design (except for 80 mg/d of soy isoflavones) among 80 Spanish postmenopausal women[55]. Improvement of fasting glucose and insulin was also reported[55]. Another 1-year clinical trial among 120 postmenopausal women with metabolic syndrome revealed that 54 mg/d genistein supplements (*n* = 60) significantly reduced HOMA-IR, fasting glucose and insulin compared to placebo (*n* = 60)[56]. However, the beneficial effects of isoflavones on glucose metabolism were not found in some short-term trials[57-60]. Since S-equol is considered the most biologically active metabolite of isoflavones, a study was specifically designed to evaluate the effects of S-equol on metabolic profiles among 54 Japanese overweight/obese men and women using a cross-over study design[61]. Significant improvement in HbA1c was observed using 10 mg/d *S*-equol for 12-wk compared to placebo[61].

As for flaxseed and lignans, a meta-analysis found that flaxseed and/or flaxseed lignan intervention significantly improved lipid profiles[62]. Two small cross-over clinical trials in overweight/obese glucose intolerant participants found that flaxseed reduced insulin resistance after 12-wk interventions[63,64]. A large intervention study in 293 Chinese adults with metabolic syndrome found that 30 g/d flaxseed significantly reduced HbA1c and glucose levels among those with central obesity at baseline[65]. A clinical trial in 55 hypercholesterolemic Chinese subjects found that 600 mg/d flaxseed lignan extract significantly lowered fasting glucose, particularly in those with a higher baseline glucose levels[66]. Another cross-over clinical trial in 22 healthy postmenopausal women reported that 500 mg/d flaxseed lignan extract significantly reduced C-reactive protein levels after 6 wk[67]. However, other studies have found null results[68-71].

Taken together, long-term intervention studies using isoflavone supplements have reported potential beneficial effects on glycemic parameters in postmenopausal women[51,53-56], while results from short-term small-size clinical trials are conflicting. Therefore, more high-quality long-term clinical trials are needed in men and premenopausal women, and to investigate the effect of lignans on glucose metabolism in humans.

Clinical trial evidence in patients with T2D

A number of clinical trials have been conducted in T2D patients to investigate the effects of phytoestrogens and related food sources on diabetes management. Jayagopal *et al*[72] found that 12-wk intervention of 30 g/d soy protein enriched with 132 mg isoflavones significantly reduced HbA1c (-0.6% *vs* 1.1% in placebo group), fasting insulin (-8.1% *vs* 9.9% in placebo group), and HOMA-IR (-6.5% *vs* 14.7% in placebo group) in postmenopausal women with T2D. Another long-term 4-year clinical trial among T2D patients with nephropathy reported a net change of -29 mg/dL in plasma glucose in the intervention group (*n* = 20; 0.8 g protein/kg body weight with 35% as soy protein, 35% as animal protein and 30% as vegetable protein) compared to the control group (*n* = 21; 70% as animal protein and 30% as vegetable protein)[73]. However, some small short-term trials among T2D patients failed to observe significant improvement for isoflavone-containing soy protein on glucose, insulin resistance or HbA1c[74-79]. On the other hand, clinical trials among T2D patients have reported improvement in lipid profiles[73,74,77,78,80], kidney function[73,78,81], endothelial function and blood pressures[76].

The effects of other isoflavone-enriched foods in T2D patients have also been tested: a 1-year intervention with 27 g/d flavonoid-enriched chocolate (containing 850 mg flavan-3-ols and 100 mg isoflavones) significantly reduced insulin resistance and improved insulin sensitivity and lipid profile compared to placebo in 93 postmenopausal women with T2D[82]. However, few studies have specifically investigated the effects of purified isoflavones supplements among T2D patients, and the available two interventions found no significant effects on glycemic control and lipid profiles[83,84], but the intervention periods were short (4 and 12 wk) and sample sizes were small (*n* = 16 and 32).

A few studies of flaxseed or lignans among diabetic patients also found promising results. Daily supplementation with 10 g flaxseed powder for 4 wk decreased fasting glucose by 19.7% and HbA1c by 15.6% in T2D patients[85], and also improved lipid profiles. Similarly, 5 g/d flaxseed gum for 12 wk significantly reduced serum glucose from 154 ± 8 mg/dL to 136 ± 7 mg/dL[86]. Moreover, 360 mg/d lignan for 12 wk slightly decreased HbA1c[36] and C-reactive

protein[87], although fasting glucose and insulin and lipid profiles remained unchanged[36]. Another study using 600 mg/d lignan for 3 mo found decreased HbA1c and glucose levels, but the results were not statistically significant after multivariate adjustment[88].

In summary, isoflavone-enriched soy products and lignin-enriched flaxseeds provide promising benefits in glycemic control, lipid profiles and other cardiovascular markers in T2D patients, but the long-term effect of purified isoflavone or lignan supplements remains unknown.

POTENTIAL MECHANISMS LINKING PHYTOESTROGENS AND PREVENTION OF T2D

The potential mechanisms linking phytoestrogens and glucose metabolism and prevention of diabetes have been extensively reviewed elsewhere[3,40,89,90], here we briefly discuss some animal studies and potential mechanisms on this topic.

A study in male C57BL/KsJ-*db/db* mice found that both genistein (0.02%, w/w) and daidzein (0.02%, w/w) supplements significantly decreased blood glucose and HbA1c levels, and this effect might be due to the suppression of hepatic glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), fatty acid synthase, β-oxidation and carnitine palmitoyltransferase activities[91]. The same effects have been observed in the female non-obese diabetic mice as well, a T1D animal model[92]. Some other studies suggested that isoflavones may exert antidiabetic effect *via* peroxisome-proliferator activated receptors (PPAR) pathway. In the obese Zucker rats, a T2D model, high-isoflavone soy protein diet improved glucose tolerance relative to low-isoflavone soy protein and casein diets[93]. It was further found that genistein or daidzein significantly increased *PPARα*- and *PPARγ*-directed gene expression by 2-4 fold in RAW 264.7 cells[93]. The increased *PPARα* gene expression was also seen in another study[94]. In streptozotocin-induced diabetic rats, 3-wk genistein supplementation decreased HbA1c levels and G6Pase activity, while increased glucokinase level and antioxidant enzyme activities[95]. In an obese nongenetic T2D mouse model, dietary intake of genistein (250 mg/kg diet) improved hyperglycemia, glucose tolerance, and blood insulin level but did not affect insulin sensitivity, suggesting that genistein may increase the number of insulin-positive β-cells in islets, promote their survival, and preserve them by preventing apoptosis[96]. Numerous studies have suggested that genistein may have direct effects on β-cell proliferation, glucose-stimulated insulin secretion and protection against apoptosis[40]. Meanwhile, some other studies have shown insulin-sensitizing effect of genistein in male and female C57BL/6 mice[97], as well as ovariectomized rats[98].

Secoisolariciresinol diglucoside (SDG), the major dietary lignan in flaxseed, considerably reduced the incidence of diabetes in streptozotocin-induced diabetic rats[99], diabetes-prone BioBreeding rats, a T1D model[100], and ZDF rats, a T2D model[101]. In these experiments, SDG significantly decreased oxidative stress by reducing malondialdehyde and pancreatic-chemiluminescence level. Sesamin, the most abundant lignan in sesame seed, showed hypoglycemic effect in a dose-dependent manner in KK-Ay mice, a T2D model[102]. Sesamin was also found to attenuate vascular dysfunction and oxidative stress in streptozotocin-diabetic rats[103].

The effects of phytoestrogens on glucose metabolism are thought to be *via* estrogen-dependent pathway and non-estrogen dependent pathways. Estrogens have been shown to modulate lipid and glucose metabolism directly through lipogenesis, lipolysis, and adipogenesis, or indirectly through their effect on central nervous system influencing appetite and energy expenditure[104]. The relationship between endogenous sex hormones and development of T2D has been well established[105,106]. Because of structural similarity, phytoestrogens could act as estrogen agonists or antagonists, depending on the target tissues[107], doses[108-110], and endogenous circulating sex hormone profile[111]. Although the binding affinity to estrogen receptors (ERs) is much lower for phytoestrogens compared to 17β-estradiol[112], the concentration of phytoestrogens in blood is much higher than endogenous estrogens[113], making it still possible to compete with 17β-estradiol to bind the ERs. Therefore, it is hypothesized that phytoestrogens may influence glucose metabolism by directly modulating concentrations of circulating sex hormones, and this estrogenic effects of phytoestrogens have been supported by some human studies[114-119]. Oxidative stress is considered as one of the causes for T2D and phytoestrogens are known to have strong antioxidant activity[120]. For example, SDG, the major dietary lignan[121] and its mammalian metabolites enterodiol and enterolactone[122], were shown to have antioxidant activity even higher than that of vitamin E. Animal experiments found that lignans decreased lipid peroxidation in rats fed with docosahexaenoic acid[123], and flaxseed increased activities of catalase, superoxide dismutase, and peroxidase[124]. Similarly, isoflavones also showed antioxidant activity *in vitro*[125] and *in vivo*[95,126]. Several clinical trials in humans also found that high-isoflavone soy products increased antioxidant capacity[127-130].

Phytoestrogens may influence glucose metabolism and insulin resistance through other non-estrogen dependent mechanisms. For example, both lignans and isoflavones were found to suppress the *PEPCK* gene expression[92,131]. PEPCK enzyme catalyzes the first committed step in hepatic gluconeogenesis, and *PEPCK* gene transcription is induced by glucagon and glucocorticoids and inhibited by insulin. Thus, suppression of *PEPCK* gene will improve hyperglycemia through reduced gluconeogenesis[132]. Furthermore, phytoestrogens, mostly isoflavones, activate

PPAR and increase the *PPARα*- and *PPARγ*-directed gene expression[93,94,133,134], which is implicated in the glucose homeostasis and lipid metabolism. In the yeast model, genistein was shown to be a reversible, slow-binding, non-competitive inhibitor of alpha-glucosidase[135], which breaks down starch and disaccharides to glucose. Therefore, the alpha-glucosidase inhibitors may reduce the postprandial glucose levels by slowing down the carbohydrate digestion and absorption. In the rabbit model, isoflavones were found to inhibit glucose uptake into rabbit intestinal brush border membrane vesicles *in vitro*[136]. Genistein also directly acted on pancreatic β-cells, leading to activation of the cAMP/PKA signaling cascade to increases rapid glucose-stimulated insulin secretion[137]. The increased insulin secretion was also reported elsewhere[138-140]. Other studies have found that isoflavones may inhibit tyrosine-specific protein kinases[141], induce adiponectin, leptin and *GLUT4* gene expressions in 3T3-L1 adipocytes[142]; promote postprandial carbohydrate oxidation and energy expenditure[143], and protect against high glucose-induced pancreatic cell damage through ERβ and Bcl-2 dependent pathways[144].

IMPLICATIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

As summarized in this review, there has been a long-lasting interest to examine the relation of phytoestrogens and related food sources with diabetes risk. Although the current evidence is promising, there are some knowledge gaps that should be addressed in future investigations.

The food composition databases of phytoestrogens have become the major concern in many epidemiological studies on the relation between phytoestrogens and diabetes risk. The phytoestrogen contents vary dramatically in different food items, and are also influenced by the geographic location, harvest time, and food preparation methods, *etc*. Therefore, it is urgent to establish accurate, up-to-date, and comprehensive databases in different countries. Particularly for lignans, there is a lack of databases available for research. To the best of our knowledge, there has been no prospective longitudinal study in Asian population investigating habitual intake of lignans and risk of developing T2D.

More prospective studies are needed to use objective biomarkers of phytoestrogens exposure, *e.g.*, urinary excretion concentrations. One methodology challenge of the dietary assessment by questionnaire data is the large measurement error from incomplete inclusion of phytoestrogen-enriched food items in the questionnaire and lack of comprehensive food composition databases. Furthermore, phytoestrogen metabolism and circulating concentrations in human body can be influenced by many other factors (*e.g.*, bioavailability and microflora function) in addition to dietary intake. In addition, phytoestrogen biomarker measurements can be easily done in epidemiological studies with archived biospecimen samples. Some large cohort studies have started to measure urinary concentrations of phytoestrogens and evaluate the relation with disease outcomes, but more investigations in different populations are still warranted. In these studies, repeated measures of phytoestrogen biomarkers are recommended to reduce measurement errors and address the issue of changes over time.

The results from clinical trials of the effects of phytoestrogens on glucose homeostasis are conflicting. Many trials have the limitations of small sample size and short intervention duration. Several recent trails in large sample size (n > 100) and longer duration ($\geqslant$ 1 year) have produced more consistent and promising evidence to support the use of phytoestrogens. However, those trials were all in postmenopausal women and used isoflavones as the intervention supplements; thus, more high-quality long-term clinical trials are needed in men and premenopausal women, and to investigate the effect of lignans on glucose metabolism. Furthermore, clinical trials in T2D patients have supported the use of isoflavone-enriched soy products and lignin-enriched flaxseed for glycemic and lipid control, but whether the beneficial effects are due to phytoestrogens or other active components in soy or flaxseed remains unclear. Therefore, long-term and high-quality trials using purified phytoestrogen supplements are necessary to explore the possibility of their routine use for diabetes management.

Some studies hypothesized that the observed variations in effect of isoflavones on osteoporosis, cardiovascular disease, or some cancers could be attributed to the equol production ability in human[145]. More investigations in epidemiological studies and clinical trials are needed to test whether this hypothesis is also true for T2D. Furthermore, some studies found that the effects of phytoestrogens on lipid profile[146], endometrial cancer[8], or breast cancer[147] could be modified by various polymorphisms in genes relevant to estrogen or sex hormone binding globulin, like *CYP1A1*, *CYP1B1*, and *COMT*. However, there are few studies that directly assess gene-phytoestrogen interaction for the T2D outcome or glucose metabolism. This line of investigation is important to help understand the potential mechanisms and design personalized interventions.

Although there are many *in vivo* and *in vitro* studies to explore the potential pathways for the effects of phytoestrogens, the exact anti-diabetic mechanisms are still unclear. Furthermore, the effective doses used in many experimental studies far exceed the physiological concentrations in human circulation. Thus, it is recommended to consider dosages applicable for human in future animal studies.

CONCLUSION

In conclusion, the current evidence of phytoestrogen and T2D from different study designs are complex and inconsistent. Findings from some high-quality prospective cohort studies and clinical trials are promising, but more

studies are needed to fill the aforementioned knowledge gaps. Although the widespread use of phytoestrogens could not be recommended due to the controversies, habitual consumption of phytoestrogens, particularly their intact food sources like soy and whole flaxseed, could be considered as a component of overall healthy dietary pattern for prevention and management of T2D.

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REVIEW

Changing trends in management of gestational diabetes mellitus

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Abstract

Gestational diabetes mellitus (GDM) is on the rise globally. In view of the increasing prevalence of GDM and fetal and neonatal complications associated with it, there is a splurge of research in this field and management of GDM is undergoing a sea change. Trends are changing in prevention, screening, diagnosis, treatment and future follow up. There is emerging evidence regarding use of moderate exercise, probiotics and vitamin D in the prevention of GDM. Regarding treatment, newer insulin analogs like aspart, lispro and detemir are associated with better glycemic control than older insulins. Continuous glucose monitoring systems and continuous subcutaneous insulin systems may play a role in those who require higher doses of insulin for sugar control. Evidence exists that favors metformin as a safer alternative to insulin in view of good glycemic control and better perinatal outcomes. As the risk of developing GDM in subsequent pregnancies and also the risk of overt diabetes in later life is high, regular assessment of these women is required in future. Lifestyle interventions or metformin should be offered to women with a history of GDM who develop pre-diabetes. Further studies are required in the field of prevention of GDM for optimizing obstetric outcome.

Key words: Gestational diabetes mellitus; Prevention; Vitamin D; Probiotic; Insulin analogs; Oral hypoglycemic agents

Core tip: To summarize, the use of probiotics and vitamin D supplementation may help in preventing gestational diabetes mellitus (GDM) in high risk women. Glycemic targets need to be lower than current recommendations. Oral hypoglycemic agents are an effective and safe alternative to insulin in managing GDM. Newer insulins, aspart, lispro and detemir, provide better glycemic control than routinely used insulin. Continuous glucose monitoring systems and insulin pumps may be of use in women who require a very high dose of insulin. Lifestyle interventions in GDM women help to reduce the subsequent development of diabetes. Further studies are required in the field of prevention of GDM for optimizing obstetric outcome.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable degrees with onset or first recognition during pregnancy. The prevalence of gestational diabetes mellitus is increasing globally. In the United States, up to 14% of pregnancies are complicated by GDM, accounting for 200000 cases annually[1]. The prevalence of GDM in Canada is 8%-18%[2] and in China it varies between 6.8% and 10.4%[3]. In India, there is an exceptionally high estimated prevalence of GDM (27.5%) when compared to 9.9% in Sri Lanka and 9.8% in Bangladesh[4]. Recently, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended new screening criteria for GDM based on the hyperglycemia and adverse pregnancy outcomes (HAPO) study. Based on these criteria, the total incidence of GDM reaches almost 15%-20%[5].

The offspring of a diabetic mother is at increased risk for fetal, neonatal and long term morbidities. They are at higher risks of developing macrosomia and stillbirths. Macrosomia is the most common morbidity, occurring in 15%-45% of infants, leading to shoulder dystocia and trauma during delivery[6-8]. Hypoglycemia, hyperbilirubinemia, respiratory distress syndrome, hypocalcemia, polycythemia and hypertrophic cardiomyopathy are complications expected in the immediate postnatal period. They are also at increased risk of developing diabetes, obesity and metabolic syndrome in childhood and adult life[9]. The magnitude of fetal and neonatal risks is proportional to the severity of maternal hyperglycemia.

With this background of increasing prevalence of GDM worldwide and its established association with adverse fetal, neonatal and their long term complications, we need to look into available options for preventing and managing GDM.

PREVENTION OF GDM

High risk groups for development of GDM are those with a high body mass index (BMI) (BMI cutoff of 30 kg/m^2 in non-Hispanic whites, 26 kg/m^2 in African Americans, 25 kg/m^2 in Chinese and 24 kg/m^2 in South Asians)[10], history of diabetes in a first degree relative, previous macrosomic infant, history of GDM in previous pregnancies and women belonging to the ethnic origin of Asia, the Caribbean and Arabia, particularly of the Middle East. Much research is underway in the field of prevention of GDM. Various modalities like exercise and diet have been found to be useful for prevention of GDM in high risk groups. More recently, probiotics and vitamin D are being studied for the same. The effectiveness of the preventive modalities in pre-pregnant and pregnant women are detailed below.

Diet

Dietary fiber and glycemic index of food: Less dietary fiber intake has been associated positively with GDM. Intake of dietary fiber, in particular cereal and fruit fiber, were strongly and inversely associated with GDM risk. Each 10 g/d increment in total fiber intake was associated with a 26% reduction in risk; each 5 g/d increment in cereal fiber and fruit fiber was associated with 23% and 26% reduction in GDM risk, respectively. Increased dietary fiber intake leads to decreased appetite and thus lowers total energy intake. It also delays gastric emptying and slows glucose absorption, resulting in less absorption of glucose leading to glucose homeostasis and less increase in insulin levels[11].

Both dietary glycemic index and glycemic load have an influence on postprandial glycemia. The combination of high glycemic load and low cereal fiber diet was associated with a 2.15 fold increased risk compared with the low glycemic index and high cereal fiber diet[11]. These findings suggest that the pre-pregnancy diet pattern might be associated with women's GDM risk. In particular, diet with low fiber and high glycemic load was associated with an increased risk.

Western diet pattern: A study was conducted comparing the Western pattern and the prudent dietary pattern. The prudent dietary pattern includes high intake of poultry, fish, fruit and green leafy vegetables. The Western pattern includes high intake of red meat, processed meat, French fries, pizza, refined grain products and sweets. There were strong positive associations between the Western dietary pattern and GDM, whereas the prudent dietary pattern was significantly and inversely associated with GDM[12].

Pre-pregnancy intakes of red and processed meats were both significantly and positively associated with GDM risk[12]. It might be related to the presence of saturated fat and cholesterol content in red and processed meats affecting insulin sensitivity[13].

Exercise

Physical activity was not encouraged during pregnancy due to the fear of adverse effect on the fetus and mother, but various studies show no adverse maternal and fetal effects on women engaged in mild and moderate physical activities[14,15].

Daily stair climbing, when compared with no stair climbing, was associated with a 49%-78% reduction in GDM risk ($P < 0.011$). The amount of hours spent in recreational physical activity during the year before the index pregnancy was associated with statistically significant reductions in the incidence of GDM, but the greatest reduction in risk was observed in women who engaged in physical activity before and during pregnancy (OR = 0.40; 95%CI: 0.23-0.68)[16]. A

study conducted among Hispanic women by Chasan-Taber *et al*[17] also showed that exercise reduces the incidence of GDM. Vigorous physical activity before pregnancy and continuation of physical activity in early pregnancy may reduce a woman's risk for developing GDM[18]. In the absence of either medical or obstetric complications, moderate exercise for ⩾ 30 min/d on most of the day of the week is recommended for a pregnant woman[19].

Pre-pregnancy BMI and GDM

Pre-pregnancy BMI has significant influence on GDM. Compared to women with a normal BMI, the odds ratio of an underweight woman developing GDM was 0.75 (95%CI: 0.69-0.82). The odds ratio of overweight, moderately obese and morbidly obese women to develop GDM were 1.97 (95%CI: 1.77-2.19), 3.01 (95%CI: 2.34-3.87) and 5.55 (95%CI: 4.27-7.21) respectively[20]. The risk of GDM is positively associated with pre-pregnancy BMI. This information is important when counseling women planning for a pregnancy.

Another parameter which is as important as pre-pregnancy weight is an acceptable weight gain during pregnancy. Guidelines for weight gain during pregnancy were released by the Institute of Medicine in 2009. These guidelines are based on pre-pregnancy BMI. Those who have a normal BMI between 18.5-24.9 kg/m² should aim for a weight gain of 25-35 lb (11-15 kg) and those who are underweight with BMI < 18.5 kg/m² should aim for 28-40 lb (12-18 kg) weight gain during pregnancy. Those who are overweight with a pre-pregnancy BMI between 26 and 29.9 kg/m² should aim for a weight gain of 15-25 lb (6.8-11.4 kg). Those who are obese with a pre-pregnancy BMI of ⩾ 30 kg/m² should aim for 11-20 lb (5-9 kg) weight gain[21]. In women who have excessive weight gain in the first trimester, there is increased risk of development of GDM[22,23]. Counseling regarding acceptable weight gain in pregnancy is also required during the pre-pregnancy period.

Probiotics

Numerous studies show that probiotics can reduce the incidence of GDM. A systematic review by Lindsay *et al*[24] demonstrated that probiotic use in pregnancy could significantly reduce maternal fasting glucose levels. Also, there is a significant decrease in the incidence of GDM and pre-eclampsia rates. By supplementing the gut bacteria, they change the metabolism in individuals and help to prevent GDM. Among the list of various organisms, Lactobacillus rhamnosus and Bifidobacterium lactis are found to have an anti-diabetic effect[25]. A randomized controlled trial was conducted in 256 pregnant women randomized to receive probiotics or placebo. Probiotics given in that study were Lactobacillus rhamnosus GG (10^{10} colony forming units) and Bifidobacterium lactis Bb12 (10^{10} colony forming units). Significantly reduced plasma glucose ($P = 0.025$) and improved insulin sensitivity ($P = 0.028$) were observed in the diet/probiotic group[26]. In yet another study, daily intake of Lactobacillus rhamnosus GG (10^{10} colony forming units) and Bifidobacterium lactis Bb12 (10^{10} colony forming units) reduced GDM frequency in the diet/probiotic group (13%) compared to the diet/placebo (36%) and control/placebo (34%) groups ($P = 0.03$)[27]. There were no adverse events in mothers or children who took probiotics during pregnancy. No significant difference in prenatal or postnatal growth rates among the study groups was detected. This shows that probiotics are a safe and cost-effective tool in preventing GDM.

The Cochrane review also states that the use of probiotics is associated with a reduction in the rate of GDM (relative risk 0.38). There was also a reduction in the birth weight of infants of women who were supplemented with probiotics[28]. All this evidence suggests that daily intake of probiotic capsules with Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb12 with 10^{10} colony forming units may be effective in pregnant women who are at high risk for GDM.

SPRING, a multicenter randomized control trial of a high-risk group of overweight and obese pregnant women, is being conducted which will provide a clear idea about the usage of probiotics in preventing GDM in that group[29].

Various studies support the beneficial relationship between fermented dairy products and a reduced risk of diabetes[30-32]. An observational study conducted in > 6500 individuals found that yogurt consumers had reduced levels of glucose and insulin resistance compared with non-consumers[33]. A study shows a daily consumption of 200 mL of a shake containing Lactobacillus acidophilus (4×10^8 CFU/100 mL) and Bifidobacterium bifidum (4×10^8 CFU/100 mL) resulted in a blood glucose reduction in type 2 diabetes individuals[34]. It has also been shown that consumption of yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis for a duration of 6 wk by type 2 diabetes individuals resulted in reduced fasting glucose, reduced glycosylated hemoglobin (HbA1c) levels and higher activity of superoxide dismutase and glutathione peroxidase when compared to a control group[35].

Probiotic food supplements are available from many sources but effectiveness is dependent on various factors like temperature, anaerobic storage conditions, the initial dose of the strain and its quality. It is not known to what extent these food sources alter the gut microbes and thereby have biological effects outside research settings.

Vitamin D

All over the world, there is a high prevalence of vitamin D deficiency irrespective of age. A study done in United States adults showed that the overall prevalence rate of vitamin D deficiency was 41.6%[36]. Vitamin D deficiency is known to cause musculoskeletal problems. Apart from that, it is associated with various cardiovascular

problems like myocardial dysfunction, heart failure and sudden cardiac deaths. Its deficiency also leads to respiratory problems, autoimmune diseases, certain cancers, hypertension and diabetes mellitus[37]. Various studies have shown that there is an increased prevalence of pre-eclampsia[38], gestational diabetes[39,40], preterm labor[41] and intrauterine growth restriction[42] in pregnant women with vitamin D deficiency. Supplementation of vitamin D during pregnancy may reduce these complications. Safe dosage of vitamin D in pregnancy has been studied. A dose of 4000 IU/d or 50000 IU every 2 wk was able to raise serum 25-hydroxyvitamin D levels to > 30 ng/mL, leading to a decrease in insulin resistance[43]. This dosage is associated with reduction in pregnancy complications without producing toxicity but, as there is insufficient evidence supporting a vitamin D role in GDM, the Cochrane database has concluded that more randomized trials are required to evaluate its role in pregnancy[44]. Results in a European multicenter study of vitamin D and lifestyle interventions are awaited, which may throw light on our dilemma about the role of vitamin D in the prevention of GDM[45].

DIAGNOSIS OF GDM

There is a dilemma whether to perform selective screening or universal screening for GDM in pregnant women. By screening only the high risk population, up to 30% of GDM women may be missed. In areas where the incidence of GDM is < 3%, selective screening in a high risk population is acceptable, but where the prevalence of GDM is > 3%, universal screening may be considered[46].

Diagnostic criteria

Various criteria are being utilized worldwide for diagnosing GDM.

American College of Obstetricians and Gynecologists (ACOG): It recommends a 1 h glucose challenge test for screening GDM. If plasma glucose value is ⩾ 140 mg/dL, a 3 h 100 g oral glucose tolerance test (OGTT) should be performed for diagnosis. Carpenter Coustan criteria are used for diagnosing GDM. Abnormal values are as follows: Fasting plasma glucose (FPG) ⩾ 5.3 mmol/L (95 mg/dL); 1 h ⩾ 10 mmol/L (180 mg/dL); 2 h ⩾ 8.6 mmol/L (155 mg/dL); 3 h ⩾ 7.8 mmol/L (140 mg/dL). GDM is diagnosed with ⩾ 2 abnormal values.

World Health Organization (WHO) 1999: (2 h 75 g OGTT). Fasting plasma glucose FPG ⩾ 6.9 mmol/L (125 mg/dL); 2 h ⩾ 7.8 mmol/L (140 mg/dL).

Australasian Diabetes in Pregnancy Society (ADIPS) 1991 Criteria: (2 h 75 g OGTT). FPG ⩾ 5.5 mmol/L (100 mg/dL); 2-h ⩾ 8.0 mmol/L (144 mg/dL).

Diabetes in Pregnancy Study Group India diagnostic criterion: Seventy-five gram oral glucose load is performed irrespective of the last meal and blood sugar level is taken 2 h later. A value of ⩾ 7.8 mmol/L (140 mg/dL) is diagnosed as GDM. This may be of use in limited resource settings and where patient compliance is doubtful.

International Association of Diabetes and Pregnancy Study Groups two-phase strategy for the detection of hyperglycemia in pregnancy

HAPO, a multicenter study, was done in a cohort of 25505 pregnant women. Participants were tested with 2 h, 75 g OGTT and their pregnancies were followed for adverse maternal and neonatal outcomes[47]. Based on the study results, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus panel formulated the new diagnostic criteria for GDM[48]. The cut-off points were derived based on infant birth weight, cord blood C-peptide and neonatal body fat composition.

At the first antenatal visit: Screening is done with either FPG, HbA1c or random plasma glucose. Based on the prevalence of GDM in the locality, either all women or only high-risk women are screened. (1) GDM is diagnosed if FPG is between 5.1 and 7.0 mmol/L (92 to 126 mg/dL). A diagnosis of overt diabetes is made with one of the following values; (2) FPG ⩾ 7.0 mmol/L (126 mg/dL); (3) HbA1c ⩾ 6.5%; and (4) RPG ⩾ 11.1 mmol/L (200 mg/dL), then confirm with FPG or HbA1c.

At 24-28 wk gestation: In all women previously not found to have GDM/overt DM, 2 h 75 g OGTT is done. If FPG ⩾ 7.0 mmol/L (126 mg/dL), overt diabetes is diagnosed. GDM was diagnosed with ⩾ one abnormal values: (1) FPG ⩾ 5.1 mmol/L (92 mg/dL); (2) 1-h ⩾ 10 mmol/L (180 mg/dL); and (3) 2-h ⩾ 8.5 mmol/L (153 mg/dL).

Australasian Diabetes in Pregnancy Society (ADIPS) criteria 2013: It also recommends the same cut-off points suggested by IADPSG. A diagnosis of GDM is made if ⩾ 1 of the following glucose levels is elevated: (1) FPG ⩾ 5.1 mmol/L; (2) 1-h ⩾ 10.0 mmol/L; and (3) 2-h ⩾ 8.5 mmol/L.

Unlike IADPSG, ADIPS does not recommend the use of the term "overt diabetes" to describe marked hyperglycemia first detected during pregnancy. Glucose intolerance of any severity with onset or first recognition during pregnancy is labelled as GDM by this guideline.

WHO 2013 recommendations[49]

In the interest of moving towards a universal standard recommendation for the diagnosis of GDM, the WHO guideline development group has decided to accept the IADPSG criteria rather than introduce another set of arbitrary cut-off values.

The difference from IADPSG guidelines is that these new WHO guidelines provide a range of plasma

glucose levels to distinguish diabetes in pregnancy and GDM. Likewise, instead of using the terminology "overt diabetes", WHO uses "diabetes mellitus in pregnancy".

For diagnosing diabetes mellitus in pregnancy, WHO recommends its 2006 criteria.

Diabetes is diagnosed if one or more of the following criteria are met: (1) FPG ⩾ 7.0 mmol/L (126 mg/dL); (2) 2 h plasma glucose ⩾ 11.1 mmol/L (200 mg/dL) following a 75 g oral glucose load; and (3) Random plasma glucose ⩾ 11.1 mmol/L (200 mg/dL).

Gestational diabetes mellitus should be diagnosed at any time during pregnancy based on any one of the following values: (1) Fasting plasma glucose = 5.1-6.9 mmol/L (92-125 mg/dL); (2) 1 h post 75 g oral glucose load ⩾ 10.0 mmol/L (180 mg/dL) (there are no established criteria for the diagnosis of diabetes based on the 1 h post-load value); (3) 2 h post 75 g oral glucose load between 8.5-11.0 mmol/L (153-199 mg/dL).

As IADPSG guidelines use only one abnormal value to diagnose GDM, its prevalence is expected to significantly increase from 5%-6% to 15%-20%.

American Diabetes Association

The American Diabetes Association (ADA) also recommends these IADPSG cut-off points in its 2011 position statement. Although ADA recognizes that with these cut-off points there would be a significant increase in the incidence of GDM, it recommends these changes with the intention of optimizing gestational outcomes for women and their babies[5].

Falavigna *et al*[50] compared IADPSG criteria with WHO 1999 criteria. The IADPSG criteria gave better results than the WHO 1999 criteria. The adoption of the IADPSG criteria instead of the WHO criteria would reduce the incidence of LGA births by 0.32% ($P < 0.001$) and of pre-eclampsia by 0.12% ($P = 0.007$).

Other evidence also suggests that adopting IADPSG criteria may be more cost effective[51,52].

As IADPSG diagnostic criteria are based on prognostic accuracy (the risk of pregnant women developing an adverse outcome in a certain period of time), these guidelines appear to be more logical and can be adopted worldwide.

MicroRNA profiling in preventing GDM

Now there is emerging evidence that the use of microRNAs (miRNAs) is useful in predicting GDM. It has been generally assumed that most genetic information is transacted by proteins, but recent evidence suggests that the majority of the genomes of mammals and other complex organisms are in fact transcribed into non coding RNAs (ncRNAs), many of which are alternatively spliced and/or processed into smaller products. These ncRNAs include miRNAs, snoRNAs and many others. They contain internal signals that control various levels of gene expression in physiology and development[53]. The miRNAs have recently been demonstrated to abundantly and stably exist in serum and to be potentially disease-specific. Specifically, miRNAs are required for pancreatic development and the regulation of glucose stimulated insulin secretion[54,55]. Serum miRNAs are differentially expressed between GDM women and controls and could be candidate biomarkers for predicting GDM. Particularly, the use of miR-29a, miR-222 and miR-132 as serum-based biomarkers in early second trimester (16-19 wk) has been shown to be effective, which warrants further evaluation and optimization[56].

TREATMENT FOR GDM

There is a greater dilemma whether it is worth treating mild cases of GDM if the outcome does not change much. The Cochrane review states that there are various benefits of diagnosing and treating mild GDM cases. There is a reduction in the proportion of infants weighing more than 4000 g (RR = 0.46, 95%CI: 0.34-0.63) and the proportion of infants weighing greater than the 90th birth centile (RR = 0.55, 95%CI: 0.30-0.99) in mothers receiving specific treatment for GDM compared to those not receiving specific treatment. Perinatal morbidity (shoulder dystocia, bone fracture, nerve palsy and death) was significantly reduced in women receiving intensive treatment for mild GDM compared to routine antenatal care (RR = 0.32, 95%CI: 0.14-0.73)[57].

Glycemic targets during pregnancy

Glycemic targets as recommended by the fifth international workshop conference on GDM[58] are capillary glucose concentrations of: (1) Pre-prandial: ⩽ 5.3 mmol/L (96 mg/dL); (2) 1 h post meal: ⩽ 7.8 mmol/L (140 mg/dL); and (3) 2 h post meal: ⩽ 6.7 mmol/L (120 mg/dL).

In the metformin in gestational diabetes (MIG) trial, analysis of glycemia and its outcome showed that a fasting capillary glucose < 4.9 mmol/L (88 mg/dL) had significantly better outcomes than women with a fasting capillary glucose between 4.9 and ⩾ 5.3 mmol/L (88 and ⩾ 95 mg/dL). Two hours postprandial, capillary glucose values of ⩽ 6.4 mmol/L (⩽ 115 mg/dL) were associated with improved outcomes. Further improvement was seen with mean postprandial capillary glucose < 5.9 mmol/L (< l06 mg/dL)[59].

A review by Hernandez *et al*[60] states that average glucose values in pregnant non-diabetic women are 4.5 mmol/L (81 mg/dL) for fasting, 6.8 mmol/L (122 mg/dL) for 1 h postprandial and 6.1 mmol/L (110 mg/dL) for 2 h postprandial.

All these studies implicate that targets for fasting and postprandial capillary glucose may need to be lower than what current guidelines recommend. Further studies are required in this field.

Adjustment of insulin therapy based on postprandial glucose values improves glycemic control better than pre-prandial glucose values and decreases the risk of neonatal hypoglycemia, macrosomia and cesarean

delivery[61].

Continuous glucose monitoring systems (CGMS) may be of use in pregnant women with overt diabetes who require a very high dose of insulin to achieve good glycemic control. CGMS during pregnancy is associated with improved glycemic control and a reduced risk of macrosomia[62]. A study in Finland showed that CGMS detects a markedly higher proportion of GDM mothers needing antihyperglycemic medication compared with self-monitoring of plasma glucose[63]. Further large scale studies are needed to evaluate whether CGMS guided initiation of antihyperglycemic therapy results in less macrosomia and perinatal complications related to GDM.

Diet

Calorie requirement for GDM women is 30-35 kcal/kg for normal weight, 25-30 kcal/kg for overweight and 35-40 kcal/kg for underweight subjects. Severe calorie restriction to < 1500 cal/d is not advisable. A study by Rizzo *et al*[64] has shown that severe calorie restriction to < 1500 cal/d is associated with increased incidence of ketonemia, resulting in lowered mental developmental index scores and average Stanford-Binet scores in the babies. The American Diabetes Association recommends a 30%-33% calorie restriction in obese women with GDM, with a minimum of 1800 cal/d[65].

Diets composed of 50%-60% carbohydrates will often result in hyperglycemia and excessive weight gain. So, calorie intake from carbohydrate has to be limited to 33%-40%, with the remaining calories divided between protein (20%) and fat (40%)[61]. Three meals and two to three snacks are recommended to distribute glucose intake and to reduce postprandial glucose fluctuations. Glycemic index of food may also be an important factor for sugar control. Low glycemic index (< 55) foods produce a better sugar control than foods with a high glycemic index (> 70). Studies have shown that the glycemic index of food has an influence on birth weight of the baby[66,67]. Studies on the effect of a high fiber diet on outcome of pregnancy in GDM have shown mixed results[68,69].

Insulin

When glycemic targets are not achieved by 1-2 wk of the diet, pharmacological treatment is recommended[70]. Short acting insulin is used to cover glucose excursions following the meal and intermediate acting insulin for hepatic glucose production in the fasting state. Regular human insulin (RHI) is a shorter acting insulin and neutral protamine hagedorn (NPH) is the intermediate acting insulin in common practice to date. Following each meal, glucose levels peak at approximately 1 h after the start of the meal and then return to pre-prandial levels within 2-3 h[71]. Short acting insulin starts its action 1/2 to 1 h after injection and its effect reaches a peak at 2-4 h. Therefore, at times, the pre-prandial administration of RHI is not able to control the peak postprandial blood glucose. At the same time, delayed peak action and a longer duration of action may result in inappropriate hyperinsulinemia before the next meal, resulting in pre-prandial hypoglycemia[72].

Rapid acting insulin analogs

In order to overcome this problem, newer rapid acting insulin analogs can be used instead of short acting insulin. These rapid acting insulin analogs start their action within 15 min, reaches a peak by 31-70 min and acts for 2-4 h. Several studies have proven the safety of insulin aspart and lispro in pregnancy. United States Food and Drug Administration (FDA) has approved both insulins for use during pregnancy. Several clinical studies have shown fewer episodes of hypoglycemia, strict sugar control and higher reduction in HbA1c levels in pregnancy.

The latest rapid acting insulin analog, insulin glulisine, is available with the same action profile as that of aspart and lispro. As there are no clinical data available, to date, United States FDA has not approved it for use in pregnancy.

Long acting insulin

Commonly used NPH insulin starts its action in 1-2 h, with peak action at 4-8 h and effective up to 12-18 h. The night dose of NPH has its peak action in the early morning hours and produces hypoglycemia[72].

Compared to NPH, newer long acting insulin analog detemir starts its action in 1-2 h and has a flatter profile with a more even distribution of metabolic effect over 24 h[73]. Insulin detemir also shows lower rates of hypoglycemia. Various studies have proved the efficacy and safety of insulin detemir in pregnancy. Moreover, the United States FDA has approved insulin detemir as class B in pregnancy. Although the insulin glargine has same pharmacodynamic properties as detemir, the use of insulin glargine is not approved in pregnancy. Well controlled trials are needed to determine its safety in pregnancy.

Premixed insulin preparations

Premixed insulin preparations are commonly used everywhere. A combination of short acting and intermediate acting injections are available in different ratios of 30/70, 25/75, 50/50. Premixed insulin analogs provide better postprandial coverage and less hypoglycemic episodes between meals. Biphasic insulin aspart (BIAsp 30) comprises rapid acting aspart combined with protamine-crystallized insulin aspart in a 30:70 ratio. It requires twice daily dosing and provides better sugar control[74]. It has been found to be safe during pregnancy.

Insulin pump

Insulin pumps allow for flexible insulin administration with a profile that resembles the physiological insulin profile of the beta cells of the pancreas very closely.

Major advantages of administering insulin by continuous subcutaneous insulin infusion include decreased variability in insulin absorption (due to small insulin doses administered at one time), decreased risk of hypoglycemia (due to the lower total doses of insulin) and improved control of the dawn phenomenon[75].

CGMS and insulin pumps used together during pregnancy help improve glycemic control. Together, these devices could potentially constitute an artificial pancreas[76]. Real-time blood glucose readings are continuously relayed to the insulin pump. Based on glucose values, the insulin pump delivers an accurate dosage of insulin needed by the patient's body. A closed-loop system with physiologically responsive insulin adjustments capable of maintaining near normal glucose levels could be of great benefit for pregnant women with type 1 diabetes or GDM with high plasma glucose values.

The Helen Murphy group evaluated closed-loop insulin delivery with a model predictive control (MPC) algorithm[77]. The basal insulin infusion rate was calculated using women's weight, basal insulin requirements (measured by continuous glucose monitoring) and total daily insulin dose during the preceding 3 d. A nurse adjusts the basal insulin infusion rate from continuous glucose measurements and feeds into the MPC algorithm every 15 min. The total daily insulin dose was reduced by 30% for conversion to insulin pump. The advantage found with a closed-loop insulin delivery system over conventional multiple injections is its ability to respond rapidly to glucose excursions with more flexible insulin infusion rates despite comparable overall insulin doses.

Pump therapy can be especially beneficial for women who require high doses of insulin and experience repeated episodes of hypoglycemia with intermittent insulin injections[78]. Large scale trials are needed before widespread use.

Oral hypoglycemic agents for GDM

The traditional management of women with GDM is to treat with insulin if diet therapy fails. However, insulin therapy has its own drawbacks. Now there is emerging evidence for the role of oral medications in these women. ACOG and National Institute for Health and Care Excellence (NICE) guidelines suggest that insulin and oral medications are equivalent in efficacy and either can be an appropriate first-line therapy[61,79].

Metformin

Su *et al*[80] conducted a systematic review of six randomized clinical trials involving 1420 subjects. They found that by using metformin in women with gestational diabetes there was no increase in adverse maternal and neonatal outcomes compared to insulin. Moreover, metformin usage in pregnancy is associated with less weight gain and neonatal hypoglycemia.

A randomized control trial by Niromanesh *et al*[81] showed that the birth weight of the neonate was lower in the metformin group compared to the insulin group, although not statistically significant. Maternal weight gain was significantly reduced in the metformin group.

A retrospective study done by Marques *et al*[82] showed that there was no statistical differences between insulin and metformin groups with regard to the rates of abortion, preeclampsia, macrosomia, prematurity, small for gestational age or large for gestational age newborns, perinatal deaths, cesarean deliveries, neonatal intensive care unit admissions and birth malformations or neonatal injuries.

There is no significant difference in postprandial glucose control between women on insulin and those taking oral hypoglycemic agents. This finding is reflected in similar rates of fetal macrosomia and mean birth weight in women receiving insulin or oral hypoglycemic agents as first-line treatment[83].

However, there are studies which show that metformin can induce neural tube defects when it is taken during first trimester of pregnancy. Expression of *Pax3* gene is essential for neural tube closure. Adenosine monophosphate-activated protein kinase (AMPK) is stimulated in embryos during diabetic pregnancy by maternal hyperglycemia[84]. This stimulated AMPK inhibits expression of *Pax3* gene and induces neural tube defects. Studies have shown that apart from maternal hyperglycemia, metformin has also been shown to stimulate AMPK activity in skeletal muscle and liver[85,86]. This stimulation of AMPK activity by metformin causes neural tube defects. However, a study by Lee *et al*[87] showed that metformin increases activated AMPK in the maternal liver but it did not have an effect on AMPK in embryos or maternal skeletal muscle. Because of the absence of AMPK activity, metformin did not inhibit Pax3 expression in embryos and thus did not cause neural tube defects. The absence of metformin responsiveness on embryos may be explained by insufficient expression of metformin transporters during neurulation. Thus, in their study, they showed that metformin does not stimulate embryo AMPK activity and consequent embryopathy.

The MIG trial by Rowan *et al*[88] showed that women receiving metformin had less weight gain between the time of enrollment and 36 wk of gestation than those receiving insulin. Similarly, there was a greater weight loss between the time of enrollment and the postpartum visit in women receiving metformin compared to insulin. There were significantly less episodes of neonatal hypoglycemia events in infants of women taking metformin. The MIG trial concluded that metformin had similar perinatal complications compared to insulin. Due to the convenience, women preferred metformin to insulin treatment.

The follow up of exposed children up to 2 years in the MiG-TOFU trial revealed that metformin-exposed infants had more subcutaneous fat and less visceral fat. This may result in an increased insulin sensitivity pattern of growth in future[89].

Glyburide

In the treatment of GDM, metformin and glyburide were equally efficacious to insulin in blood sugar control[90-92]. The only significant difference in outcome between the 2 drugs was that maternal weight gain during pregnancy was lower with metformin. A few studies showed that the incidence of macrosomia[91,93] and neonatal hypoglycemia[91] is higher in babies of GDM mothers treated with glyburide. In contrast to this, a meta-analysis showed that there is no consistent evidence for increase in any adverse maternal or neonatal outcomes with the use of glyburide or metformin compared with the use of insulin[94].

Oral agents are better in pregnancy because they are patient friendly and convenient, resulting in increased compliance with treatment regimens. Oral agents do not require instruction at the time of initiation of therapy. Glycemic control and perinatal outcomes produced by oral hypoglycemic agents (OHAs) were comparable to insulin. Insulin is costlier, inconvenient to use and needs ideal storage conditions, which makes it difficult in developing countries. So, OHAs should be considered safe alternatives to insulin which should be reserved as a second-line agent for patients in whom oral treatment does not achieve glycemic control[83].

FETAL SURVEILLANCE

There is an increased risk of fetal demise in patients with poor glycemic control. To improve fetal outcome, all women with GDM should be instructed to monitor fetal movements during the last 8-10 wk of pregnancy. They should report immediately if there is any reduction in the perception of fetal movements. Antepartum fetal surveillance should be started from 32 wk in insulin treated GDM women. Non-stress testing should be done after 32 wk gestation in women on insulin. Biophysical profile testing and Doppler velocimetry to assess umbilical blood flow may be considered in those with associated intra uterine growth restriction, macrosomia or in those with co-morbid conditions, such as pre-eclampsia. There is no consensus regarding antepartum testing in women with GDM well controlled with diet.

INDUCTION OF LABOR

The timing and the mode of delivery is still debatable. For the timing of delivery, the ADA in 2004 recommended delivery at 38 wk unless obstetric considerations dictated alternative management[65]. Similarly, NICE in 2008 recommended that pregnant women with diabetes should be offered elective birth through induction of labor after 38 completed weeks[79]. The ACOG did not recommend routine delivery before 40 wk in GDM women well controlled with diet or medication[61]. Induction of labor at term helps to reduce the incidence of shoulder dystocia in women with gestational diabetes[95]. The option of induction of labor in GDM is still controversial. Further studies are needed in this field.

For the mode of delivery, a cesarean delivery would only be suggested for an estimated fetal weight of $\geqslant$ 4500 g in mothers with GDM[79,96].

GLYCEMIC CONTROL DURING LABOR

During labor and birth, capillary blood glucose should be monitored on an hourly basis in women with diabetes and maintained at 4-7 mmol/L. Intravenous dextrose and insulin infusion is recommended during labor and birth in women with diabetes whose blood glucose is not maintained between 4-7 mmol/L.

FUTURE PREVENTION

Women with a history of GDM have a greatly increased subsequent diabetes risk and should be followed up with subsequent screening for the development of pre-diabetes or diabetes. Women who had GDM in pregnancy should have a 75 g 2 h OGTT (WHO criteria), preferably at 6-12 wk post-partum. Women with a normal result should be reassessed once in 3 years. For those who are diagnosed with impaired fasting glucose or impaired glucose tolerance, annual testing should be done.

For women who had GDM in the first pregnancy, there is 13.2 fold increased risk (95%CI: 12.0-14.6) of developing GDM during the second pregnancy. The recurrence risk of GDM in the third pregnancy was stronger when women had GDM in both prior pregnancies (25.9 fold increased risk; 95%CI: 17.4-38.4)[97]. The cumulative proportion of women developing diabetes at 1 year postpartum was 1.7%. At the end of 10 years, 17% of women developed diabetes and at the end of 15 years, 25% developed diabetes[98].

Lifestyle interventions or metformin should be offered to women with a history of GDM who develop pre-diabetes. Subsequent diabetes risk after a history of GDM was significantly lower in women who followed healthy eating patterns[99]. In the diabetes prevention program, administering metformin and intensive lifestyle modification in women with a history of GDM led to 50% reduction in diabetes risk[100]. Metformin therefore might reasonably be recommended for very high-risk individuals (those with a history of GDM or very obese women)[5].

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Prediabetes diagnosis and treatment: A review

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Abstract

Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold. While, the diagnostic criteria of prediabetes are not uniform across various international professional organizations, it remains a state of high risk for developing diabetes with yearly conversion rate of 5%-10%. Observational evidence suggests as association between prediabetes and complications of diabetes such early nephropathy, small fiber neuropathy, early retinopathy and risk of macrovascular disease. Several studies have shown efficacy of lifestyle interventions with regards to diabetes prevention with a relative risk reduction of 40%-70% in adults with prediabetes. While there is increasing evidence to prove the efficacy of pharmacotherapy in prevention of diabetes in adults with prediabetes, pharmaceutical treatment options other than metformin are associated with adverse effects that limit their use for prediabetes. There are no reports of systematic evaluation of health outcomes related to prediabetes in children. The effects of pharmacotherapy of prediabetes on growth and pubertal development in children remains unknown. Secondary intervention with pharmacotherapy with metformin is advocated for high-risk individuals but criteria for such consideration benefit of early intervention, long term cost effectiveness of such interventions and the end point of therapy remain unclear. Pharmacotherapy must be used with caution in children with prediabetes. Prediabetes is a condition defined as having blood glucose levels above normal but below the defined threshold of diabetes. It is considered to be an at risk state, with high chances of developing diabetes. While, prediabetes is commonly an asymptomatic condition, there is always presence of prediabetes before the onset of diabetes. The elevation of blood sugar is a continuum and hence prediabetes can not be considered an entirely benign condition. This aim of this review is to describe the challenges associated with diagnosis of prediabetes, the possible adverse medical outcomes associated with prediabetes and the treatment options and rationale for their use in context of prediabetes.

Key words: Impaired fasting glucose; Impaired glucose tolerance; Diabetes; Metformin; Lifestyle intervention; Prediabetes

Core tip: Prediabetes is a state of intermediate hyperglycemia. While there are several controversies about the diagnosis of prediabetes, it remains an at-risk state for development of diabetes. Several adverse health outcomes have been associated with prediabetes. This review provides a detailed description of the current literature regarding diagnosis, health consequences and treatment of prediabetes and also

provides an insight for clinical management.

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DIAGNOSIS OF PREDIABETES

Various organizations have defined prediabetes with criteria that are not uniform. The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia using two specific parameters, impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) of 6.1-6.9 mmol/L (110 to 125 mg/dL) and impaired glucose tolerance (IGT) defined as 2 h plasma glucose of 7.8-11.0 mmol/L (140-200 mg/dL) after ingestion of 75 g of oral glucose load or a combination of the two based on a 2 h oral glucose tolerance test (OGTT)[1]. The American Diabetes Association (ADA), on the other hand has the same cut-off value for IGT (140-200 mg/dL) but has a lower cut-off value for IFG (100-125 mg/dL) and has additional hemoglobin A1c (HbA1c) based criteria of a level of 5.7% to 6.4% for the definition of prediabetes[2]. Several studies have shown poor correlation between HbA1c and IFG and IGT[3-5]. The usefulness of diagnosis of diabetes or prediabetes on basis of IFG and IGT have been challenged due to inability of these blood glucose cut points to capture pathology related to diabetes and probability of developing diabetes in future[6]. These cut-offs further loose their credibility due to poor reproducibility of these tests in adults and children[7,8]. Although, HbA1c is believed to represent an average blood sugar level and should ideally represent hyperglycemia more accurately, this may not be entirely true. HbA1c is substantially determined by genetic factors independent of blood glucose levels and may be an imprecise tool to measure average blood sugar[9,10]. While there are valid concerns about diagnostic criteria of prediabetes, prediabetes remains to have a lower reproducibility (approximately 50%) than diabetes (approximately 70%). Based on the available evidence, it appears that prediabetes defined by various alternative criterions consists of an overlapping group of individuals with one or more abnormalities in their glucose excursions. It is possible that presence of IFG and IGT identifies subjects with different pathological abnormalities in their glucose metabolism and presence of both of these signifies more advanced impairment in overall glucose homeostasis.

PREVALENCE OF PREDIABETES

There have been reports of increased mean FPG and prevalence of diabetes in developed as well as developing countries[11]. The Centers of Disease Control and Prevention National Diabetes Statistics Report suggested that 37% of United States adults older than 20 years and 51% of those older than 65 had prediabetes in 2009-2012 defined by fasting glucose or HbA1c levels[12]. When applied to the entire United States population in 2012, these estimates suggest that, there are nearly 86 million adults with prediabetes in United States alone[12]. The world wide prevalence of IGT in 2010 was estimated to be 343 million (7.8%) ranging from 5.8% in South East Asia to 11.4% in North American and Caribbean Countries of the nation's population[13]. International Diabetes Federation projects an increase in prevalence of prediabetes to 471 million globally by 2035[13].

HEATLH RISKS ASSOCIATED WITH PREDIABETES

Progression to diabetes

The conversion rate of individuals from prediabetes to diabetes changes with population characteristics and the criteria used to define prediabetes[14,15]. In a meta-analysis evaluating the progression of prediabetes to diabetes published in 2007, the annual incidence rate of diabetes was found to be 4%-6% for isolated IGT, for isolated IFG 6%-9% and for both IGT and IFG was 15%-19%[16]. This meta-analysis only consisted of studies published prior to 2004. In subsequently reported major studies, the annual incidence rates of conversation from prediabetes to diabetes were similar. In the Diabetes Prevention Program (DPP) Outcomes Study, the incidence of diabetes was noted to be 11% in the control group[17]. In the United States Multi-Ethnic Study of Atherosclerosis the annual incidence of diabetes in IFG group slightly above 4%[18]. In the The Toranomon Hospital Health Management Center Study the incidence of diabetes was reported as 7% in the group with an HbA1c 5.7%-6.4% and 9% in the IFG group[19]. In the China Da Qing Diabetes Prevention Study (CDQDPS), the cumulative incidence of diabetes over a 20 years period, was noted to be higher than 90% among subjects with IGT defined by repeated OGTT in the control group[20]. The use of ADA *vs* WHO criteria to define prediabetes has also been shown to affect the incidence rate of diabetes with lower incidence in individuals defined by ADA criteria compared to WHO criteria[21].

According to an expert panel, continuous rather than dichotomous risk scores are more useful for predicting the risk of developing diabetes[22]. A diabetes risk score based more easily accessible variable such as age, sex, ethnicity, fasting glucose, systolic blood pressure, HDL cholesterol, BMI and history of diabetes in parents or siblings has been shown to have better predictive value than either IFG or IGT[23].

Nephropathy and kidney disease

Several studies have shown an association of increased risk of chronic kidney disease and early nephropathy with

prediabetes[24-28]. The causal nature of this relationship remains unclear as this association may be due increased incidence of diabetes in this group or the presence of other factors associated with both hyperglycemia and nephropathy rather than the effect of prediabetes itself[29,30].

Neuropathies

Prediabetes is found to be associated with dysfunction of cardiac autonomic activity, reflected by reduced heart rate variability[31-35], decreased parasympathetic modulation of the heart[35] and increased prevalence of male erectile dysfunction in individuals with prediabetes[36]. Non-invasive evaluation of neural impairment in subjects with IGT has shown significantly greater abnormalities detected by four of five cardiovascular reflex tests, increase prevalence of both hyperesthesia and hypoesthesia, and increased heat detection thresholds[37]. There is also increasing evidence to demonstrate a higher frequency of idiopathic polyneuropathy, painful sensory neuropathy[38-43] and small fiber neuropathy[38,40,41] among prediabetic individuals with IGT. These findings suggest an involvement of the small unmyelinated nerve fibers that carry pain, temperature, and regulate autonomic function during prediabetes, prior to development of diabetes.

Retinopathy

Nearly 8 percent of participants with prediabetes in the DPP study were found to have evidence of diabetic retinopathy[44]. While prediabetes has been associated with an increased risk of diabetic retinopathy in some studies, these findings vary depending on the method used for detection[24,45-49].

Macrovascular disease

Prediabetes has been associated with increased risk of developing macrovascular disease but whether this elevated risk is due to prediabetes itself or due to development of diabetes remains unclear[50,51]. While cross sectional studies have shown an increased prevalence of coronary heart disease in individuals with prediabetes[52,53] but this relationship may be confounded by the common risk factors present between cardiovascular diseases and prediabetes.

TREATMENT OPTIONS FOR PREDIABETES

Lifestyle interventions

The encompassing theme of lifestyle intervention programs is to change the modifiable risk factors of prediabetes and diabetes by targeting obesity with increase in physical activity and dietary changes. The two largest diabetes prevention studies, the United States DPP and the Finnish Diabetes Prevention Study (DPS) have both shown beneficial effects of lifestyle interventions[54,55]. In the DPP study, after a 3 year follow-up, intensive lifestyle interventions (ILS) lead to a 58% risk reduction. The ILS involved changes in diet and physical activity aimed at producing weight. The biggest determinant of risk reduction was note to be weight loss. This study showed that for every 1 kg decrease in weight, the risk of developing diabetes in future was reduced by 16%[56]. In the DPS, the benefits were found to be dependent on achievement of the number of pre-defined goals of the intervention by the participant. These goals consisted of weight reduction greater than 5 percent, total fat intake less than 30 percent of energy intake, saturated-fat intake less than 10 percent of energy intake, fiber intake greater than or equal to 15 g per 1000 kcal , and exercise greater than 4 h/wk[55]. While both of these studies were largely among Caucasians, studies in Asian population have also shown similar benefits[57,58].

Pharmacotherapy

Several groups of antidiabetic drugs such as Biguanides, Thiazolidinediones, α-Glucosidase Inhibitors, GLP-1 analogies and non-antidiabetic drugs and therapies such as anti-obesity drugs, and bariatric surgery have been studied in context of prediabetes.

Metformin has been used for several decades for treatment of diabetes and has been noted to have additional favorable outcomes such as body mass index (BMI) reduction and improved cholesterol profile. The collective evidence of trials among subjects with IGT, suggests a 45% risk reduction for development of type 2 diabetes[59]. Metformin was noted to be less effective than lifestyle in the United States DPP trial but in the indian DPP (IDPP) trial it was noted to be as effective as lifestyle intervention[54,57]. Metformin has been found to be more beneficial to individuals with higher BMI and higher FPG[54]. Metformin has also been studied in obese children by several investigators. The collective evidence shows a slight benefit in BMI reduction over lifestyle interventions, while the benefit was statistically significant it was noted to be only short term with the greatest benefit at 6 mo and no difference at 12 mo duration[60].

The glitazones are synthetic ligands for peroxisome proliferator-activated receptors-γ. They increase glucose uptake and utilization in the peripheral organs and decrease gluconeogenesis in the liver, thereby reducing insulin resistance[61]. In the double blind placebo controlled Diabetes Reduction Assessment with Ramipril and rosiglitazone Medication study, rosiglitazone was found to be effective in decreasing incidence risk of diabetes by 60% over a 3 year period but was associated with significant side effects such as an additional average weight of 2.2 kg in intervention group compared to controls and a higher incidence of heart failure (0.5% *vs* 0.1%) and total cardiovascular events (2.9% *vs* 2.1%)[62,63]. Pioglitazone was found to decrease the risk of diabetes by > 70% in obese subjects with IGT in the ACT NOW study. Some of the added benefits were, decrease

in diastolic blood pressure, reduction in rate of carotid intima-media thickness and a greater increase in HDL cholesterol but it was associated with increased weight gain (approximately 3 kg more than placebo) and edema (13% *vs* 6% in controls)[64]. In the double blinded placebo controlled 3 years prospective IDPP-2 study, there was no difference in incidence of diabetes between subjects receiving lifestyle intervention and placebo and subjects receiving lifestyle intervention and pioglitazone[65]. In the more recent Canadian Normoglycemia Outcomes Evaluation trial, low does combination of rosiglitazone and metformin was tested against placebo to investigate whether low dose combination therapy would decrease the incidence of type 2 diabetes with a lower risk of adverse events. Incident diabetes occurred in significantly fewer individuals in the active treatment group (14%) than in the placebo group (39%). The relative risk reduction was 66% and the absolute risk reduction was 26%, and 80% subjects in the treatment group reverted to normoglycemia compared to 53% in the control group, but the subjects in active treatment group had increased reports of diarrhea (16% *vs* 6% in controls)[66]. Overall, there are safety concerns for thiazolidinedione such as weight gain, liver toxicity, increased cardiovascular risk and possible link with bladder cancer which have limited the use of these mediations for treatment of prediabetes.

α-glucosidase inhibitors such as acarbose and voglibose, prolong the overall carbohydrate digestion time, and reduce the rate of glucose absorption, thus decrease the postprandial rise in blood glucose[67]. In the STOP-NIDDM trial, acarbose was found to decrease the relative risk for diabetes by 25% among subjects with IGT during a 3.3 years of follow-up[68,69]. The medication was associated with several gastrointestinal side effects such as flatulence and diarrhea and 31% of the participants in the acarbose arm dropped out before completion of the study[68]. A Japanese trail found a 40% risk reduction in incidence of diabetes in high-risk individuals with IGT with voglibose over a 48 wk period. Voglibose was noted to have a similar side effect profile as acarbose but only 7% subjects discontinued the use of drug due to adverse effects[70].

GLP-1 analogs exploit the physiological effects of GLP-1, they have been shown to augment post prandial insulin secretion, suppress glucagon and hepatic glucose production, slow gastric emptying, and reduce appetite[71]. Exenatide and liraglutide have been demonstrated to have long term efficacy for sustained weight loss in obese subjects and reduce prevalence of prediabetes over a follow-up period of 1-2 years. The most common side effects with these drugs are nausea and vomiting and they remain injectable preparations[72-74].

Anti-obesity drugs Orlistat has also been studied in context of prediabetes. Orlistat is a gastrointestinal lipase inhibitor used for treatment of obesity that acts by inhibiting the absorption of dietary fats by approximately 30%. Research has shown that over a 1.5 year follow-up period, use of Orlistat in conjunction with low energy diet is associated with greater weight loss as compared to placebo (6.7 kg *vs* 3.8 kg) and a decrease in conversion rate from IGT to overt diabetes (7.6% *vs* 3.0%) in obese adults[75]. Similar finding have also been reported by the XENDOS trail regarding the efficacy of Orlistat with a 37% relative risk reduction in development of diabetes after 4 years of treatment[76].

Bariatric surgery

Bariatric surgery includes a variety of procedures aimed at either creating a mal-absorptive state, a restrictive state or a combination of the two to limit caloric intake. The procedures commonly used include Roux-en-Y gastric bypass, Laparoscopic adjustable gastric banding, Sleeve gastrectomy, and Duodenal switch with biliopancreatic diversion. In the Swedish Obese Subject, bariatric surgery was found to result in sustained weight loss (23.4% at 2 years and 16.1% at 10 years) and a 75% relative risk reduction of diabetes compared to controls[77]. Bariatric surgery was also associated with a lower 2 year and 10 year rate of development of type 2 diabetes, cardiovascular disease and reduced number of cardiovascular deaths in obese adults[77,78]. A previous study had demonstrated that after gastric bypass surgery, 78% subjects with previous diabetes and 98% subjects with IGT reverted to normoglycemia[79].

PROS AND CONS FOR TREATMENT OF PREDIABETES

The rationale behind treatment of prediabetes includes, prevention of development of diabetes, prevention of consequences of diabetes and prevention of the consequences of prediabetes itself. Several research studies have shown success of interventions designed for treatment of prediabetes with sustained reduction in incidence of diabetes[20,54,80-82]. The CDQDPS study, with lifestyle intervention and 20 year follow-up showed nearly 50% relative risk reduction in incidence of severe retinopathy, but there was no difference between the intervention and control groups in the risk of developing other microvascular complications, such as neuropathy and nephropathy[83]. The evidence regarding effects of interventions on macrovascular complications is inconsistent. The Malmo Preventive Project with a 12 year follow-up, showed reduce mortality in subjects with IGT after a long-term lifestyle intervention program, with emphasis on dietary counseling and physical activity, but this was not a randomized trial[84]. The Collective evidence of all randomized control trails among prediabetic subjects with lifestyle and drug based interventions in a recent meta-analysis showed that these interventions resulted in reduction in stroke risk but did not result in any risk reduction for all-cause mortality, cardiovascular death or myocardial infarction over a mean follow-up period of 3.8

years[80]. While, the current evidence suggests efficacy of several treatment modalities regarding prevention of progression to diabetes, the long term benefits on microvascular or macrovascular complications remains debatable. There is no evidence to suggest that early intervention is better than late intervention and long term studies looking at cost *vs* benefit and long terms of outcomes related to the point at which glycemic intervention should begin are lacking. Majority of published literature and guidelines support that lifestyle interventions focusing on dietary modification and increased physical activity should be the foundation of therapy for diabetes prevention in patients with prediabetes. Although, lifestyle interventions are safe and have proven efficacy in prevention of diabetes, these programs are not reimbursed by most health care insurance plans. There is increasing evidence to prove the efficacy of pharmacotherapy and support its use in adults with prediabetes. Due to the favorable long term safety profile and observed positive outcomes with metformin, organizations such as ADA have recommended the use of metformin in certain high risk individuals[85] but the end point of pharmacotherapy is yet to be defined. The concept of prediabetes or its treatment has not been systematically studied in children with prediabetes. Long term effect of common medications used for prediabetes on growth and pubertal development in children have not been studied. Moreover in children, due to puberty related insulin resistance, incidence of diabetes may be over all inflated. There is lack of evidence with regards to long term efficacy as well as safety for use of pharmacotherapy in children with prediabetes.

CONCLUSION

In conclusion, there remains a need of systematic evaluation of the health outcomes of prediabetes and benefits if any with its early treatment. It is very important to choose the right outcomes for such a study. Moreover, the criteria used to define prediabetes needs to be refined in accordance to the long term medical outcomes. While, these studies seem essential, the length of duration needed to study the adverse outcomes of prediabetes and low frequency of some of these outcomes may be a limiting factor for such studies. At present there is no concrete evidence to formulate clinical guidelines for treatment of prediabetes. Lifestyle interventions remain an essential part of management of prediabetes. The use of pharmacotherapy should be on an individual case based approach. When pharmacotherapy is used to treat prediabetes, such treatment plan should be initiated with predefined goals and end points by the physician. A cautious approach is warranted for used of pharmacotherapy in children and youth.

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Economic development and diabetes prevalence in MENA countries: Egypt and Saudi Arabia comparison

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Abstract

Diabetes is increasing in epidemic proportions globally, exhibiting the most striking increase in third world countries with emerging economies. This phenomena is particularly evident in the Middle East and North Africa (MENA) region, which has the highest prevalence of diabetes in adults. The most concerning indirect cost of diabetes is the missed work by the adult population coupled with the economic burden of loss of productivity. The major drivers of this epidemic are the demographic changes with increased life expectancy and lifestyle changes due to rapid urbanization and industrialization. Our focus is to compare MENA region countries, particularly Egypt and Saudi Arabia, in terms of their economic development, labor force diversity and the prevalence of diabetes.

Key words: Diabetes; Obesity; Health-related behavior; Burden of disease; Middle East and North Africa region

Core tip: The prevalence of diabetes across the Middle East and North Africa (MENA) region has been significantly rising with an increasing burden of healthcare costs. The economic changes occurring in the past decade throughout the MENA region have directed more of the labor force towards the service sector and low physically active lifestyle.

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INTRODUCTION

The Middle East and North Africa (MENA) region countries spans from the Pacific Ocean to the Persian Gulf and extending from the North Africa shores to the sub-Saharan desert. The Gulf Cooperation Council (GCC) region is part of MENA region but exclusive to countries surrounding the Persian Gulf Sea. The GCC consists of Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and the United Arab Emirates. The climate, traditions, religious, and dietary habits are intertwined and shared across the region for eons[1].

The MENA region had the highest comparative prevalence of diabetes in the world in 2012, with four countries in the region among the top ten worldwide

in terms of prevalence[2]. The International Diabetes Federation (IDF) estimates that by 2030, patients with diabetes will double to current estimates of up to 59.9 million in the MENA region[3]. Even though epidemiological studies document high diabetes prevalence in each country, there are specific regions within each country that have higher prevalence of diabetes than originally stated. For instance, one study reported a prevalence of diabetes in Basra, Iraq of more than 19%[4], while, the national prevalence of Iraq is 7.4%[2]. Thus, the diabetes epidemic in the region could be much worse than anticipated and the differences between rural or urban centers are yet to be investigated.

A number of reports have documented a relationship between increased per capita income and economic development on the drastic increase in diabetes prevalence[5]. There is evidence that urbanization and economic progress in emerging economies, such as China, have also led to a drastic reduction in overall and occupational physical activity[6,7]. In West Africa, major urbanization have enabled physical inactivity and diabetes is a major health concern[8,9]. However, it is important to question the contributions of the sedentary lifestyle or economic development on the epidemic of diabetes *per se*. Developed countries have many similarities including demographic, economic, and population genetics. However, these countries also display varying proportions of diabetes prevalence, as the United States is leading with 12.3%[10] while Japan has a diabetes prevalence of 7.6%[2] with very similar economic status and urbanization demographics. These countries have high-income economies with very high Human Development Index. Yet, with their high economic development, they are not regarded as having a rapidly growing diabetes epidemic. The Middle East has the highest prevalence of diabetes in the world with Egypt leading the MENA region in the number of patients with diabetes and with Saudi Arabia leading in the highest prevalence of diabetes in its the adult population.

In this paper we review the economic development, physical activity, and prevalence of diabetes across the MENA and specifically its largest two states, Egypt and Saudi Arabia. We will attempt to understand whether the economic development and westernization of the region is a curse or a blessing concerning diabetes and its epidemic progression?

RESEARCH

We searched PubMed and Google for articles, reports, and major organization statistics related to diabetes mellitus in the MENA region published from 1990 to 2014 without language restriction. We used the following keywords: diabetes, MENA region, physical inactivity, and noncommunicable diseases and found 46 relevant publications. Linear correlation coefficient and statistical analysis was assessed by Minitab (State College, Pennsylvania).

FINDINGS

Diabetes prevalence varies greatly across the MENA region

Egypt and Saudi Arabia are on the separate ends of the spectrum with regards to diabetes prevalence in the MENA region with Egypt having the lowest rate of 7.2% and Saudi Arabia the highest 21.8% (Figure 1)[11]. There is no significant difference in diabetes prevalence between genders within the Egyptian and Saudi population. The urban population within Egypt have higher prevalence of diabetes compared to the rural population (4.9%), regardless of higher (20%) or lower socioeconomic statues (13.5%)[12]. The increased prevalence of diabetes in urban areas could be partly explained by higher socioeconomic status was associated with decreased physical activity and increased prevalence of obesity[13]. In Saudi Arabia, the same trend can be observed where diabetes was more prevalent among Saudis living in urban areas (25.5%) compared to rural Saudis (19.5%). Despite the readily available access to healthcare facilities, a large number of diabetics (27.9%) were unaware of having diabetes[14]. Thus, not only diabetes prevalence varies greatly between countries but also among urban and rural regions. This may suggest that lifestyle changes associated with urbanization plays a pivotal role in the progression of the diabetes epidemic.

Arabs are genetically predisposed to diabetes

The incidence of type 2 diabetes is determined by the complex interplay between multiple genetic, epigenetic and environmental factors[15]. The prevalence of diabetes was highest in the Eastern Mediterranean Region, which includes most MENA region countries, (11% for both sexes) and lowest in the World Health Organization (WHO) European Region (7% for both sexes)[11]. Whether genetics are a contributing factor remains unknown but recent research may shed some light on this issue.

Approximately 40 single-nucleotide polymorphisms (SNPs) that display genome-wide associations with type 2 diabetes have been identified[16]. How these SNPs predispose to type 2 diabetes is still limited and is only available for a few variants that mainly affect insulin secretion and sensitivity[17]. However, there are patterns of SNPs across the MENA region which may bear a role in the diabetes prevalence diversity across the region and on the differences between Arabs and Caucasians.

A unique risk of SNPs for type 2 diabetes that are exclusive to Arab ethnicities has been identified, through a comparative meta-analysis of previously identified SNPs among Arabs and Caucasians[18], The study demonstrated diversity in the MENA region with countries on the Mediterranean Sea such as Egypt, Palestine, and Tunisia having similar SNPs as Caucasians. Whether the low prevalence of diabetes in those countries is

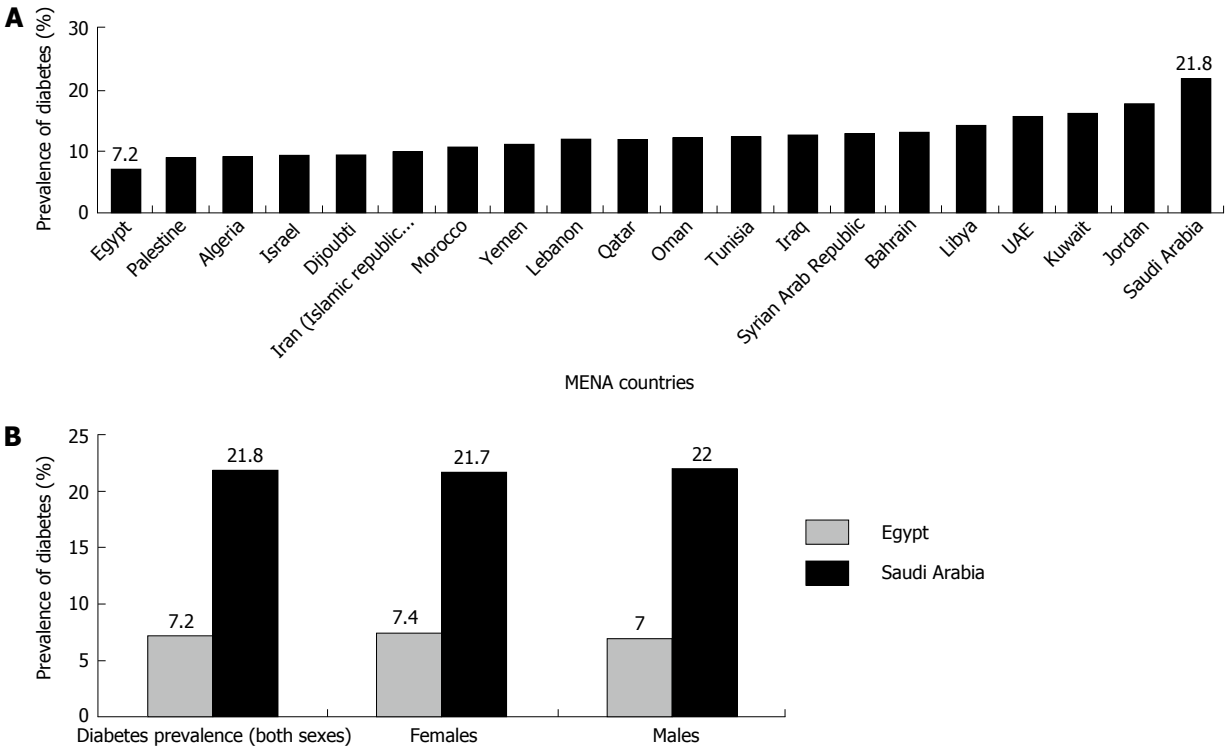

Figure 1 Prevalence of diabetes in Middle East and North Africa region. A: Middle East and North Africa (MENA) countries as defined by the World Bank arranged in increasing prevalence of diabetes; B: Prevalence of diabetes in both genders of Egyptian and Saudi Arabia population. From World Health Organization, 2008. UAE: United Arab Emirates.

influenced by genetic factors is yet to be determined. Conversely, countries from the GCC have different SNPs from Caucasians. North African countries have lower prevalence of diabetes than GCC nations and a similar pattern with the Organization for Economic Co-operation and Development (OECD) countries, which are predominately Caucasian. Egypt, with its low prevalence of diabetes, has a different pattern of SNPs to Saudis.

Other clinical studies comparing Arabs to Caucasians have confirmed that Arabs are more susceptible to diabetes[19,20]. In particular, when comparing Iraqi immigrants (a state located on the Persian Gulf but not a member of GCC) with native Swedes, type 2 diabetes mellitus was twice as prevalent in Iraqis and the people were at a higher risk of developing diabetes[21]. To underscore the implications of genetics on diabetes, consanguinity is prevalent in the Arab culture and that may enhance the diabetic genetic predisposition[22]. A recent study investigated possible mechanisms of consanguinity on the etiology of diabetes in a Saudi population by genotyping SNPs associated with a higher risk of diabetes and measuring other risk factors. It concluded that consanguinity might increase the risk of diabetes by an earlier onset of the disease and by strengthening possible genetic effects on fasting blood glucose[23]. It may explain why a family history of diabetes increases the risk of diabetes by 1.6, 1.8, and 2.4 times in studies among Palestinians, Iranians, and Kuwaitis, respectively[24-26]. However, there are variations in the degree consanguinity across the MENA region. In Saudi Arabia, the prevalence of consanguinity is as high as 60%[22]. On the other hand, Egypt ranges from 8.3% to 17.2% for the urban and rural regions respectively[27].

MENA region westernization and lifestyle changes due to its economic growth

Three out of the world's top five oil exporting countries are located in the GCC region[28], with the world's second largest exporter of natural gas, Qatar. The diabetic population is increasing overall across the MENA region[2] but the GCC states in particular are increasing at a staggering rate compared to others[29]. Some have proposed that the degree of westernization and economic development of the GCC has reached comparable levels to OECD with respect to per capita income and have fueled this diabetes epidemic. However, the economic development in the OECD countries is based on industrialization, manufacturing, and other manpower dependent activities. On the other hand, the major driver of GCC economic development is export of raw materials, primarily crude oil and natural gas.

These resources varying allocation within the region created great disparities among MENA region countries. Not only is there a large difference between Egypt and Saudi Arabia with respect to diabetes prevalence but there is also a wide economic gap. Saudi Arabia has more than double the Gross National Income (GNI) of Egypt (Figure 2). Overall, the gap is wide across the

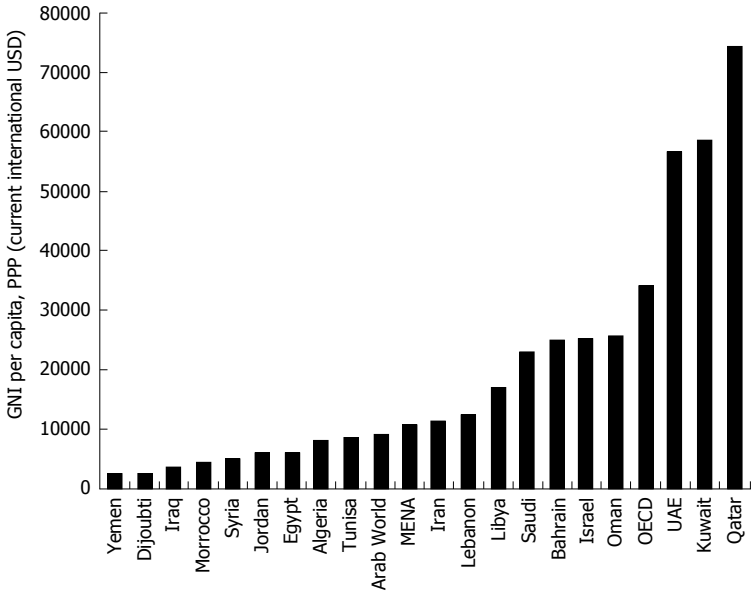

Figure 2 Gross national income per capita based on purchasing power parity of Middle East and North Africa countries. Egypt falls bellows Arab world average ($5710 *vs* $8882) where Saudi Arabia is well above average ($22760). OECD: Organization for Economic Co-operation and Development. From World Bank, 2008. UAE: United Arab Emirates; PPP: Purchasing power parity; GNI: Gross national income.

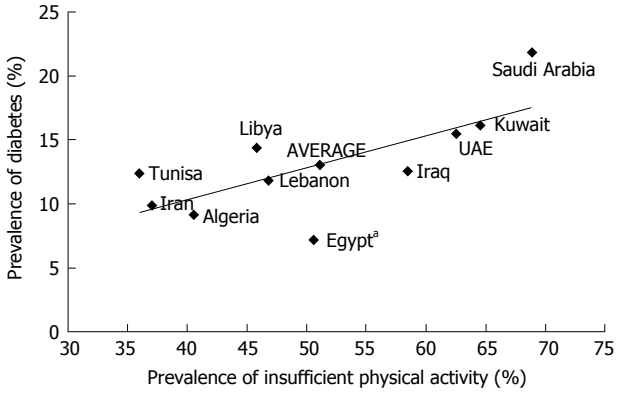

Figure 3 Prevalence of insufficient physical activity in Middle East and North Africa countries compared to prevalence of diabetes. The region's average of prevalence of insufficient physical activity is estimated to be 51.1%. Tunisia and Saudi Arabia are on the two ends of prevalence of physical inactivity in the region (35.9% *vs* 68.8%). Egypt's prevalence of insufficient physical activity is approximately at the region's average (50.6%). The correlation coefficient of the relationship between prevalence of insufficient physical activity and prevalence of diabetes indicate a moderate positive linear relationship (R^2 = 0.51). Regression Analysis of prevalence of Insufficient physical activity *vs* prevalence of diabetes showed statistical significance (P = 0.01). From World Health Organization, 2008. [a]Ministry of Health and population, Egypt Preventive Sector Central Epidemiology and Disease Surveillance (ESU). Non-Communicable Disease Surveillance Unit Community based survey study on non-communicable diseases and their risk factors, Egypt, 2005-2006. UAE: United Arab Emirates.

region with Qatar's GNI over USD 70000 while Yemen is as little as USD 2000, creating financial strains for some nations and lavish lifestyles for others. Furthermore, this overall economic progress has altered the lifestyle of the region. Where the Arab population once frequently participated in outdoor activities, everyone is now almost always inside in an air-conditioned environment, and physical activity has declined[30].

Increased service sector jobs contributes to higher prevalence of physical inactivity and diabetes

Figure 3 shows that the prevalence of diabetes in a MENA country correlates with a higher prevalence of physical inactivity. Countries with the higher prevalence of physical inactivity and diabetes are mostly GCC countries which are also high income countries. We speculate that the exclusive reliance on export of raw resources by these GCC countries may have detrimental effects on the health of the adult population by decreasing their physical activity.

The statistics on the labor force complied by the Arab Labor Organization, confirm the diversity of labor in countries that have a lower diabetes prevalence compared with the Gulf region with an economy that is entirely reliant on the service sector with low physically demanding occupations (Figure 4). In contrast, Egypt depends on manufacturing, fishing, and agricultural labor. Egypt's agricultural sector is reliant predominantly on manual labor and old farming techniques that require heavy manpower; mechanical farming is not prevalent nor invested in as labor cost is inexpensive. The labor pattern across the MENA region impacts the gross national income of the countries illustrating the economic disparity between Egypt and Saudi Arabia. The service sector tends to predominate in countries with higher GNI, whereas in Egypt, there is a demand for hard laborious jobs for the underprivileged class.

To emphasize the impact of physical inactivity on mortality, a recent study utilized life-table analysis to estimate gains in life expectancy if physical inactivity were to be eliminated[31]. Saudi Arabia, with the highest prevalence of physical inactivity and diabetes, had the highest gain in the region, with an estimated additional 1.5 years of life expectancy (1.0 average for region).

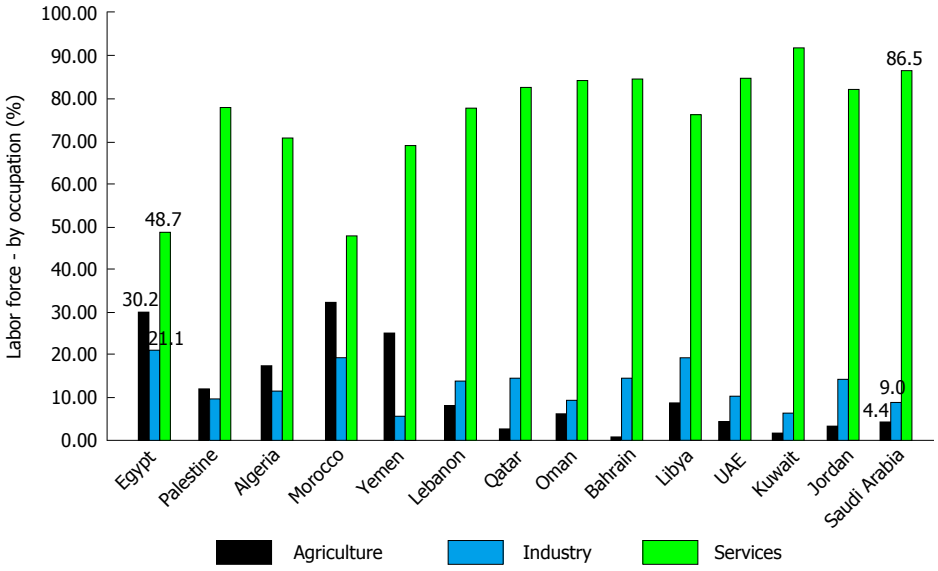

Figure 4 Middle East and North Africa countries labor force by occupation. Diversity of the workforce of Middle East and North Africa countries arranged horizontally from lowest diabetes prevalence (left) to highest diabetes prevalence (right). Data from Arab Labor Organization, but data collected from each country varies from 2006 -2008 (Egypt, 2008; Saudi Arabia, 2007). UAE: United Arab Emirates.

Tunis, with the region's lowest prevalence of physical inactivity, benefited the least with only 0.6 years gain of life expectancy. Iran, second in terms of lowest prevalence of physical inactivity in the region, only gained 0.7 years of life expectancy. Other studies have confirmed the magnitude of health benefits of physical activity with respect to the prevalence and complications of diabetes on the MENA region population. One large study, with an average 3.2 years follow up, has reported that diabetes can be prevented solely by lifestyle changes even in high risk subjects[32]. Currently we cannot identify which labor force sector contributed significantly to the increasing diabetes prevalence in the MENA region due to lack of epidemiological studies. However, a study identified the highest prevalence of diabetes was in the service sector above all sectors in the sub-Saharan country of Namibia, also a developing country[33].

Serious consideration need to be taken to diversify the labor force in countries heavily affected by the diabetes epidemic. This will not only maintain economic growth and provide jobs but may also induce a physically active lifestyle that will eventually enhance the longevity of the adult population and decrease morbidity, mortality, and health care expenditure.

Direct healthcare cost of diabetes

Diabetes presents a severe economic burden as patients with diabetes require at least 2-3 times the health care resources compared to people who do not have diabetes[34]. The direct diabetes healthcare expenditure varies across the MENA region due to economic inequalities. In the United Arab Emirates (UAE) treatment costs are USD 1605 per person[35] *vs* USD 175[36] in Sudan. The economic disparity and the different prevalence of diabetes between Egypt and Saudi Arabia affects their healthcare expenditure for diabetes. Based on a large population study on global health care in diabetes[37], Saudi Arabia's healthcare expenditure for diabetes per person has reached USD 682, which is 21% of total healthcare expenditure. On the other hand, Egypt's healthcare expenditure for diabetes has reached only USD 116 per person which is 16% of total healthcare expenditure. It is difficult to assess the exact economic burden of diabetes in each individual country across the region because not a single country has optimally invested in accordance to the magnitude of its diabetic epidemic. Further investigations needs to be conducted to identify risk factors within the region that propagate the diabetes epidemic and optimal healthcare strategies and investments tailored specifically to each country.

MENA region underinvests in diabetes related healthcare expenditure

When comparing OECD and GCC countries, there is a large gap in healthcare expenditure even though some GCC states surpass OECD's GDP per capita. High incomes states such as, Kuwait, the UAE, and Qatar's per capita health expenditures were USD 1500, 1640, and 1776, respectively, compared to an average of USD 4593 in OECD countries[38].

This underinvestment in health care is likely the responsible for diabetes being the fifth leading cause of death in 2010 in the MENA region compared with 11th place in the 1990s[39]. Epidemiological studies have noted that in high income countries, such as Oman, only 2% of the diabetes population surveyed had their blood glucose levels controlled[40]. Such studies indicate the dire need for MENA countries, especially the GCC states, to invest in primary healthcare, outreach programs, lifestyle coaching, and self-management

education to produce long term healthy gains. This will not only enhance the longevity and quality of life of patients with diabetes but also ultimately reduce the economic burden of healthcare expenditure.

Improvement in life expectancy across MENA region despite increase in noncommunicable diseases

Economic development across the region has dramatically improved the overall life expectancy of many MENA countries. According to WHO, Lebanon, with the region's highest life expectancy at 80 years in 2012, has sharply risen from 67 years in 1990. Lebanon falls below the MENA region's average prevalence of diabetes (11.9% *vs* 12.4%) and physical inactivity (46.8% *vs* 51.1%) contributing to the high life expectancy[11]. Even countries with the lowest life expectancy in the region have dramatically improved. For example, Yemen had a life expectancy of 58 years in 1990 but has risen to 64 years in 2012[41]. When comparing GCC countries with lower middle income countries in the MENA region, there is also a difference in the life expectancy based on differences in overall economic development. Egypt's life expectancy is lower than Saudi Arabia, 71 compared to 76 in 2012, despite the higher prevalence of diabetes and physical inactivity. However, Egypt has an overall higher noncommunicable disease death rate than Saudi Arabia[42].

Increased work hours does not increase risk of developing diabetes

A study was conducted on 40861 employees from four large companies in Japan to determine the effect of long work hours on the prevalence of diabetes[43]. It was hypothesized that longer work hours would predispose employees to diabetes. Surprisingly, there was a decreasing trend of diabetes prevalence with increasing hours of overtime of up to 100 h/mo. In the participating companies, however, occupational physicians recommended employers to shorten working hours for patients with advanced disease, including diabetes. Exclusion of workers taking medication for diabetes did not appreciably change the results. Of the measured risk factors, short sleep duration and leisure time physical inactivity were associated with long working hours, but BMI was not. The physical demand of the job description was not considered in the study. Whether shorter periods of sleep and longer working hours (implying a more vigorous lifestyle) would have a direct causality in prevention of diabetes mellitus is yet to be determined. Taking these studies in account it is important not only to diversify the labor force in MENA region but to consider prolonging work hours for possible additional health benefits.

Dietary habits and prevalence of diabetes

Egypt is one of the few countries in the MENA region that has strictly adhered to the Mediterranean diet[44] which consists primarily of raw vegetables, fruits, moderate amount of fat, and low amounts of meat[45]. It has been suggested that the Mediterranean diet has numerous health benefits including diabetes prevention and management[46,47]. The Egyptian population has a low alcohol consumption of 0.5% which will further decline to 0.3% by 2015[48]. Low alcohol consumption is not specific to Egypt but is characteristic of the MENA region. This eliminates the risk of alcohol attributable and nonattributable diseases such as diabetes, cancers, and liver cirrhosis. However, infection with hepatitis B and C viruses (HCV) continues to be a risk factor[49]. As the prevalence of diabetes is increased in cirrhosis due to HCV infection (compared to cirrhosis to other etiologies), this may be conducive to the development of type 2 diabetes *via* insulin resistance[50]. The prevalence of HCV in Egypt is ten-fold higher than that in other countries[51]. The contribution of HCV infections in Egypt on the prevalence of diabetes is not precisely known and remains to be investigated.

In contrast, the Saudi population, with its desert environment, infrequently consume vegetables and fruits and has been reliant on meat and heavy carbohydrates, primarily dates, as its main diet. When taken in the context of the country's current westernization, this unbalanced diet has progressively worsened to include high carbonated drink such as soda and processed food. Whether the dietary contrast of Saudi Arabia to Egypt may have direct causality to the large difference of diabetes prevalence between the two countries remains unknown but is an interesting speculation.

CONCLUSION

Diabetes inflicts unacceptable high human, social and economic costs on MENA region countries at all income levels. Increased economic development and the subsequent adaptation of a Western lifestyle and the emergence of low physically demanding job sectors on the expense of manual labor may have causal effects on the increased diabetes prevalence in the population independent of other lifestyle habits. Diabetes is more than a health issue and serious consideration need to be taken to initiate diversity in the labor force in the MENA region that might require change in current economic policies. This strategy would produce direct health benefits with lower healthcare expenditure and improved quality of life on the overall population.

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MINIREVIEWS

Current knowledge on diabetic retinopathy from human donor tissues

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Abstract

According to the American Diabetes Association, diabetes was the seventh leading cause of death, and diabetic retinopathy the leading cause of blindness in working age adults in the United States in 2010. Diabetes is characterized by hyperglycemia associated with either hypoinsulinemia or insulin resistance, and over time, this chronic metabolic condition may lead to various complications including kidney failure, heart attacks, and retinal degeneration. In order to better understand the molecular basis of this disease and its complications, animal models have been the primary approach used to investigate the effects of diabetes on various tissues or cell types of the body, including the retina. However, inherent to these animal models are critical limitations that make the insight gained from these models challenging to apply to the human pathology. These difficulties in translating the knowledge obtained from animal studies have led a growing number of research groups to explore the diabetes complications, especially diabetic retinopathy, on tissues from human donors. This review summarizes the data collected from diabetic patients at various stages of diabetic retinopathy and classifies the data based upon their relevance to the main aspects of diabetic retinopathy: retinal vasculature dysfunction, inflammation, and neurodegeneration. This review discusses the importance of those studies to discriminate and establish the relevance of the findings obtained from animal models but also the limitations of such approaches.

Key words: Retina; Diabetic retinopathy; Human donor; Physiopathology; Vascular disease; Inflammation; Neurodegeneration

Core tip: This review summarizes the current state of the knowledge on the physiopathology of diabetic retinopathy directly obtained from the analysis of tissues from human patients strongly complementing what has been gathered from animal models. The review discusses the vascular, inflammatory and neurodegenerative aspects of the disease, their inter-relation and the advantages and limits of such studies compared to the ones using animal models. Altogether, these analyses clearly demonstrate the complexity of the disease mechanisms but also the somewhat

still limited knowledge and the need for additional complementary studies.

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INTRODUCTION

Diabetic retinopathy (DR) is one of the most prevalent complications of diabetes, and while it is now well recognized that this disease involves perturbations of all the components of the retinal tissue, its diagnosis still mostly relies on the detection of damages associated with the vascular network. Based on those clinical features, diabetic retinopathy has been subdivided into two main stages: non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR). In the primary stages of NPDR, microaneurysms, or the swelling of small blood vessels, are the first detectable clinical signs of DR, and are associated with hypoperfusion of discrete regions of the retina. While the clinical gradation is still made based on vascular pathology, recent studies have shown that ganglion cell sensitivity as well as dark adaptation is altered in patients with NPDR, thus confirming early alteration of neuronal function[1] and raising questions regarding the relative causality of those phenotypes. The progression of swelling can also be associated with leakage of fluid into the eye and increased inflammation, which can ultimately lead to swelling of the macula, the most sight-threatening stage of NPDR called diabetic macular edema (DME). While not detectable on fundus photographs, several studies showed that DME was also characterized by dramatic loss of neuronal function and increased inflammation, confirming the progression of the non-vascular alterations at this stage of the disease[2,3]. In some cases, it is believed that progression of NPDR yields a severe ischemic state in specific areas of the retina, causing production of various factors leading to growth of new blood vessels in a process called neovascularization, marking the transition to PDR[4,5]. These new vessels grow in a less controlled manner and disturb vision by developing in normally avascular regions, such as the vitreous cavity, often leading to hemorrhage, or the macula, leading to dramatic and persistent vision loss. Lastly, scarring or gliosis associated with retinal neovascularization can cause traction between the vitreous and the retina, which can ultimately lead to detachment of the neural retina from the retinal pigment epithelium, a phenomenon called tractional detachment. When taking place in the macula, this detachment is a major cause of severe vision loss in DR patients.

While the diagnosis and associated grading of DR has been more clearly defined over the years, the molecular mechanisms responsible for the different stages of this disease have been more difficult to characterize. Various studies have been conducted using animal models of diabetes to explore the molecular mechanisms of retinal vascular changes and neurodegeneration central to this disease. In contrast, and despite the limitations of the studies based on animal models, only a small number of studies have been conducted focusing specifically on the human pathology. This review will summarize what is presently known about DR from studies using human donor tissues. We will describe how the use of post-mortem tissues has allowed investigation of the vascular, inflammatory, and neuronal aspects of the disease and how it compares to data collected from animal models. Lastly, this review will briefly discuss how this approach enables a better understanding of the pathological mechanisms associated with the individual stages of DR and will explore both the advantages and limitations of using human donor tissues.

VASCULAR ALTERATIONS OF DR

In order to comprehend how changes in the vascular integrity can lead to DR, it is important to understand the basic properties and function of the neuroretina and its vasculature. Retinal capillaries are composed of endothelial cells located on the basement membrane, which is surrounded by pericytes. Connecting neighboring endothelial cells are tight, gap, and adherens junctions, which are responsible for maintaining the integrity of the blood-retinal barrier (BRB). The BRB tightly regulates the permeability of those vessels and is responsible for allowing nutrients and certain elements to reach neuroretinal cells. Blood flow is in turn mostly controlled by the surrounding pericytes through the regulation of the diameter of the vessels. Chronic conditions, such as diabetes, can lead to the disruption of the retinal vasculature over time, leading to the blockage of vessels, leakage of capillaries, and other vascular complications, ultimately resulting in conditions like DR.

Retinal microvascular cell death

Progressive retinal microvessel obliteration marks one of the most significant effects of DR in its early stages, resulting in increased vascular permeability as demonstrated clinically on retinal angiograms. The association between DR and pericyte or endothelial cell death was first described by Mizutani *et al*[6] using trypsin digest preparations and retinal cross-sections from diabetic patients and age-matched non-diabetic controls[6]. Combining TUNEL staining on retinal cross-sections and histological analysis of trypsin digests, the authors first demonstrated an increased number of dying cells in the vascular network of diabetic patients. Morphological analysis coupled with cell-specific immunostaining

demonstrated that both pericytes and endothelial cells were dying in tissues from diabetic patients. To control for structural and functional changes primarily due to aging, rather than diabetes, or even simply post-mortem artifacts, the authors reproduced those findings in two rodent models. The authors reproduced those findings in two rodent models, the alloxan-induced diabetic and galactose-fed rats. This study was one of the first demonstrating the death of endothelial cells and pericytes in retinal tissue from human diabetic patients, and that this phenomenon occurred during the early stages of DR, even before clinical signs could be detected. Subsequently, several studies confirmed those results in independent sets of postmortem human tissues from diabetic patients[7,8]. Detection of a decreased pericyte/endothelial cell ratio was also found, suggesting a higher sensitivity of pericytes compared to endothelial cells that could be associated with their lower capacity to replicate[7].

Neo-angiogenesis

Endothelial and pericyte cell death and the subsequent retinal microvessel degeneration results, among other things, in ischemic foci, which, by way of unmet metabolic needs, can ultimately lead to the initiation of angiogenesis. Previous studies investigating the molecular mechanisms of vascular growth and permeability in DR have identified some of the growth factors involved in angiogenesis in response to diabetes[9]. Non-human primate models involving retinal hypoxia were among the first in which increased mRNA levels of vascular endothelial growth factors (VEGFs) were detected in retina and intraocular fluids after vaso-obliteration[10,11]. Soon after, VEGF was reported to be almost exclusively detected in the intraretinal and choroidal vasculature of diabetic patients[12]. To acquire further insights specific to human vasculature, Mathews *et al*[13] analyzed the retinal vasculature of human post-mortem eyes to determine if a correlation existed between VEGF-positive vessels and vascular permeability. Supporting this hypothesis, the authors observed an increase in the number of VEGF-positive vessels in diabetic eyes compared to non-diabetic eyes. Furthermore, the greatest concentration of VEGF-positive vessels were found in the central retina of diabetic eyes, consistent with prior observations that angiogenesis occurs mainly in this region[14]. Conversely, non-diabetic eyes displayed higher numbers of VEGF-positive vessels within the peripheral retina, results reported in previous studies and likely attributed to degeneration of the eye caused by age or other vascular diseases[15]. Additionally, the authors reported that vascular permeability, assessed by human serum albumin levels, was far greater in diabetic eyes than in non-diabetic eyes. Upon further statistical analysis, the levels of VEGF and human serum albumin in diabetic eyes were directly correlated, whereas no pattern existed in non-diabetic eyes. This represented some of the first evidence that increased permeability of diabetic retinal vessels correlated with increased VEGF levels, even prior to the detection of clinical signs of PDR[13].

While VEGF is a central player in PDR and DME, some evidence points to other factors being involved, including the cytokines tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β or other growth factors such as platelet-derived growth factor[16]. Analysis of vitreous fluid from diabetic patients with PDR showed increased levels of TNF-α[17] which could be part of the pathological mechanism of DR due to its known role in vascular and inflammatory regulation that will be expanded upon later in this review. As for TGF-β, it has previously been shown to have a critical role in the maintenance of the BRB and endothelial cell barrier function[18]. However, one study reported that TGF-β was detected in the photoreceptor layer of both diabetic and non-diabetic human retinal tissue[19], and thus, its exact role in DR remains to be clarified.

Angiogenesis relies not only on different growth factors such as VEGF, TGF-β and TNF-α, but also cell adhesion molecules. The role of cell adhesion molecules is to connect the cell to the external environment as well as neighboring cells by binding proteins that make up the extracellular matrix (ECM) *via* membrane-associated proteins on the cell surface. One class of cell adhesion molecules, integrins, is critical for cell-cell and cell-ECM interaction, thus significantly influencing cellular responses and physiology. Interaction of integrins with the ECM has been shown to influence angiogenesis through regulation of intracellular signaling that affects replication and differentiation of endothelial cells and pericytes. A study conducted by Friedlander *et al*[20] in animal models of angiogenesis suggested that since $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins were involved in angiogenesis, these specific integrins could be critical in PDR. Ning *et al*[21] utilized this data to expand upon and determine the co-localization of five different integrins with the retinal vascular endothelium of four patients with PDR. Contrary to what was found in the animal models, no staining was observed for $\alpha v\beta 5$ integrins, possibly attributed to inter-species variability. As for the other integrins tested, $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins were detected in all tissues, at moderate level and without co-localization with endothelial cells. Only $\alpha v\beta 3$ and $\beta 3$ integrin proteins were found to be moderately induced and specifically co-localized with endothelial cell markers in two of the four patients with PDR tested. While further validation is required, these data suggest a specific impact of PDR on a subset of integrins, which could reflect their role in pathological angiogenesis[21].

Perturbations of the retinal vasculature associated with diabetes have long been known from animal models, but studies using tissues from human donors continue to be key to our understanding of the molecular mechanisms underlying the vascular changes associated with the different stages of DR. These studies highlight the complexity of those mechanisms and the interconnection of the vascular pathology with other aspects of the disease

such as the inflammation and neurodegeneration.

INFLAMMATION ASSOCIATED WITH DR

Astrocytes and Müller glial cells, the main macroglial cells of the retina, represent another contributing factor to the diabetes-associated pathology of the neuroretina. In their position between the vasculature and retinal neurons, glial cells play important roles in retinal physiology including regulating permeability of the BRB, supporting neuronal cells, and sensing the extracellular environment, the latter being critical to their regulatory function of retinal inflammation during chronic diseases such as diabetes.

Glial cells dysfunction

Müller cells have long been thought to be affected by diabetes, mostly in their capacity as a support network for the rest of the retina. The first study to investigate the impact of PDR on Müller cells was conducted by Nork *et al*[22] almost 30 years ago, using four post-mortem human eyes. The authors reported the first evidence in human tissues from PDR patients of reactive gliosis demonstrated by the formation of intra-retinal bridges between cystic spaces; and Müller cell dysfunction as suggested by disorganization and migration of their nuclei[22]. More recently, two independent studies sought evidence of abnormalities within Müller cells during the early stages of NPDR. Both studies confirmed reactive gliosis, as demonstrated by increased glial fibrillar acidic protein (GFAP) immunoreactivity in tissues from patients with no to mild NPDR[23,24]. The two studies, however, reported different outcomes regarding the expression of apoptotic regulatory markers, which will be discussed later in this review. GFAP upregulation in diabetic eyes has been demonstrated in various animal models of diabetes and is classically recognized as an indication of cell and tissue reactivity to environmental stress[25,26]. Collectively, these early studies not only confirmed that DR affects Müller cells, but that diabetic conditions promote increased glial cell activation. One of the main functions of glial cells is to regulate the communication between blood vessels and neurons to respond to changes in the environment and maintain retinal homeostasis. Previous studies have suggested that in the early stages of DR, the number of Müller cells increases as astrocyte population decreases, likely as a reaction to the increase in vascular permeability[25,27]. The reported loss of astrocytes in response to diabetes could be enhancing the increased vascular permeability both through mechanical perturbations induced by their absence and loss of the important role they play in the induction of tight junctions within inner retinal blood vessels and the maintenance of the BRB[28,29]. However, the astrocyte loss could also play a pivotal role in the increase in the number of Müller cells, and thus, the enhanced inflammatory response that occurs in the retina in DR.

Inflammatory response

In their original work, Rungger-Brändle *et al*[25] showed that the number of microglial cells, the innate immune cells of the retina, was increased in diabetic rodents. As importantly, they showed that those cells became activated, a state characterized by morphological changes as well as an increase in cytokine production and secretion[25]. While resting microglial cells have multiple, long processes to sense the surrounding environment, activated microglial cells become compact and respond to stress signals by producing various signaling molecules including pro-inflammatory cytokines such as TNF-α, proteases, and reactive oxygen species. There is increasing evidence that inflammation in general, and microglial cell activation in particular, may play an important role in the pathogenesis of DR. One of the premier findings that hinted to this was a study in 1964, when diabetic patients on a salicylate diet, a known anti-inflammatory drug, for treatment of rheumatoid arthritis, reported lower incidence of DR[30]. Subsequent studies displayed similar results, wherein inflammation increased in retinas in response to diabetes; however, most of those have been done in animal models rather than human donor tissues. One such study used streptozotocin-induced diabetic rats to support previously reported findings of an increase in diabetes-associated pro-inflammatory cytokines. Interestingly, and for the first time, this study specifically reported the increase of chemokine (C-C motif) ligand 2 (CCL2) mRNA in the retina of diabetic rats[31]. To this day, data from human retinas remain to be obtained to confirm these observations; however it is supported by human data that were collected by multiple groups from vitreous samples. interleukin-1β and TNF-α, two pro-inflammatory cytokines have been shown to be increased in vitreous samples from patients with DR, even more so in patients with PDR[17]. Several studies also reported CCL2, also known as monocyte chemotactic protein 1 (MCP1), and other cytokines to be elevated in the vitreous of patients with DR[2,32-37]. Muramatsu *et al*[37] also reported that increased levels of cytokines such as MCP-1 correlated with increased VEGF and complement factors levels in the vitreous of PDR patients, while Funatsu *et al*[2] demonstrated an association of cytokine levels with DME, both providing evidence of a link between increased vascular permeability and the inflammatory response in PDR. Of note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, a known negative regulatory mechanism of cytokine signaling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye[38].

In addition to these inflammatory mediators, enhanced expression of intracellular adhesion molecule-1 (ICAM) and P-selectin has been linked to the progression of

DR[39]. ICAM is believed to play a critical role in leukocyte adhesion, one of the initial steps of the inflammatory response allowing leukocytes to cross the BRB in response to increased stress signals. Increased expression of these molecules has been shown to promote the release of inflammatory cytokines, which interfere with endothelial cell tight junction integrity, and thus, increase vascular permeability[40]. While not clearly established, this could be linked with the basement membrane thickening, known to be associated with increased permeability and changes in ECM content in DR patients[41].

As suggested by the observation made by Powell *et al*[30] in 1964, for diabetes as for a wide range of chronic pathologies, a proper control of inflammation is critical to maintaining cellular and tissue homeostasis. While inflammation is a protective mechanism of most complex organisms in response to injury or infectious disease, an uncontrolled inflammatory response becomes part of the pathological mechanism in a variety of chronic diseases such as multiple sclerosis and diabetes. In a study conducted by Krady *et al*[42], the anti-inflammatory drug, minocycline, was suggested to have therapeutic potential in treating/preventing the progression of DR. The authors showed that minocycline treatment leads to a decrease in diabetes-induced cytokine production and reduces microglial cell activation in a rodent model of diabetes. These results were the first to suggest that regulating the inflammatory response could be an important strategy for DR treatment, and that minocycline may be a viable drug to prevent the advancement of DR[42]. More recently, a proof of concept study reported that minocycline oral administration had been associated with improved visual function and regression of central macular edema and vascular leakage in diabetic patients[43] providing further evidence that regulating the inflammatory response can be beneficial to preventing irreversible vascular and neuronal degradation over time.

Overall, the data collected from tissues from human donors along with the data from animal models of diabetes strongly support a role of inflammation in the progression of DR. Seminal work has now been performed that suggests that anti-inflammatory drugs could represent a key component of future therapies for the treatment of DR in order to protect retinal function from the adverse effects of diabetes-associated conditions.

NEURODEGENERATION IN DR

While DR has generally been considered a micro-vascular complication of diabetes, degeneration of the neuroretina has been known for over 50 years. The primary histological analysis of retinal tissue from patients with DR resulted in reports characterizing the loss of cell bodies in every nuclear layers of the retina suggesting a significant loss of retinal neurons in response to diabetes[44,45].

Neuronal cell death

Ganglion cell atrophy and inner nuclear layer (INL) degeneration in retinal tissues from diabetic patients was first described in 1961 and was suggested to happen even prior to vascular changes[46,47]. Soon after, the degeneration of the INL and ganglion cell layers was confirmed in a study using 295 postmortem human eyes and documented the fragmentation of ganglion cell nuclei, which is a characteristic of cells undergoing apoptosis[45]. Following these seminal reports, numerous studies only focused on understanding the vascular aspect of the disease, and additional investigation regarding the neurodegenerative mechanisms only occurred recently. The nature of neuronal cell dysfunction and death associated with diabetes began to be better characterized in a study by Barber *et al*[48] when they reported reduced thickness of the retinal inner nuclear and plexiform layers in 7.5 mo diabetic rats. Retinal ganglion cell (RGC) survival was also decreased by 10% compared to non-diabetic rats. The authors discovered that apoptosis began soon after the induction of diabetes and that the population of cells undergoing apoptosis potentially included ganglion and photoreceptor neuronal cells[48]. More recent findings using rodent models of diabetes have confirmed the diabetes-associated alteration of RGC function and survival (reviewed in[49]). These findings are interesting with regards to reports analyzing flat-mounts and cross-sections of human retinas by immuno-based assays. Consistent with the first report of apoptotic cells in retinas from diabetic human donors distinct from vascular lesions[48], expression of the pro-apoptotic protein Bax was shown to be increased in RGCs of diabetic patients[50]. This study supported the previous findings wherein the progression of diabetes paralleled increased levels of the pro-apoptotic protein Bax in diabetic human retinas, specifically in the inner retina and concentrated in ganglion cells[51]. A subsequent study revealed an increase in levels of Bax, cleaved caspase-3, and caspase-9 in RGCs of diabetic patients supporting the hypothesis that RGCs specifically are undergoing apoptosis during diabetes and providing evidence of neurodegeneration during the early stages of DR[52]. This activation of the apoptotic pathway could be associated with a loss of trophic factors. While the exact impact of diabetes on local insulin signaling in the human retina remains to be fully characterized, rodent models strongly suggest that it is affected as demonstrated by significant reduction of kinase activity of the whole signaling cascade - insulin receptor (IR), insulin receptor substrate 2, phosphoinositide 3-kinase, Akt, and mechanistic target of rapamycin[53,54]. While this could be directly due to loss of insulin signaling, it could also be a result of loss of activation by insulin-like growth factors (IGF). Retinal IR is known to be a spliced variant that is equally susceptible to insulin and IGFs, and IGF1 levels of expression was shown to be decreased in retina from rodent models and human diabetic patients[51]. These

data support a diabetes-associated decrease of trophic factors and subsequent molecular signaling that could lead to retinal cell death.

Apoptosis regulation

In addition to their role in the regulation of inflammation, glial cells and more specifically Müller cells, are involved in supporting neuronal cells, including the regulation of their survival. Regulation of the intrinsic apoptotic pathway is dependent upon expression of members of the anti-apoptotic Bcl-2 protein family. Two independent groups reported Bcl-2 expression in the human retina to be confined to Müller cells; however, one study by Mizutani *et al*[23] reported no change in the expression of the anti-apoptotic protein while the second study by Abu-El-Asrar *et al*[24] reported a small but significant induction of Bcl-2 in samples from diabetic patients compared to age-matched non-diabetic patients. The authors of the second study also reported changes in expression of other anti-apoptotic proteins, which suggests activation of survival pathways, but also increased expression of the cytotoxic effector Fas ligand, which reflects the ambiguous and complex role of Muller glial cells in the molecular mechanisms leading to neuronal cell death[24].

Neurodegeneration is a cumulative process resulting from the complex interplay of several independent stress mechanisms, one of which is oxidative stress. In animal models of diabetes, various cellular stresses have been shown to alter the oxidative state of retinal cells and lead to accumulation of reactive oxygen species (ROS), promoting damages to the cell machinery and ultimately leading to increased cell death. Several studies have reported increased superoxide production, a known marker of oxidative stress, in diabetic rats[55,56]. One of these reported further increased production of superoxide in hypertensive rats in response to diabetes. Additionally, this study showed that those animals presented with increased levels of gliosis and neuronal apoptosis suggesting a link between superoxide production and neurodegeneration in an animal model of diabetes, especially under hypertensive conditions[56]. Despite the growing number of reports suggesting a direct role for oxidative stress in promoting retinal cell death in animal models of diabetes, evidence of such a link between increased oxidative stress and neurodegeneration associated with DR has not been directly shown in human tissues. Indeed, the only data collected so far and supporting this hypothesis come from indirect measures of oxidative stress *via* analysis of the levels of ROS in the retinas of diabetic human donors[57,58]. It is interesting to note that an anterior study using retina samples from twelve human donors with varying durations of diabetes, showed that, within the retina, rod photoreceptors are the most vulnerable to oxidative stress, likely attributable to the high concentration of polyunsaturated fatty acids within their membranes[59]. High lipid concentration, specifically low-density lipoprotein, was shown to be associated with the progression of DR, and recent studies theorize that the retina is highly susceptible to oxidative stress due to its composition of polyunsaturated fatty acids and high oxygen usage[60,61].

Altogether these data strongly support that retinal neurons are highly affected by diabetes and that neurodegeneration is a key aspect of diabetic retinopathy. While it suggests that oxidative stress and loss of trophic factors could play an important role in the induction of apoptosis, it also clearly shows how little we still know about this aspect of the disease and the need for additional studies.

ADVANTAGES AND LIMITATIONS OF POST-MORTEM TISSUES

Advantages

The primary advantage to using human donor samples is that the data collected are directly representative of the disease pathology as opposed to mechanisms uncovered in artificially-induced or genetically-modified animal models of the disease. In addition, the retinas from the majority of the animal models of diabetes do not have the same structural and cellular properties as the human retina. For example, rabbit and guinea pig models are more similar to human in regards to the type and repartition of photoreceptors, but lack an intraretinal vascular network, as opposed to rodent models that possess this vascular network but have a very different rod/cone repartition and properties. Additionally, neither of those models has a macula and thus does not allow investigation of the specific impact of diabetes on this central and key region of the human retina. Another reason of the limited success in translating data obtained from animal models is the heterogeneous nature of the human pathology[62]. In addition to the human-specific variable environmental conditions, none of the animal models routinely used recapitulate the anatomical and regional specificity of the human retina and how it is impacted by diabetes; *i.e.*, peripheral vascular hypoperfusion, non-homogenous visual field impairment, local hemorrhage and lipid exudates, and macular edema.

Limitations

However, it is also important to note the limitations when working with post-mortem human tissues. The first and main concern is the difficulty in promptly processing fresh tissues from human donors compared to animal models, where no consent is needed, and experiments can be planned ahead. Moreover, there is less control over inter-sample experimental variability due to the very diverse background and health history of human donors. In contrast, studies using animal models can be tightly controlled by using inbred strains that have identical

genetic background and are maintained in identical experimental conditions.

CONCLUSION

The goal of this review was to produce an overview of the current state of knowledge regarding the human specificity of the pathophysiology and molecular mechanisms of DR. This review summarizes what has been discovered regarding the impact of diabetes on the vascular, inflammatory, and neuronal components of human retinal tissue. Overall, this review demonstrated that while animal model-based studies can be utilized to address a variety of disease-related questions, studies using human donor tissues are necessary to validate the conclusions from animal models, as well as characterize different molecular mechanisms associated with the individual stages of DR pathology. It also demonstrates the importance for continuous evaluation of the various disease models to assess their efficacy and limitations for investigating specific pathological mechanisms. Finally, this review underscores the gaps in our knowledge concerning even the basic mechanisms regulating vascular alteration, retinal cell survival, and the interplay of various components of the retina in response to diabetes that underline the progression of DR.

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MINIREVIEWS

Hepatic glycogenosis: An underdiagnosed complication of diabetes mellitus?

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Abstract

Hepatic glycogenosis (HG) is characterized by excessive glycogen accumulation in hepatocytes and represents a hepatic complication of diabetes that particularly occurs in patients with longstanding poorly controlled type 1 diabetes (T1D). HG has been reported to be a very rare disease, although it is believed to be extremely underdiagnosed because it is not possible to distinguish it from non-alcoholic fatty liver disease (NAFLD) unless a liver biopsy is performed. In contrast to HG, NAFLD is characterized by liver fat accumulation and is the more likely diagnosis for patients with type 2 diabetes and metabolic syndrome. The pathogenesis of HG involves the concomitant presence of insulin and excess glucose, which increases glycogen storage in the liver. HG is characterized by a transient elevation in liver transaminases and hepatomegaly. Differentiating between these two conditions is of the utmost importance because HG is a benign disease that is potentially reversible by improving glycemic control, whereas NAFLD can progress to cirrhosis. Therefore, HG should be suspected when liver dysfunction occurs in patients with poorly controlled T1D. The aim of this article is to review the epidemiology, clinical characteristics, pathogenesis and histology of HG.

Key words: Hepatic complications; Diabetes mellitus; Type 1 diabetes; Hepatic glycogenosis; Non-alcoholic fatty liver disease

Core tip: Hepatic glycogenosis (HG) is a complication of diabetes mellitus that is often underdiagnosed. It is defined as pathological glycogen storage in hepatocytes with hepatomegaly and elevated liver enzymes and mainly occurs in patients with longstanding poorly controlled type 1 diabetes. HG cannot easily be distinguished from non-alcoholic fatty liver disease (NAFLD) by history, physical examination or ultrasound; only liver biopsy can provide a definitive diagnosis. The hallmark of this condition is its reversibility with improved glycemic control; in contrast, NAFLD can progress to fibrosis.

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INTRODUCTION

Diabetes mellitus (DM) is associated with various structural and functional liver abnormalities, including non-alcoholic fatty liver disease (NAFLD) and hepatic glycogenosis (HG). NAFLD represents the most common liver disease associated with DM, especially in patients with type 2 diabetes (T2D) and metabolic syndrome[1]. HG involves pathological glycogen storage in hepatocytes and is associated with poorly controlled DM, particularly type 1 diabetes (T1D). This condition is believed to be extremely underdiagnosed because it is indistinguishable from NAFLD in the absence of a liver biopsy. However, the distinction between these two diabetes-related complications is important: whereas NAFLD may progress to fibrosis and cirrhosis, HG is potentially reversible with sustained glycemic control[1,2]. This review aims to provide an overview of the clinical characteristics and pathological features of HG to improve recognition of this diabetes-related complication.

DEFINITION AND EPIDEMIOLOGY

HG is defined as pathological excessive glycogen accumulation in hepatocytes and is characterized by hepatomegaly and a transient elevation in liver transaminases[2]. Glycogen accumulation in the liver was first described in children by Mauriac[3] in 1930 as a component of Mauriac's syndrome. He observed glycogen accumulation in a child with T1D and poor diabetic control that was associated with hepatomegaly and abnormal liver enzymes as well as other features such as growth retardation and/or dwarfism, delayed puberty, cushingoid features and hypercholesterolemia. Currently, HG is a well-recognized disease that occurs at any age and can be present without the full spectrum of features described for Mauriac's syndrome. Although numerous case reports and several series have been published[2,4-24], the exact prevalence of HG is unknown, but it is considered to be the primary cause of hepatomegaly in children and adolescents with T1D[24]. This condition has been given many labels, such as hepatic or liver glycogenosis[4,11,12,15,16], glycogenic hepatopathy[2,5,17,19,22,23], liver glycogen storage [6,13], and DM-associated glycogen storage hepatomegaly[10].

HG was first described in association with acute ketoacidosis or recurrent hypoglycemia[7-10,15] in cases presenting with excessive insulin and/or elevated glucose. However, hepatic glycogen accumulation also develops in diabetic patients with long-term poor control and several hospitalizations for diabetic ketoacidosis[4,5,11,16-21]. Although HG is more common in patients with T1D, it has also been described in insulin-dependent type 2 diabetic patients during ketosis or poor diabetic control requiring increasing amounts of insulin[25]. In addition, HG has also been reported in other clinical settings, such as in three children after short-term, high-dose steroid therapy without insulin treatment[26] and in a patient with dumping syndrome associated with gastrostomy[27].

PATHOGENESIS

Although the underlying mechanisms by which HG develops have not yet been fully elucidated, wide fluctuations in glucose and insulin concentrations seem to be essential for its pathogenesis[28]. Blood glucose and insulin levels often fluctuate in diabetic patients with poor metabolic control, thereby promoting hepatic glycogen accumulation. High plasma glucose levels cause an insulin-independent glucose influx into hepatocytes by facilitated diffusion. In the cytoplasm of hepatocytes, glucose is irreversibly converted into glucose-6-phosphate by glucokinase, an enzyme regulated by glucose and insulin. Then, glucose-6-phosphate is converted into glycogen by glycogen synthase, which is converted from the inactive form into the activated form by a phosphatase. This phosphatase plays a key role in regulating this step in the pathway: its concentration is maintained by insulin, and its activity depends on the presence of glucose (Figure 1). Therefore, the synthesis of hepatic glycogen is the consequence of the combination of high blood glucose levels (which promote the flow of glucose into hepatocytes) and hyperinsulinemia (which stimulates the conversion of glucose to glycogen)[2,5,11,12]. This situation is frequently observed in patients with unstable diabetes who are treated with insulin for marked or prolonged hyperglycemia.

CLINICAL PRESENTATION

The clinical presentation is not specific and can include abdominal pain that is sometimes associated with nausea, vomiting and anorexia[2,11,12]. The key clinical features are hepatomegaly and a mild to moderate increase in transaminases, although in some cases, the transaminase levels can be dramatically elevated[2,5]. Alkaline phosphatase levels can be elevated, and liver synthetic function is usually normal. Ascites has rarely been reported[2,11]. The clinical and pathological features are similar in adults and children.

Torbenson *et al*[2] reported 14 patients (range, 8 to 25 years old) with HG. All had T1D with poor glycemic control. The clinical presentations included hepatomegaly, abdominal pain and elevated transaminases. Ascites was present in 1 patient. In 3 cases, the transaminase levels were markedly elevated to 10 times greater than the upper limit of normal. All the biopsies revealed

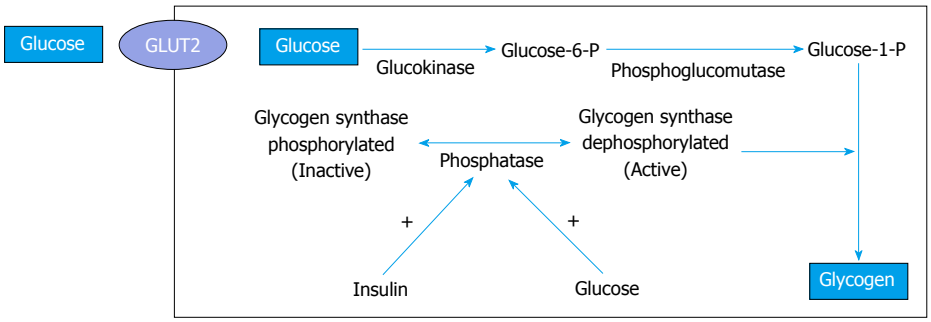

Figure 1 Pathogenesis of hepatic glycogenosis. Glucose from the blood enters hepatocytes by facilitated diffusion independent of insulin and is converted into glycogen via glucose-6-phosphate. Glycogen synthesis depends on insulin and glucose (modified from Munns *et al*[12]). GLUT2: Glucose transporter 2.

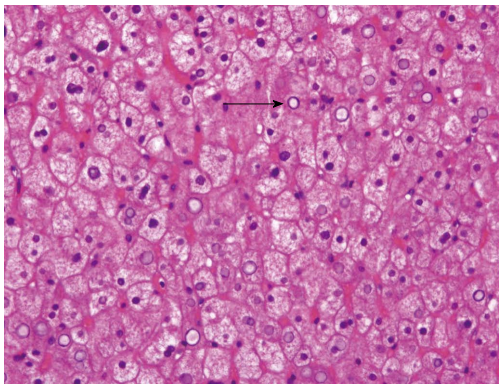

Figure 2 Liver biopsy, haematoxylin and eosin staining. The hepatocytes are swollen with pale cytoplasm and accentuation of the cell membranes. Sinusoids appear compressed by the swollen hepatocytes. Glycogen nuclei are present (black arrow).

excessive glycogen accumulation. Another large cohort was reported that included 11 patients (8 adults between 19 and 70 years of age and 3 children) with poorly controlled insulin-dependent diabetes (T1D or T2D was not specified)[11]. Nine patients (6 adults and 3 children) had hepatomegaly as evidenced by physical examination or ultrasonography. Ascites was present in 1 patient, and serum transaminases were markedly elevated in 4 patients.

DIAGNOSIS AND HISTOLOGY FINDINGS

HG cannot be distinguished from NAFLD by history, physical examination or laboratory blood tests. In addition, ultrasound cannot distinguish fatty liver from glycogen accumulation[2,12]. The usefulness of abdominal computed tomography (CT) in distinguishing HG from NAFLD was reported by Sweetser *et al*[19]. A low density liver is usually observed by CT in patients with fatty liver, whereas the liver density by CT is typically increased in patients with HG. However, a low liver density by CT may be not observed in the early stages of NAFLD[28], and CT only provides qualitative information. Recently, it has been reported that gradient dual-echo magnetic resonance imaging (MRI) can effectively discriminate glycogen from fat in the liver[28,29]. A gradient dual-echo MRI sequence, as well as magnetic resonance spectroscopy, can quantify the intrahepatic lipid content and detect even small amounts of fat accumulation[30].

The differential diagnosis of HG, as opposed to NAFLD, must consider several other potential causes of liver damage, such as infection (*e.g.*, viral hepatitis), metabolic disorders (*e.g.*, α1-antitrypsin deficiency and Wilson's disease), obstruction, autoimmune liver disease and celiac disease[12]. On the other hand, there is an increasing evidence that focal, but sometimes also diffuse, HG is a potential preneoplastic lesion[31-33]. Investigations in animal's models of chemical, viral and hormonal hepatocarcinogenesis and some observations in humans suggest that focal HG, represents a critical early event in the pathogenesis of benign and malignant hepatocellular neoplasm[34,35]. Although the exact mechanism remains elusive, recent data suggest that oncogenic agents have an early insulin-like effect[35-37]. It is noteworthy that a number of epidemiology studies have shown that DM is a risk factor for the development of hepatocellular carcinoma[38,39]. However, no relationship has been described between diabetes-related HG and hepatocellular neoplasms, but further studies are warranted in order to clarify this point.

HG is only diagnosed by liver biopsy. In general, HG is characterized by several histological features: (1) marked glycogen accumulation. After conventional tissue preparation (fixation by formaldehyde-solution and staining with haematoxylin and eosin) the glycogen is usually removed from the hepatocytes. Thus, the hepatocytes are diffusely swollen with a pale cytoplasm and accentuation of the cell membranes, frequently with displacement of the nuclei to the cell periphery (Figure 2), the sinusoids are compressed by swollen hepatocytes, and glycogenated nuclei and giant mitochondria are present; glycogen accumulation within hepatocytes is demonstrated by periodic acid-Schiff staining (Figure 3A) which disappeared after digestion with diastase (Figure 3B); (2) no or a minimal change in fat content; (3) the absence of or minimal inflammation; (4) the absence or minimal presence of spotty lobular necrosis; and (5) intact liver architecture without or with minimal fibrosis[2,5,11].

In contrast to NAFLD, which can progress to cirrhosis, HG is potentially reversible with optimal diabetes control. The abnormal transaminase levels and hepatomegaly

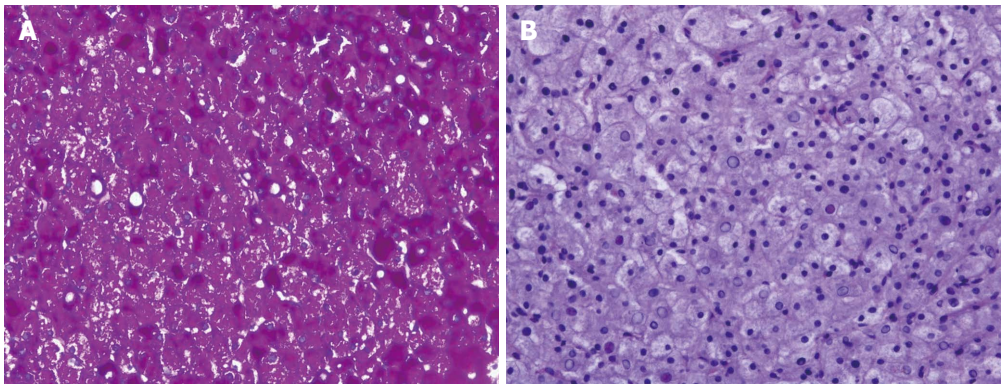

Figure 3 Liver biopsy, periodic acid-Schiff staining. A: Periodic acid-Schiff (PAS) demonstrates abundant cytoplasmic deposits. An intense reaction is also found in the nucleus; B: The hepatocyte cytoplasm is not stained with PAS after diastase treatment confirming the present of glycogen.

have been described to be reversible after improving glycemic control with insulin treatment, usually within 2 to 14 wk[11,12,16]. In a large series published by Chatila *et al*[11], hepatomegaly was resolved in all the patients within 2 wk of stabilizing the blood sugar levels. Aminotransferases rapidly decreased, but remained moderately elevated in some patients during the 14-wk follow-up. In 2011, our group published a case of HG in a 31-year-old woman with poorly controlled T1D. During admission for acute ketoacidosis, she presented with hepatomegaly and markedly elevated transaminases. Liver glycogen storage was diagnosed by biopsy. After optimal glycemic control, transaminase levels rapidly decreased, but the hepatomegaly remained after 6 mo[20]. In two previously published severe cases, pancreatic transplantation was reported to be effective[40].

CONCLUSION

HG most likely represents an underdiagnosed hepatic complication of diabetes that is difficult to distinguish from NAFLD. For this reason, a diagnosis of HG should be considered in diabetic patients, especially in those with T1D, who exhibit poor metabolic control and present with a transient elevation of liver transaminases and hepatomegaly. Although HG is definitively diagnosed histologically, a gradient dual-echo magnetic resonance imaging sequence combined with CT of the liver is a powerful methodology for distinguishing HG from NAFLD. The correct diagnosis of this disease is important given its potential resolution after improved glycemic control.

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Role of oxidative stress in endothelial insulin resistance

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Abstract

The International Diabetes Federation estimates that 316 million people are currently affected by impaired glucose tolerance (IGT). Most importantly, recent forecasts anticipate a dramatic IGT increase with more that 470 million people affected by the year 2035. Impaired insulin sensitivity is major feature of obesity and diabetes and is strongly linked with adverse cardiometabolic phenotypes. However, the etiologic pathway linking impaired glucose tolerance and cardiovascular disease remains to be deciphered. Although insulin resistance has been attributed to inflammatory programs starting in adipose tissue, emerging evidence indicates that endothelial dysfunction may represent the upstream event preceding peripheral impairment of insulin sensitivity. Indeed, suppression of reactive oxygen species-dependent pathways in the endothelium has shown to restore insulin delivery to peripheral organs by preserving nitric oxide (NO) availability. Here we describe emerging theories concerning endothelial insulin resistance, with particular emphasis on the role oxidative stress. Complex molecular circuits including endothelial nitric oxide synthase, prostacyclin synthase, mitochondrial adaptor p66^{Shc}, nicotinamide adenine dinucleotide phosphate-oxidase oxidase and nuclear factor kappa-B are discussed. Moreover, the review provides insights on the effectiveness of available compounds (*i.e.*, ruboxistaurin, sildenafil, endothelin receptor antagonists, NO donors) in restoring endothelial insulin signalling. Taken together, these aspects may significantly contribute to design novel therapeutic approaches to restore glucose homeostasis in patients with obesity and diabetes.

Key words: Endothelium; Insulin resistance; Oxidative stress; Obesity; Cardiometabolic risk; Vascular disease

Core tip: We present here the most recent advances in the understanding of endothelial insulin resistance, with a particular focus on the role of oxidative stress. The molecular pathways described may be instrumental for the development of mechanism-based therapeutic strategies to prevent maladaptive endothelial insulin signalling in patients with cardiometabolic disturbances.

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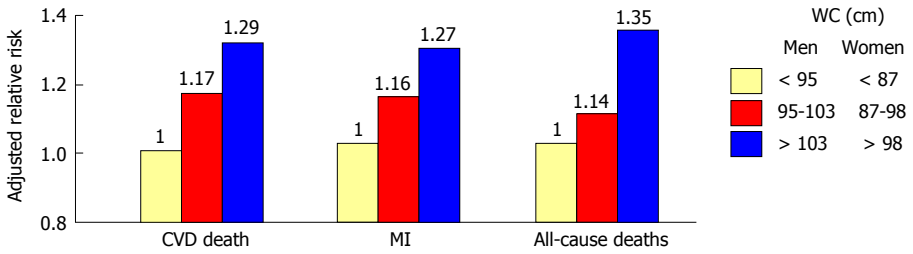

Figure 1 Association between abdominal obesity and cardiovascular disease. Subjects in the upper tertile of waist circumference had an increased adjusted relative risk of 29% for cardiovascular (CV) death, 27% for myocardial infarction (MI), and 35% for total mortality, suggesting a strong association between abdominal obesity and CV events. Adapted from Dagenais *et al*[9]. WC: Waist circumference; CVD: Cardiovascular disease death.

PREVALENCE OF IMPAIRED GLUCOSE TOLERANCE

The most recent update of the International Diabetes Federation shows that 6.9% of the global population (316 million people) is currently affected by impaired glucose tolerance (IGT) and, most importantly, forecasts anticipate a dramatic IGT increase with more that 470 million people affected by the year 2035[1]. Such pandemic of metabolic syndromes and obesity-related disorders hints a proportional increase in the prevalence of type 2 diabetes (T2D), a major driver of morbidity and mortality worldwide[2]. Currently, 382 million people are affected by T2D, with a global age-adjusted prevalence of 10%. If these trends continue, 592 million people, or one adult in 10, will have diabetes by 2035[1]. The link between environmental factors (pollution, caloric intake, sedentary lifestyles), obesity and subsequent dysglycemia indicates that the progression to diabetes is not linear and involves different cellular mechanisms including alterations of insulin signalling, changes in glucose metabolism, free fatty acids oxidation as well as dysregulation of genes relevant to endothelial integrity[3,4]. The progression from obesity to T2D may take many years to occur, leading to different intermediate phenotypes with progressive changes in glucose parameters and shifts in glucose tolerance category. Yet, the etiologic pathway linking increased body weight, altered insulin signaling and subsequent hyperglycemia remained to be understood. Novel insights in this area may be instrumental to identify novel mechanism-based therapeutic strategies for the preservation of insulin signaling and, hence, diabetes development.

IMPACT OF INSULIN RESISTANCE ON CARDIOVASCULAR OUTCOME

Mortality from cardiovascular disease (CVD) is significantly higher in subjects with T2D than in those without[5]. Notably, the risk of macrovascular complications seems to be proportional to the impairment of glucose homeostasis[6]. Among different diabetes-related conditions, insulin resistance (IR) and hyperglycemia are major precursors of atherothrombotic events and poor CV outcome[7]. Waist circumference, an hallmark of IR, is a strong independent predictor of CVD[8]. Dagenais *et al*[9] showed that subjects in the upper tertile of waist circumference had an increased adjusted relative risk of 29% for CV death, 27% for myocardial infarction, and 35% for total mortality, suggesting a strong association between abdominal obesity and CV events (Figure 1). Along this line, elevated insulin and glucose concentrations are associated with increased CVD risk, regardless of diabetes[10,11]. Impaired insulin signalling is a key feature of the metabolic syndrome (MetS), defined by the presence of hyperglycemia, central obesity, low high density lipoprotein cholesterol level, high triglyceride level and elevated blood pressure or antihypertensive drugs use. MetS is highly represented in patients with type 1 diabetes (T1DM) (38% in men and 40% in women) and is an important predictor of CV events[12]. Indeed, MetS was associated with a 2.1-fold increased risk of CV events and a 2.5-fold increased risk of CV-related mortality after 5.5 years follow-up in 3783 patients with T1DM[13]. The main issue when it comes to insulin resistance is how to measure it in clinical practice. The Homeostasis Model Assessment IR (HOMA-IR) is emerging as well-established marker of IR with a high predictive value for incident coronary events and stroke[14-16]. This is likely due to the fact that HOMA-IR includes in its formula both fasting glucose and insulin levels thus showing a stronger association with cardiovascular disease than glucose or insulin alone[14]. Despite many investigations confirmed a potential predictive value of IR, the mechanisms underlying this phenomenon still remain poorly understood. First, it is not clear whether IR is an active process or rather the consequence of the inflammatory milieu observed in obese and diabetic patients. Second, it remains unknown if the impairment of insulin signaling occurs simultaneously in all insulin-sensitive organs or whether tissue-specific IR has a primary role in triggering maladaptive insulin responses in other tissues. In order to answer these complex questions, many researchers are now exploring the pathophysiology of IR in different organs as well as its impact on metabolic features and

longevity.

ENDOTHELIAL INSULIN RESISTANCE: AN EMERGING CONCEPT

Evidence accumulated over the last decade has shown that loss of insulin signaling in the endothelium accelerates atherosclerotic lesions and vascular dysfunction in mice[17-21]. Noteworthy, these effects occur regardless of concomitant CV risk factors, suggesting a central role of endothelial IR[4]. Although IR has been attributed to inflammation in adipocytes, recent work has overturned such "adipocentric paradigm"[22]. The novel concept that IR may primarily starts in the endothelium squares with the notion that endothelial cells are highly represented within the entire vascular system and, hence, within different organs[18,20,23]. Recent experimental work has demonstrated that the transcription factor nuclear factor kappa-B (NF-κB) is a key determinant of endothelial insulin resistance in mice[17]. NF-κB is a well known molecular complex involved in inflammatory programs enabling transcription of cytokines, activation of stress kinases and dysregulation of insulin-related pathways[24,25]. Of note, genetic disruption of IκB prevents inflammation and insulin resistance in obesity and T2D[26]. Hasegawa *et al*[17] have shown that mice with endothelium-specific suppression of NF-κB signaling (E-DNIκB) were protected against IR in adipose tissue and skeletal muscle. These mice displayed reduced oxidative stress markers, decreased macrophage infiltration of adipose tissue as well as increased blood flow and muscle mitochondrial content. Of note, capillary recruitment and subsequent insulin delivery were explained by restoration of nitric oxide (NO) levels in E-DNIκB animals[17]. This latter observation is important since endothelial nitric oxide synthase (eNOS) dysfunction may lead to a reduction in microcirculatory blood flow and, hence, reduced delivery of insulin within hormone-sensitive organs. Indeed, insulin-mediated glucose uptake is reduced in $eNOS^{-/-}$ as compared with WT mice[27]. In other words, microvascular dysfunction occurring in liver, adipose tissue and skeletal muscle explains the progressive decline of peripheral insulin distribution[28,29]. Of note, restoration of eNOS functionality due to suppression of endothelial NF-κB signaling was capable to rescue aging-associated insulin resistance and, most importantly, to prolong lifespan in mice[17]. In line with these experimental data, studies in humans demonstrated that insulin-dependent vasodilation may represent a significant contributor to insulin-stimulated glucose uptake[30-32]. Muris *et al*[33] proposed that approximately 40% of insulin-mediated glucose uptake by skeletal muscle can be attributed to capillary recruitment; according to this hypothesis, microvascular dysfunction not only precedes and predicts the development of T2D but also constitutes one of the links between IR and hypertension in MetS. Consistently, improvement of insulin sensitivity in patients with cardiometabolic disturbances is associated with restoration of flow-mediated vasodilation[34,35]. Another study showed that disruption of endothelial insulin signaling by genetic deletion of insulin receptor substrate-2 (IRS-2) alters insulin delivery in muscle thus affecting glucose tolerance in mice[18]. Of interest, endothelial IRS-2 and ApoE knockout mice showed a more severe atherosclerotic disease progression as compared to controls[18]. Further work demonstrated that knockout of three major FoxO isoforms in endothelial cells attenuates endothelial IR and atherosclerosis in low density lipoprotein receptor knockout mice, suggesting that FoxO inhibition may represent a potential therapeutic approach to prevent CVD and IR in patients with diabetes[36]. Accordingly, activity of protein kinase C (PKC)β2 and NF-κB in endothelial cells isolated from insulin resistant subjects was markedly enhanced and this finding was associated with blunted eNOS phosphorylation, reduced nitric oxide availability and impaired endothelial function[23].

Research discussed so far implies that reprogramming detrimental pathways in the vascular endothelium may be considered a novel approach to prevent metabolic disease.

ROLE OF OXIDATIVE STRESS IN MALADAPTIVE INSULIN SIGNALING

In obese subjects, exposure to environmental cues triggers many pathological processes including reprogramming of oxidant genes and subsequent redox changes in different tissues[37,38]. The importance of reactive oxygen species (ROS) has been claimed over the last 50 years since these mediators are likely the most pervasive precursors of maladaptive intracellular signalling[39]. Previous seminal work carried out in conditions of hyperglycemia has elegantly demonstrated how ROS accumulation can easily boost activation of detrimental downstream pathways such as advanced glycation end products (AGEs), polyol and hexosamine pathways as well as proinflammatory transcriptional programs initiated by NF-κB[40]. These ROS-sensitive molecular events are being translated to endothelial dysfunction and, hence, micro and macrovascular complications[7]. While the relation between hyperglycemia and oxidative stress has been clearly delineated, the etiologic path linking insulin resistance to ROS generation remains to be deciphered. Recent evidence suggests that oxidative stress may contribute to alter insulin sensitivity in the vascular endothelium. Du *et al*[19] have shown that enhanced oxidation of free fatty acids (FFAs) in aortic endothelial cells increases the production of superoxide by the mitochondrial electron transport chain thus triggering molecular pathways of maladaptive insulin signalling. Indeed, FFAs-induced overproduction of superoxide was able to activate an array of proinflammatory signals while hampering the activity of key anti-atherogenic enzymes such as prostacyclin synthase (PGIS) and

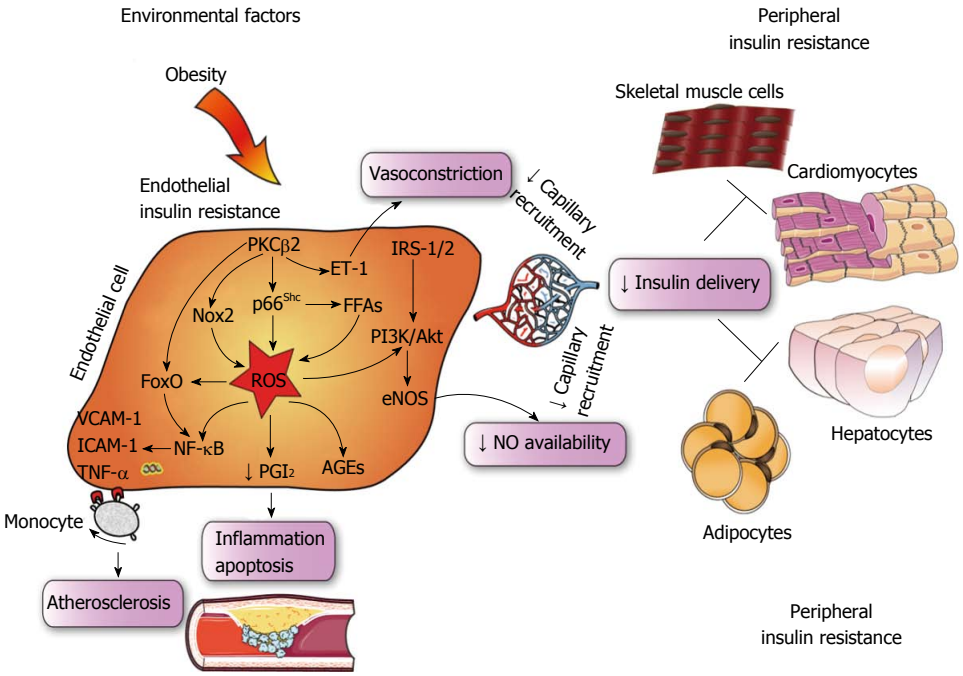

Figure 2 Central role of endothelial insulin resistance. Schematic representing intricate inflammatory and ROS-sensitive pathways responsible for maladaptive insulin signalling in the vascular endothelium. In obese subjects, environmental stimuli favour progressive impairment of endothelial cell function due to ROS accumulation and reduced NO bioavailability, leading to defective capillary recruitment and hampered insulin delivery to hormone sensitive organs. PKC: Protein kinase C; NF-κB: Nuclear factor kappa-B; IRS; Insulin receptor substrate; ROS; Reactive oxygen species; AGEs; Advanced glycation end products; PGI_2; Prostacyclin; VCAM-1; Vascular cell adhesion molecule-1; ICAM-1; Intercellular cell adhesion molecule-1; NO; Nitric oxide; TNF-α: Tumor necrosis factor α; FFA: Free fatty acid; ET-1: Endothelin-1; Nox2: NADPH oxidase 2; FoxO: Forkhead box O; eNOS: Endothelial nitric oxide synthase. NADPH: Nicotinamide adenine dinucleotide phosphate.

eNOS. The importance of ROS in this setting was outlined by experiments in obese mice showing that inactivation of PGIS and eNOS was prevented by inhibition of FFAs release from the adipose tissue[19]. For the first time, this study demonstrated that ROS may actively participate to impaired endothelial signalling thus favouring a pro-atherosclerotic phenotype in subjects with IR. Consistently, in *ApoE*$^{-/-}$ mice with endothelium-specific IR, generation of superoxide was strongly linked to hampered insulin sensitivity, vasorelaxation and atherosclerotic lesions[41]. A further study recently showed that nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase 2 (Nox2) may also be implicated in maladaptive insulin response by inducing a detrimental rearrangement of insulin receptors with subsequent deregulation of downstream kinase effectors, and eNOS dysfunction. Interestingly, obese mice with genetic disruption of Nox2 were protected against ROS accumulation and endothelial IR, suggesting that targeting Nox2 could represent a valuable therapeutic strategy in the context of prediabetes[42] (Figure 2). On such a background, we have recently explored the possibility that the mitochondrial adaptor p66^{Shc} might participate to ROS-driven IR in the endothelium. The adaptor p66^{Shc} is a pivotal modulator of mitochondrial ROS through oxidation of cytochrome c[43,44]. We have previously reported that genetic deletion of p66^{Shc} protects against vascular dysfunction and oxidative stress in diabetic mice[45]. Moreover, p66^{Shc} expression is increased in patients with T2D and correlates with plasma isoprostane levels, a reliable *in vivo* marker of oxidative stress[46]. We have recently found that *in vivo* gene silencing of p66^{Shc} restored endothelial insulin response by affecting the IRS-1/Akt/eNOS pathway[47]. Furthermore, p66^{Shc} knockdown in endothelial cells isolated from obese mice attenuated ROS production, FFAs oxidation and prevented dysregulation of redox-sensitive pathways such as NF-κB, AGE precursor methylglyoxal and PGI2 synthase. Collectively, our results show that p66^{Shc} may contribute to the pathogenesis of IR and increased vascular risk in the context of obesity and T2D. Selective targeting of p66^{Shc} may restore endothelial insulin sensitivity thus preventing adverse cardiometabolic phenotypes. In line with our findings, a recent work has shown that endothelium-specific overexpression of PKCβ2, a key molecular event eliciting ROS production, suppressed insulin-dependent pathways in *APOE*$^{-/-}$ mice[21]. Interestingly, expression of the potent vasoconstrictor endothelin-1 was highly increased in vessels isolated from *APOE*$^{-/-}$ animals with PKCβ2 overexpression (Figure 2). Taken together, these results indicate that p66^{Shc} stands along a detrimental signalling cascade involved in ROS generation, microvascular dysfunction and, hence, peripheral insulin resistance. The clinical relevance of these experimental findings is supported by the notion that oxidative stress is significantly increased in cardiometabolic disorders. A cross-sectional study from the LIPGENE cohort revealed that levels of total nitrite, lipid peroxidation products, hydrogen peroxide (H_2O_2), superoxide dismutase

and glutathione peroxidase activities were all strongly associated with metabolic syndrome traits[48]. Despite such evidence brings enthusiasms toward the possibility of targeting oxidative stress in humans, we are still far from having achieved satisfactory results in term of intermediate endpoints such as endothelial function and atherosclerotic lesions. Indeed, available antioxidants may not fully scavenge cellular ROS since they are unable to target intracellular enzymes involved in redox signalling. This notion is confirmed by the negative results of major trials with oral supplementation of high-dose vitamins[49].

FUTURE PERSPECTIVES

The possibility to target specific machineries in the vascular endothelium may represent an attractive challenge to prevent or delay systemic features of IR favouring adiposity and related comorbidities. There are several examples suggesting that mechanism-based therapeutic approaches might be tested over the next decades. High doses of salicylates have been shown to ameliorate IR and improve glucose tolerance by suppressing NF-κB activity in patients with T2D[50]. Moreover, selective pharmacological inhibition of PKCβ with LY379196 in freshly isolated endothelial cells from T2D patients reduced basal eNOS phosphorylation and improved insulin-mediated eNOS activation[23]. Consistently, the Food and drug administration-approved PKC inhibitor ruboxistaurin ameliorates functional endothelial IR and smooth muscle cell hypersensitivity to insulin in experimental obesity and diabetes[51]. In conditions of IR also the phosphodiesterase 5 inhibitor sildenafil has shown to improve NOS activity in human endothelial cells, thus suggesting the potential therapeutic use of this compound to warrant glucose homeostasis[52]. Furthermore, preclinical work demonstrated that dual ET(A)/ET(B) receptor blockade enhanced endothelium-dependent vasodilatation in individuals with IR, thus restoring vascular recruitment and insulin delivery to peripheral organs[53]. Yet, strategies to drive compounds specifically to the vascular endothelium are still far to be applied in humans. The main problem when it comes to tissue-specific treatment is represented by drug delivery. It is clear that selective rearrangement of maladaptive pathways in the endothelium would provide invaluable to restore microvascular dysfunction and insulin distribution to the liver, adipose tissue and skeletal muscle. An alternative option may be represented by NO donors or administration of eNOS cofactors in order to improve tissue capillary recruitment. Unfortunately this approach has failed many times due to the high oxidative burden in patients with metabolic disease which rapidly inactivates NO, thus favouring accumulation of peroxinitrite ($ONOO^-$), protein nitrosylation and cellular dysfunction. In this respect, an example is provided by a recent clinical trial where oral treatment with eNOS cofactor tetrahydrobiopterin (BH4) has shown limited effectiveness on endothelial function due to systemic oxidation and poor uptake into the vascular wall[54]. These latter results highlight the need for more mechanistic understanding and alternative strategies to counteract pathways triggering eNOS dysfunction in patients with IR. We have recently showed that *in vivo* RNA interference may represent a valid approach to target specific ROS-generating enzymes in the endothelium[55]. Distribution studies showed that *in vivo* delivery of small interfering RNA together with a cationic transfection reagent is able to target the vascular endothelium while sparing surrounding tissues. Indeed, we demonstrated that *in vivo* gene silencing of the adaptor $p66^{Shc}$ restores insulin-dependent vasorelaxation in obese mice, suggesting that blunting endothelial oxidant pathways may be efficient for the maintenance of glucose homeostasis[47]. This work will be instrumental to understand the efficacy and safety of such technology in humans, and whether other candidates may be considered for gene therapy in the setting of endothelial IR.

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MINIREVIEWS

Antioxidant role of zinc in diabetes mellitus

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Abstract

Chronic hyperglycemia statue noticed in diabetes mellitus favors the manifestation of oxidative stress by increasing the production of reactive oxygen species and/or by reducing the antioxidant defense system activity. Zinc plays an important role in antioxidant defense in type 2 diabetic patients by notably acting as a cofactor of the superoxide dismutase enzyme, by modulating the glutathione metabolism and metallothionein expression, by competing with iron and copper in the cell membrane and by inhibiting nicotinamide adenine dinucleotide phosphate-oxidase enzyme. Zinc also improves the oxidative stress in these patients by reducing chronic hyperglycemia. It indeed promotes phosphorylation of insulin receptors by enhancing transport of glucose into cells. However, several studies reveal changes in zinc metabolism in individuals with type 2 diabetes mellitus and controversies remain regarding the effect of zinc supplementation in the improvement of oxidative stress in these patients. Faced with the serious challenge of the metabolic disorders related to oxidative stress in diabetes along with the importance of antioxidant nutrients in the control of this disease, new studies may contribute to improve our understanding of the role played by zinc against oxidative stress and its connection with type 2 diabetes mellitus prognosis. This could serve as a prelude to the development of prevention strategies and treatment of disorders associated with this chronic disease.

Key words: Diabetes mellitus; Type 2; Oxidative stress; Zinc; Superoxide dismutase; Metabolism

Core tip: Type 2 diabetes mellitus is a metabolic disease characterized by the presence of chronic hyperglycemia which favors the manifestation of oxidative stress due to high production of reactive oxygen species and/or induced by the reduction of the antioxidant defense system activity. Zinc plays a relevant role in antioxidant defense in type 2 diabetic patients by acting through different protection mechanisms. Zinc for instance is an essential cofactor for superoxide dismutase enzyme. This mineral also facilitates reduction and neutralization of free radicals. The aim of the present review is to examine the antioxidant role of zinc in type 2 diabetic patients.

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INTRODUCTION

Type 2 diabetes mellitus is a metabolic disease characterized by the presence of glucose intolerance and hyperglycemia. The main pathophysiological effect is to induce a peripheral resistance to insulin action associated with a relative deficiency of secretion of this hormone in response to glucose[1,2].

Chronic hyperglycemia statue in diabetes favors the manifestation of oxidative stress due to high production of reactive oxygen species and/or a decrease of the antioxidant defense system activity linked to lipid peroxidation and oxidative cellular injury themselves resulting in damages in the metabolism of lipids, proteins and DNA and from changes in cells functions[3-5].

Hormonal, biochemical and nutritional disorders present in type 2 diabetic individuals have been subject to researches with the aim of clarifying the mechanisms involved in the pathogenesis of this disease. Regarding both biochemical and nutritional disorders, studies show changes in the mineral metabolism and the activity of antioxidant enzymes such as zinc and superoxide dismutase[6,7].

Zinc plays a relevant role in antioxidant defense in patients with type 2 diabetes mellitus. This mineral may act by different protection mechanisms by notably being an essential cofactor for more than 300 enzymes, such as superoxide dismutase. This mineral also facilitates reduction and neutralization of free radicals[8,9].

Considering changes in zinc metabolism and in superoxide dismutase enzyme activity present in type 2 diabetic patients simultaneously with the importance of these compounds in antioxidant defense, the aim of this review is to examine the antioxidant role of zinc in this type of patients.

RESEARCH

The bibliographical survey was conducted in the data base of Pubmed, Scielo and Lilacs, without limit of year of publication, considering the following inclusion criteria: studies that evaluated the effect of zinc supplementation on markers of oxidative stress in type 2 diabetes mellitus. Articles were selected for their originality and relevance, considering both the accuracy and adequacy of the experimental design, sample size, type of physiological and the performance measures undertaken. Classic and recent works were preferentially used.

The search of literature references was performed using the following keywords: "diabetes mellitus type 2", "zinc", "oxidative stress", "superoxide dismutase". The bibliographical survey included the following types of studies: randomized controlled clinical trials, cohort, case control study, being surveyed in 80 articles of which 36 were used, all of them related with this literature.

ZINC, OXIDATIVE STRESS AND TYPE 2 DIABETES MELLITUS

Recently, several researches have been conducted from the perspective of clarifying the connection between the metabolic and biochemical aspects involved in the pathogenesis of type 2 diabetes mellitus and the metabolism of minerals such as zinc. In this way, studies reveal changes in the metabolism of this nutrient and the results are still limited and controversial[6,10,11]. The Table 1 shows studies that evaluate participation of zinc in diabetes mellitus.

Saharia *et al*[6], Basaki *et al*[7] and Jansen *et al*[17] found reduced plasma concentration of zinc in type 2 diabetic patients. These results are associated with a high amount of the mineral lost in the urine. Such loss is influenced by glycemic control in these patients not compensated neither by an increase in its absorption by intestinal cells nor the concomitant reduction of intestinal excretion. Jayawardena[18] affirms that hyperglycemia interferes in active transport of zinc into the renal tubular cells promoting hyperzincuria.

Agte *et al*[10] found reduced zinc concentrations in the erythrocytes of type 2 diabetic patients compared to the control group, which seems to be related to the high osmotic fragility of erythrocytes resulting in oxidative stress. Percentage of hemolysis of theses cells also showed significant negative correlation with values of glycated hemoglobin.

On the other hand, study of Lima *et al*[11] found increased erythrocyte and plasma concentrations of zinc in type 2 diabetic patients compared to the control group. The authors suggest that plasma values observed are linked to the time of diagnosis of the disease, being higher at the beginning of its manifestation. About the erythrocyte concentration of the mineral, the authors have highlighted the role of metallothionein as a regulator of homeostasis of zinc. The oxidative stress present in type 2 diabetic patients indeed favors both the release of the mineral of this protein and the increase in intracellular zinc content.

Another factor that may favor the increase in zinc concentration in erythrocytes is the fact that oxidative damages induced by type 2 diabetes mellitus seem to be more prominent in erythrocytes, favoring increased concentration of antioxidants as a compensatory mechanism to protect these cells[11,19].

It is appropriate to draw attention to the antioxidant role of zinc. This mineral acts as a cofactor for superoxide dismutase enzyme, regulates the glutathione metabolism and the metallothionein expression, competes with iron and copper in the cell membrane and also inhibits the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase) enzyme[20,21].

Another important point is the action of a group of

Table 1 Studies that evaluate participation of zinc in diabetes mellitus

Ref.	Samples	Results
Aly *et al*[12]	Diabetics rats	Zinc chloride supplementation (5 mg/kg) during one month, helped maintain serum concentration of glucose; preserved hepatic tissue; diminished NO, MDA, and PEPCK and increased SOD, GSH, LDH, pyruvate kinase and hexokinase
Zhang *et al*[13]	Diabetics mice (n = 12) and control groups (n = 14)	Reduced hepatic zinc concentration were found in diabetics mice Zinc deficiency has contributed to increase serum concentrations of ALT and deposit of lipids in the liver of the mice. Furthermore, this deficiency stimulated expression of inflammatory cytokines PAI-1, TNF-α and ICAM-1 and the oxidative damage markers (3-NT e 4-HNE)
Gunasekara *et al*[14]	Diabetics adults: (n = 96) Group A (n = 29): Zinc and multivitamin/mineral complex supplementation Group B (n = 31): Multivitamin/mineral complex supplementation Group C (n = 36): Placebo	Zinc and multivitamin/mineral complex supplementation decreased serum concentrations of HbA1c, fasting glucose, postprandial glucose and serum cholesterol. This supplementation also decreased cholesterol/HDL ratio
Yoshikawa *et al*[15]	Diabetics mice (n = 8)	Bis(aspirinato)Zn complex supplementation improved glycemia, insulin resistance, leptin resistance, hypoadiponectinemia and arterial hypertension
Foster *et al*[16]	Women with type 2 diabetes mellitus (n = 48)	Zinc supplementation (40 mg/d) during 12 wk did not alter HbA1c, insulin and HOMA-IR values. Also, this supplementation did not change metallothionein and zinc transporters gene expression

ALT: Alanine aminotransferase; GSH: Reduced glutathione; HbA1c: Glycosylated haemoglobin; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model of assessment–insulin resistance; LDH: Lactate dehydrogenase; MDA: Malondialdehyde; NO: Nitric oxide; PAI-1: Plasminogen activator inhibitor type 1; PEPCK: Phosphoenol pyruvate carboxykinase; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor-α; 3-NT: 3-nitrotyrosine; 4-HNE: 4-hydroxynonenal; ICAM-1: Intercellular adhesion molecule-1.

antioxidants enzymes called superoxide dismutase, which regulates the detoxification of reactive oxygen species and catalyzes the dismutation of superoxide anion into hydrogen peroxide and oxygen[22,23]. Mammals have three isoforms of this enzyme, but only isoforms 1 (CuZnSOD) and 3 (SOD extracelular) need zinc as a cofactor for its enzymatic activity and to act predominantly and respectively in the intracellular space and extracellular fluids[20,24,25].

A study by Zhu *et al*[22] with diabetic mice shows that the zinc supplementation increased the activity of superoxide dismutase and reduced malondialdehyde concentrations in both serum and pancreas. According to the authors, low levels of zinc in the organism impair the action of the antioxidant defense system. Corroborating previous findings, Li *et al*[3] verified that zinc supplementation increased the activity of superoxide dismutase and decreased lipid peroxidation in the liver of diabetic rats, emphasizing that zinc can protect the liver from oxidative damage.

However, Anderson *et al*[26] did not find any increase in superoxide dismutase activity after supplementation with 30 mg of zinc for 6 mo in type 2 diabetic patients. Roussel *et al*[27] supplemented type 2 diabetic patients with 30 mg of zinc gluconate over 6 mo and noticed a reduction in the production of reactive substances to the thiobarbituric acid, but did not find any increase in the activity of superóxido dismutase.

Action of zinc on glutathione metabolism is significant and as such must be mentioned. Zinc indeed influences the expression of glutamate-cysteine ligase enzyme involved in the synthesis of glutathione, which directly acts on the neutralization of free radicals and indirectly as a cofactor of glutathione peroxidase[20,28].

Karatug *et al*[29] performed zinc sulfate supplementation in diabetic rats and found both an increased concentration of glutathione and a diminution of the lipid peroxidation. The non-enzymatic glycosylation in renal tissue substantiate the relevant antioxidant properties of this mineral in reducing the risk of renal complications associated with type 2 diabetes mellitus.

In terms of zinc action on metallothionein expression, numerous studies indicate that zinc supplementation increases both mRNA levels and the activity of such enzyme in type 2 diabetic individuals. The induction of metalloprotein being one of the explanations for the protective effect of supplementation with zinc in these patients[30,31].

Wang *et al*[32] evaluated the effects of zinc supplementation in diabetic rats and found reduced concentrations of blood glucose and malondialdehyde, as well as an increased expression of metallothionein in the liver. No changes in serum zinc levels were observed, implying a beneficial effect of supplementation in the reduction of oxidative stress.

A study by Özcelik *et al*[31] showed that zinc supplementation increased the concentrations of both metallothionein and zinc, and decreased the lipid peroxidation in renal tissue of diabetic rats, showing the performance of the mineral acting as an antioxidant nutrient and its role in the prevention of renal damages in type 2 diabetes mellitus.

On the other hand, Seet *et al*[33] evaluated the effect of an intake of 240 mg zinc/d in type 2 diabetic

patients with normozincemia and observed that the supplementation with this nutrient did not change the concentration of markers of oxidative stress and vascular function, suggesting that high doses of zinc have no beneficial effect on diabetics who do not have hypozincemia.

Another mechanism that explains the antioxidant role of zinc in type 2 diabetes mellitus, refers to its ability to compete with iron and copper for binding sites on the cell membrane. The iron and copper ions can catalyze the production of lipid peroxides, and the replacement of these metals for zinc in the plasma membrane could prevent lipid peroxidation in diabetic patients[28].

The literature has shown that zinc also regulates the production of free radicals in neuronal cells in type 2 diabetic individuals. This mineral is known for its inhibiting effect on N-methyl-D-aspartate (NMDA) receptors involved in calcium transportation from the extracellular medium to the cytosol. Therefore, in case of zinc deficiency, NMDA receptors activation promotes and increase intracellular calcium concentration. In return, NADPH-oxidase and nitric oxide synthase enzymes are activated, favoring the production of reactive oxygen and nitrogen species[28].

Liu *et al*[34] noticed that zinc supplementation decreased malondialdehyde concentration and stimulated the transcription of metallothionein genes in peripheral nerves of diabetic mice. This suggests that this mineral may improve peripheral neuropathy associated with type 2 diabetes. Such protective effect seems to be mediated by the reduction of oxidative stress.

Zhu *et al*[22] observed that zinc supplementation in diabetic rats caused an increase in glutathione peroxidase enzyme activity as well as a drop in concentrations of malondialdehyde and nitric oxide. The nitric oxide synthase activity in both pancreas and serum of these rats also demonstrates the protective action of zinc against oxidative stress present in type 2 diabetes mellitus. Moreover, the authors observed that the intake of this mineral improved liver functions and also prevent damage to pancreatic tissue induced by the diabetes.

Oxidative stress found in type 2 diabetes is improved by the action of zinc because it also reduces chronic hyperglycemia. It is important to point out that this oligoelement takes part in insulin inventory, secretion and action processes for being a catalytic cofactor for carboxypeptidase H enzyme which catalyzes the conversion from proinsulin (inactive) into insulin (active). Zinc also promotes phosphorylation of insulin receptor by enhancing glucose transport into cells[30,35]. In this perspective, Vashum *et al*[36] demonstrated the role of zinc in reducing chronic hyperglycemia in type 2 diabetes mellitus by considering that patients with higher serum concentration of the mineral improved their insulin sensitivity.

Considering the biochemical and nutritional aspects presents in type 2 diabetes mellitus pathophysiology, important is the participation of zinc in mechanisms involved in this process, for instance, its relevant role as an antioxidant nutrient that improve metabolic control in these patients.

CONCLUSION

Scientific evidences highlighted in this review point out changes in zinc metabolism which contributes to an oxidative stress manifestation in patients with type 2 diabetes mellitus. Several researches have found controversial results regarding zinc supplementation and its positive impact on oxidative stress in these patients. Faced with the serious challenge of the metabolic disorders related to oxidative stress in diabetes in addition to the importance of antioxidant nutrients in the control of this disease, the carrying out of studies may contribute to improve our understanding of the role played by zinc against oxidative stress and its connection with type 2 diabetes mellitus prognosis. This could serve as a prelude to the development of prevention strategies and treatments of disorders associated with this chronic disease.

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MINIREVIEWS

Effects of maternal diabetes on trophoblast cells

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Abstract

Diabetes mellitus (DM) is a health condition characterized by hyperglycemia over a prolonged period. There are three main types of DM: DM type 1 (DM1), DM2 and gestational DM (GDM). Maternal diabetes, which includes the occurrence of DM1 and DM2 during pregnancy or GDM, increases the occurrence of gesttional complications and adverse fetal outcomes. The hyperglycemic intrauterine environment affects not only the fetus but also the placental development and function in humans and experimental rodents. The underlying mechanisms are still unclear, but some evidence indicates alterations in trophoblast proliferation, apoptosis and cell cycle control in diabetes. A proper coordination of trophoblast proliferation, differentiation and invasion is required for placental development. Initially, increased expression of proliferative markers in junctional and labyrinth zones of rat placentas and villous cytotrophoblast, syncytiotrophoblast, stromal cells and fetal endothelial cells in human placentas is reported among diabetics. Moreover, reduced apoptotic index and expression of some apoptotic genes are described in placentas of GDM women. In addition, cell cycle regulators including cyclins and cyclin-dependent kinase inhibitors seem to be affected by the hyperglycemic environment. More studies are necessary to check the balance between proliferation, apoptosis and differentiation in trophoblast cells during maternal diabetes.

Key words: Diabetes; Placenta; Proliferation; Apoptosis; Differentiation; Trophoblast

Core tip: This review article focuses on current knowledge about the effects of diabetes on trophoblast function such as proliferation, apoptosis and cell cycle control during placental development in human and rodent animal models. It also briefly discusses some placental pathological findings as a consequence of altered metabolic environment during diabetes.

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. DM is a

public health problem worldwide which is increasing mainly because of the high prevalence of obesity and sedentarism. In 2000, a previous study including all age groups, estimated the global prevalence of DM at 2.8% expecting to rise up to 4.4% by 2030[2].

There are three main types of DM: DM type 1 (DM1), DM2 and gestational DM (GDM). About 10% of all diabetes cases are DM1: an autoimmune disease in which an absolute deficiency of insulin resulting from the pancreatic β cells destruction occurs. This destruction can be caused by an autoimmune process (type 1a) or it can be idiopathic (type 1b). The former represents the vast majority of DM1 cases and usually manifests itself before 30 years of age, being more common in individuals of European origin. On the other hand, the vast majority of cases of diabetes existing in the population is DM2, characterized by peripheral resistance to insulin action and relative insulin deficiency[3].

In general, the overall prevalence of DM has increased in recent years due to the aging population and lifestyle changes. Parallel to this trend, the number of pregnant women with pre-existing diabetes (DM1 and DM2) has been increasing worldwide, and in some countries, this numbers are even doubling[4,5]. This increase is closely related to the high number of diabetic patients in reproductive age as well as to advances in clinical care available for pregnant diabetic women. Until the mid-20th century, DM1 women either had not reached the child-bearing age or had had serious health problems that contraindicated pregnancy. The discovery and commercial availability of insulin changed this scenario and a better glycemic control for women with diabetes led to a considerable reduction in the rates of maternal and fetal complications[6].

GDM is the principal metabolic disorder that occurs during pregnancy and can affect 3% to 30% of pregnant women depending on the population studied and the diagnostic criteria used[7]. It is also defined as any degree of glucose intolerance of variable severity which arises or is diagnosed during pregnancy. Besides, it is characterized by the maternal pancreas inability to meet the growing demand of insulin as from the second trimester of gestation[8].

Maternal diabetes, which includes the occurrence of either DM1 or DM2 in pregnancy and GDM, creates an unfavorable environment for embryonic and fetoplacental development. Despite the several developmental and morphological differences between rodents and women placenta, the alterations induced by maternal diabetes are similar in diabetic patients and diabetic experimental models[9]. Several works have been published addressing the impact of diabetes on placental weight and growth and materno-placental oxygen supply[10,11].

As it is known, the placenta is a highly specialized organ in the interface between maternal and fetal circulation with fundamental functions for pregnancy. It permits the fetus anchorage to the uterus, O_2/CO_2 exchange, the nutrition and the waste products removal during embryonic and fetal development[12]. Also, it acts as a protective barrier against xenobiotics and releases a variety of steroids, hormones and cytokines[13]. Therefore, placental dysfunction has deleterious effects on adequate pregnancy support. Among placental cells, trophoblasts permit the embryo implantation and nutrition in the early pregnancy and thereafter they will contribute considerably to the development and function of the placenta. The underlying mechanisms of placental pathology during diabetes are still unclear, but some evidence indicates changes in trophoblast proliferation, apoptosis and cell cycle control.

MATERNAL DIABETES EFFECTS ON TROPHOBLAST PROLIFERATION

A proper coordination of trophoblast proliferation, differentiation and invasion is required for placental development. Initially, cell proliferation should be tightly controlled for proper tissue growth and differentiation. Throughout gestation, growth factors such as epidermal growth factor, vascular endothelial growth factor, platelet-derived growth factor, placental growth factor, colony stimulating factor 1, insulin-like growth factor Ⅰ (IGF-Ⅰ), or IGF-Ⅱ are abundantly secreted from diverse cell types of the fetal-maternal interface and have been to promote proliferation, adhesion and/or invasion[14-17].

In diabetes, the enlargement of the junctional zone (JZ) in the diabetic rat placenta is described as the increased number of glycogen and giant trophoblast cells[10]. Indeed, our previous stereological study confirmed a greater volume of spongiotrophoblast/glycogen cells in diabetic rats compared with controls[18]. Also, other works have shown changes in the size and organization of the spongiotrophoblast and glycogen cells in rat models of diabetes[10,19,20], suggesting the JZ as the placental compartment most sensitive to the diabetic condition[18,21].

Studies on the effects of maternal diabetes on placental development have solely reported increased expression of proliferative markers in the JZ and labyrinth zone (LZ) of rat placentas[22,23]. Zorn *et al*[23] showed that diabetes promotes an increased cell proliferation rate, detected by Ki67 immunostain, especially of spongiotrophoblast cells at gestational day 14 (gd 14), and of labyrinth cells, spongiotrophoblast and trophoblast giant cells at gd 17. Also, intense proliferating cell nuclear antigen (PCNA) immunostain in labyrinth and spongiotrophoblast cells on 17 d and in spongiotrophoblast and trophoblast giant cells on 21 d of pregnancy were noted in diabetic groups than in control groups, indicating deregulated cell proliferation in hyperglycemic condition which may explain the placentomegaly observed in diabetic animals at gd 20[22].

In humans, the placentas of GDM pregnancies are heavier than those of control patients[24,25] and the

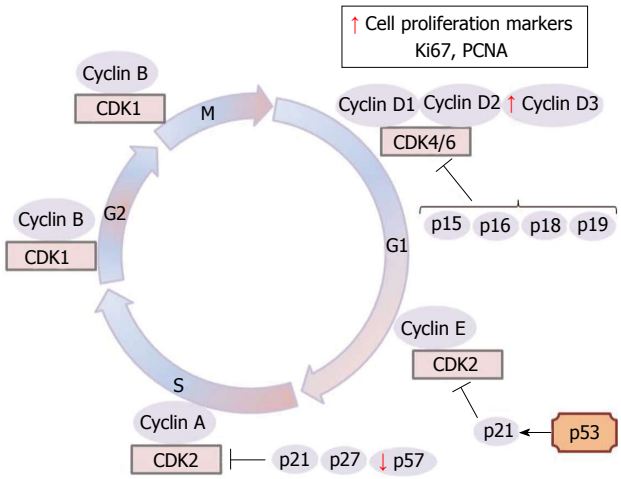

Figure 1 Schematic representation of eukaryotic cell cycle and key regulatory proteins which allow the transition from one cell cycle phase to another. CDKs inhibitors, such as p15, p16, p18, p19 (INK4 group), p21, p27 and p57 (CIP/KIP class) and proliferative markers are also showed. The arrows in red indicate increased or decreased expression of some trophoblast key regulatory proteins, CDKs inhibitors and proliferative markers in maternal diabetes. G1: Gap 1 phase; S: synthesis phase; G2: Gap 2 phase; M: Mitosis; CDKs: Cyclin-dependent kinases; PCNA: Proliferating cell nuclear antigen; p53: Tumor protein p53.

mechanism accounting for this increased placental mass is unknown. Enlargement of the capillary surface area with capillary proliferation and penetration of newly formed vessels have also been shown in DM[26]. Villous immaturity is present in 60% of diabetic placentas and is characterized by an increase in the number of mature and immature intermediate villi[27]. At the same time, a higher number of villous cytotrophoblast, villous stromal fibroblasts, macrophages, endothelial cells and syncytiotrophoblast nuclei in diabetes were noted[28-30]. As in diabetic animals, it was also reported, increased proliferative activity in villous cytotrophoblasts compared to normal placentas[27,30,31]. Leach *et al*[32] reported higher PCNA immunoreactivity in endothelial cells of diabetic placentas. In addition to PCNA staining, Ki67 and cyclin D3 (Figure 1) staining of villous cytotrophoblast, syncytiotrophoblast, villous stromal cells and fetal endothelial cells increased in diabetic placentas compared to controls[33].

TROPHOBLAST APOPTOSIS IN MATERNAL DIABETES

The occurrence of apoptosis is shown during normal placental development and in morbid states[34-44]. In the normal human placenta, the presence of apoptotic cells could be associated with many events like trophoblast attachment and invasion[45,46], spiral artery remodelling[47,48], trophoblast differentiation[47-49] and labor[34,50]. However, the rates of placental apoptosis, even in normal human gestations, are still controversial. A predominance of apoptosis during early gestation, diminishing after the second trimester[41,51] and a significant increase in apoptosis as pregnancy progresses were reported[34,38,44].

Some works showed reduced apoptotic index, by TUNEL assay, in placentas from GDM[52,53] and DM1[36] patients compared to control placentas. Increased placental weight in GDM was associated with significantly reduced trophoblast apoptosis[52,53]. On the other hand, some authors reported increased apoptosis of villous cytotrophoblasts and syncytiotrophoblast nuclei in diabetic placentas *in vivo*[54] or *in vitro*[55]. Some technical differences such as the mode of delivery, the placental sampling or differences in gestational age could be responsible for study discrepancies[52]. Therefore, more studies are necessary to check the balance between proliferation and apoptosis in human diabetic placentas.

In the placenta, conditions like low oxygen and oxidative stress could induce to apoptosis that may be initiated by intrinsic or extrinsic pathways resulting in the activation of central apoptotic effectors, the caspases[56]. The extrinsic pathway involves members of the tumor necrosis factor (TNF) death receptor family, whose ligands include TNF-α, Fas ligand, Apo3 ligand (Apo3L) and Apo2L[44]. The activation of death receptors results in receptor aggregation and recruitment of adaptors molecules Fas-associated death domain or TNF-R-associated death domain[57]. As a consequence, procaspase-8 and procaspase-10 are recruited and become activated, initiating the cleavage of downstream effector caspases[58-60]. Caspase-8 could occasionally cleavage BH3-interacting domain death agonist that activates the intrinsic pathway[61].

The intrinsic pathway could be initiated by toxins, radiation, DNA damage and reactive oxygen species that lead to cellular stress and deficiency of growth factors[62]. This pathway induces mitochondrial membrane permeability modification by changes in the association of pro- and anti-apoptotic B-cell lymphoma 2 (BCL2) proteins[63]. The outer membrane permeabilization leads to the release of cytochrome c from the mitochondrial intermembrane space to the cytosol[64]. Them, the cytochrome c binds to the protease activating factor-1 forming the apoptosome[65]. The apoptosome cleaves procaspase-9, activating the terminal pathway of apoptosis. Additionally, the Smac (Second mitochondria-derived activator of caspase) is released from the mitochondria and eliminates the inhibitory effect of inhibitor of apoptosis proteins on caspases[66,67]. Both pathways culminate in a terminal pathway involving the cleavage and activation of caspase-3, -6, and -7, initiating cell destruction by activating DNAses and cleaving DNA repair enzymes such as poly (ADP-ribose) polymerase (PARP)[68,69].

Concerning proteins associated with cell death pathways reduced expression of BCL2 has been reported in placentas from diabetic patients compared to normoglycemic women[54]. Furthermore reduced

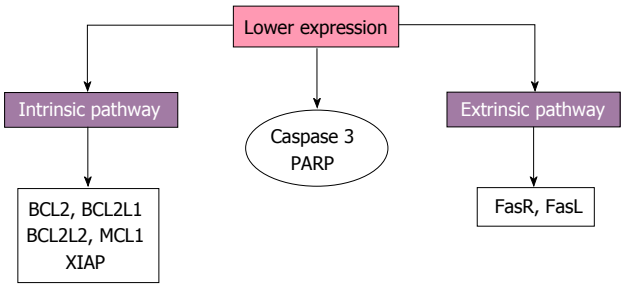

Figure 2 Schematic representation of trophoblast apoptosis findings in maternal diabetes. Reduced expression of apoptotic components from both intrinsic and extrinsic pathways, caspase-3 and poly (ADP-ribose) polymerase (PARP) are reported by some works. FasR: Fas receptor; FasL: Fas ligand; MCL1: Myeloid cell leukemia 1; BCL2: B-cell lymphoma 2; BCL2L1: BCL2-like 1; XIAP: X-linked inhibitor of apoptosis; BCL2L2: BCL2-like 2.

gene expression of *BCL2*, *BCL2L1*, *BCL2L2*, myeloid cell leukemia 1 and X-linked inhibitor of apoptosis and reduced protein expression of the Fas receptor (FasR), FasL, caspase-3 and its PARP has been reported, indicating extrinsic and intrinsic pathways downregulation in placentas with GDM[50,53] (Figure 2).

According to Rudge *et al*[70] (2012), severe diabetes in mice decrease placental TUNEL index from day 18 to 21 of pregnancy, at the same time that small for pregnancy age fetus and increased placental weight are also found. A GDM animal model (*db/+* mice), when treated with TNF-α at gd 11.5, a pro-apoptotic peptide, there was an increased number of apoptotic cells, detected by cleaved caspase-3 immunostaining, in both labyrinth and trombospongium, at gd 18.5[71]. Unfortunately, little is known about the cleaved caspase-3 placental activity from other's models of animal diabetes or even human diabetic pregnancies.

CELL CYCLE CONTROL OF TROPHOBLAST IN MATERNAL DIABETES

The appropriate development of an organism depends on the balance between cell cycle exit and the differentiation process in all tissues. The cell cycle exit is required for terminal differentiation of many cell types and cell cycle progression is regulated by a series of cyclin-dependent kinases (CDKs) that consist of catalytic subunits, designated CDKs, and activating subunits, designated cyclins[72,73]. The activation and inactivation of different cyclin-CDKs at adequate moments is necessary for precise progression into the cell cycle[74,75].

Although placental growth is essentially a result of the coordination of trophoblast proliferation and differentiation, there is little information about the mitotic regulators that provide the synchronization of trophoblast proliferation and differentiation[76]. In the rat term placenta, cyclin D1 and cyclin D3 are expressed in placental fetal cells, whereas the G1/S cyclin E are present only in the spongiotrophoblast and labyrinthine trophoblast cells[77]. The D-type cyclins serve as growth factor sensors that integrate extracellular signals with the cell cycle machinery. Together with their partner kinases, CDK4 and CDK6, they operate in early-to-mid G1 to promote progression through the G1-S restriction point[73]. The nuclei expression of cyclins D1 and D3 in mesenchymal and labyrinthine trophoblast cells could infer a role in the differentiated state maintenance in late gestation[77]. In the human placenta, cyclins D1 and D3 have been observed in endothelial cells[78]. However, cyclin activity during diabetes has been little explored in placental cells. Only one work reported cyclin D3 staining intensities significantly increasing in villous parts, basal plates and chorionic plate of a diabetic group when compared to control placentas[33]; perhaps prominent cyclin D expression could contribute to the increased cell proliferation observed in diabetic placentas (Figure 1).

There are two families of CDK inhibitors that act to inhibit cell cycle progression. The INK4 family (p15ink4b, p16ink4a, p18ink4c, p19ink4d) inhibits the CDK4, CDK6 and cyclin D activities in the G1 phase and G1/S transition of cell cycle. In turn, the CIP/KIP family (p21waf/cip1, p27kip1 and p57kip2) inhibits the cell cycle at many checkpoints by acting on multiple cyclin-CDK complexes[74,75].

The altered metabolic environment in maternal diabetes could affect the expression of genes that control the cell cycle events as was observed for reduced p57 expression in diabetic rat placentas on days 17 and 21 of pregnancy[79] (Figure 1). In the normal rat placenta, immunostaining intensities of cell cycle inhibitors p27, and p57 were observed to be higher in the JZ compared to the LZ close to term[77]. Accordingly, since p57 is a cell cycle inhibitor and tumor suppressor, lack of p57 activity can lead to a loss of cell cycle control and hyperproliferation[33]. Therefore, less p57 expression may explain the reason why diabetic placentas are heavier and bigger[33].

In fact, abnormal placental development is present in p27 and p57 knockout mice[80]. The LZ is less vascularized and contains more trophoblasts than those from wild type placentas. Moreover, Takahashi *et al*[81] demonstrated that placentomegaly was observed in p57 deficient mice in which the numbers of placental cells in the LZ and spongiotrophoblasts were twice the number of those of the wild type. In addition to placentomegaly, deregulation of the cell cycle can result in the development or progression of some trophoblastic diseases like preeclampsia that can occur in diabetic women. In humans, p57 staining index of villous parts decreases significantly in diabetes[33]. Another CIP/KIP family member, p27, has no difference in staining intensity in the villous part between diabetic and control groups of human patients, but has different staining patterns in different placental cell types[33]. Some studies indicated that p27 and p57 have different functions in human placental development[82,83].

In summary, this article reviewed the current knowledge about the effects of hyperglycemia on trophoblast proliferation, apoptosis and cell cycle control

during pregnancy. More detailed studies are required to check the balance between proliferation, apoptosis and differentiation in trophoblast cells during maternal diabetes.

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MINIREVIEWS

Impact of glucose level on morbidity and mortality in elderly with diabetes and pre-diabetes

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Abstract

The prevalence of type 2 diabetes mellitus (DM) increases with age and reaches 25% in those older than age 65 years. Pre-diabetes status is also very common in the elderly, and is present in about half of those age 75 years and older. Many physicians care for elderly patients with diabetes and pre-diabetes, dealing with the challenge of controlling glucose levels and improving health with minimal adverse events. Over the last decade, research on diabetes among the elderly population has proliferated, adding new information on this topic. This review summarizes the updated medical literature on diabetes and pre-diabetes in the elderly, including the significance of pre-diabetic conditions, new-onset DM in the elderly and long-standing DM. The role of therapeutic intervention and the level of glycemic control for this population are discussed in particular.

Key words: Diabetes mellitus; Elderly; Old age; Pre-diabetes; Glycemic control

Core tip: The prevalence of diabetes mellitus (DM) and pre-diabetes in old age is very high. However, clinical guidelines do not provide complete information to the clinician managing patients with these conditions. Pre-diabetes status in the elderly increases the risk for DM, but probably does not increase the risk of cardiovascular morbidity and mortality. The role of therapeutic interventions in elderly patients with pre-diabetes is not yet proven. New-onset DM in older age is associated with better glycemic control and better prognosis compared to long standing DM in this population. Nevertheless, higher glucose levels in elderly with new-onset DM are associated with increased all-cause mortality. The benefits of tight glycemic control in elderly with long standing DM are doubtful and may cause more harm than good. To conclude, more research in this field is needed. Currently, the clinical approach for DM and pre-diabetes in the elderly should be tailored to meet individual needs.

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INTRODUCTION

The concept of individualized treatment for type 2 diabetes mellitus (DM) is becoming established and replaces previous recommendations for tight glucose control for all diabetic patients. One of the main criteria in constructing personalized care for the patient is the chronologic and biologic age.

The incidence and prevalence of DM increase with age (www.cdc.gov/diabetes/statistics). Pre-diabetes states, including impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and elevated HbA1c are even more prevalent among the elderly[1-3].

However, the clinical impact of glucose levels on microvascular and macrovascular complications, and mortality is not well established. The commonly used clinical guidelines do not provide separate recommendations for elderly individuals with pre-diabetes, and do not differentiate between elderly with long-standing or new-onset DM[4].

This review summarizes the data in the literature regarding the effect of glycemia in different stages on morbidity and mortality in the elderly population. It will address the aspects of the clinical impact of glucose levels in pre-diabetes, diabetes that was first diagnosed in old age and long-standing diabetes separately.

PRE-DIABETES IN THE ELDERLY

According to American Diabetes Association (ADA) guidelines, pre-diabetes may be diagnosed as IFG, IGT and/or by elevated HbA1c values of 5.7%-6.4%[4]. The rates of pre-diabetes states, including IFG and IGT, are very common in the general population, and increase with age[1-3].

It is well-established that pre-diabetes states are a significant risk factor for developing type 2 DM, as well as for diabetic complications and mortality in younger adults[5,6]. It is also well known that lifestyle changes, including loss of at least 7% of body weight and $\geqslant$ 150 min/wk of activity, delay or even prevent development of DM, and may potentially reduce its complications among persons with pre-diabetes[7]. Thus, pre-diabetic patients are an important target group for primary prevention interventions.

Understanding the clinical impact of pre-diabetes in older adults is very important, as the prevalence of pre-diabetes increases with age and reaches about 50% in those age 75 years and older[2]. Moreover, lifestyle interventions are more successful in decreasing hyperglycemia in the elderly than in younger adults. This was demonstrated in the Diabetes Prevention Program (DPP) trial, which included subjects with combinations of IGT and IFG, considered to be at high risk for developing DM. The oldest age group, 60-85 years at enrollment, had the greatest benefit from the program, both in terms of weight loss and decreased incidence of DM over time[7,8].

Yet, there are some important, unanswered questions for the clinician. First, what is the clinical impact of pre-diabetes state in the elderly? Second, do glucose lowering interventions improve morbidity and mortality in this population?

The answer to the first question, regarding the clinical significance of pre-diabetes in elderly subjects, is based on a small number of studies (Table 1). A prospective, observational study followed 1466 elderly subjects with IGT and compared their mortality rate to subjects with normal glucose levels and overt diabetes. The age of enrolled participants was 55-74 years and median follow-up was 8.8 years. Mortality rates were almost equal in the pre-diabetes and normal glucose groups. Nevertheless, within the non-diabetic range (*i.e.*, normal and pre-diabetic glucose levels), a J-shaped association was demonstrated between glycemia and all-cause mortality, even after adjustment for multiple risk factors. The lowest mortality rates were documented in subjects with fasting plasma glucose 88-93 mg/dL and HbA1c 5.4%-5.5%. Participants with glucose levels at the upper pre-diabetes range had a higher mortality rate[9].

On the other hand, a recent prospective cohort study of 8365 older subjects, 50-74 years old, revealed that the increased cardiovascular risk in pre-diabetes (defined as IFG or HbA1c 5.7%-6.4%) can mainly be explained by other concurrent cardiovascular risk factors and not by the hyperglycemia itself[10]. Similar results arose from the Cardiovascular Health Study of 4602 community-dwelling elderly participants, 65 years-of-age and older. This study found no evidence that pre-diabetes is an independent risk factor for a variety of cardiovascular outcomes, including heart failure, myocardial infarction, stroke and all-cause mortality[11]. Pre-diabetes increased the risk of developing DM, but the absolute rate was low and not related to increased cardiovascular risk.

An interesting pooled analysis examined the age specific effect of different metabolic risk factors on cardiovascular diseases. The analysis for plasma glucose included 372000 participants in 116 cohorts. The authors calculated the impact of mildly elevated glucose on the relative risk of ischemic heart disease and stroke. They concluded that the proportional effect of elevated fasting glucose declined with age[12].

For the second question - whether glucose lowering intervention would improve morbidity and mortality outcomes in elderly population - there is still no satisfactory answer. The DPP study, mentioned above, found a better response of elderly subjects to lifestyle interventions, in particular weight loss and DM prevention. However, clinical outcome data were not reported[7,8]. The effect of lifestyle

Table 1 Studies comparing cardiovascular morbidity and mortality in elderly with pre-diabetes and normal glucose tolerance

Ref.	No. of participants	Age at inclusion	Length of follow up (yr)	Population	Results
Kowall *et al*[9]	1466	55-74	8.8 (median)	German	Mortality rates were almost equal in the pre-diabetes and NGT groups
Schöttker *et al*[10]	8365	50-74	7.9 (median)	German	Major CV event[1] rates were almost equal in the pre-diabetes and NGT groups
Deedwania *et al*[11]	4602	⩾ 65	13 (median)	United States: 87% Caucasians, 13% African American	Major CV event[2] rates were almost equal in the pre-diabetes and NGT groups

[1]Major cardiovascular events including non-fatal stroke, non-fatal MI and cardiovascular mortality; [2]Major cardiovascular events including heart failure, MI, angina pectoris, stroke and all-cause mortality. CV: Cardiovascular; NGT: Normal glucose tolerance; MI: Myocardial infarction.

interventions on mortality and cardiovascular disease in pre-diabetes patients is questionable, even among younger adults[13].

In summary, existing data regarding the clinical impact of pre-diabetes on morbidity and mortality among elderly individuals are limited and study results are conflicting. There is an association between pre-diabetes and mortality, which increases with higher glucose levels within the pre-diabetic range. However, it is unclear whether pre-diabetes is a marker of poor metabolic condition or an independent risk factor. There is almost no information regarding the influence of lifestyle or medical interventions on morbidity and mortality in this population.

The ADA does not have separate clinical guidelines for pre-diabetes in the elderly population[4]. The 2012 consensus report of the ADA and the American Geriatrics Society provided vague advice on this issue. They recommended screening for pre-diabetes in elderly patients who are likely to benefit from diagnosis of pre-diabetes and from subsequent intervention. Similarly, they recommended lifestyle interventions for elderly individuals with pre-diabetes who are able to participate and are likely to benefit from DM prevention[14].

NEW-ONSET DIABETES IN THE ELDERLY

The pathophysiology of the appearance of DM in the elderly is a combination of age-related changes in carbohydrate metabolism, pancreatic endocrine dysfunction and adverse lifestyle factors[15,16]. The epidemiology of incident DM in relation to population age is interesting. The incidence of new-onset DM increases with age until age 65 years, after which both incidence and prevalence of DM seem to level off (www.cdc.gov/diabetes/statistics).

The natural history of new-onset DM in the elderly seems to have a benign course in comparison to that of long-standing DM. Interesting information comes up from a study of centenarian subjects, ages 100-109, compared to elderly subjects aged 65-84 years. The centenarians had relatively low prevalence of DM (7.64%), and almost exclusively had senile DM, that is DM diagnosed after 65 years of age. The authors suggest that long-standing DM is not compatible with extreme longevity, while senile DM does not change the clinical outcomes significantly[17]. New onset DM in older age is associated with better glycemic control[18] and with less frequent microvascular complications compared to long standing DM[19]. Data from the National Health and Nutrition Examination Survey database found that although elderly with new-onset DM were 5 years older in average, they had much lower prevalence of retinopathy and a similar burden of macrovascular disease compared with long-standing DM. The difference in retinopathy rate may reflect the difference in DM duration between the two groups[20].

The few studies that compared elderly subjects with new-onset DM to non-diabetic patients demonstrated short term elevation in all-cause and cardiovascular mortality and long term elevation of microvascular and macrovascular complications[21-25]. None of these studies checked the association of glucose levels with mortality or diabetic complications and did not consider the influence of other cardiovascular risk factors on morbidity and mortality.

A large, observational study focused on the association between glycemic control and mortality in elderly patients with new-onset DM. This study followed almost 3000 elderly patients with new-onset DM for 7 years. A J-shaped relationship was found between HbA1c level and mortality rate. A HbA1c level above 7.5% was associated with significantly higher all-cause mortality, while the lowest mortality rate was found in subjects with HbA1c levels from 6.5% to 6.99%. This association remained statistically significant after adjustment for other conventional cardiovascular risk factors[26].

In summary, the existing data suggest that new-onset DM in the elderly is associated with better glycemic control and better prognosis compared to long-standing DM in this population. However, when compared to elderly people with normal glucose levels, the new-onset DM patients have higher rates of morbidity and mortality (Table 2). There is some evidence that higher glucose levels within the diabetic range are associated with increased mortality.

The ADA guidelines do not deal separately with new-onset DM in elderly individuals, but mention the

Table 2 Studies comparing cardiovascular morbidity and mortality in elderly subjects with new-onset diabetes mellitus and subjects with normal glucose tolerance and long-standing diabetes mellitus

Ref.	No. of participants	Glycemic status	Age at inclusion(yr)	Length of follow up (yr)	Population	Results
Wang *et al*[19]	155	New-onset DM and long-standing DM	⩾ 65	-	China	Microvascular complication rate was higher in long-standing DM
Selvin *et al*[20]	2809	New-onset DM and long-standing DM	⩾ 65	-	United States	Microvascular complication rate was higher in long-standing DM
Smith *et al*[21]	1119	NGT and new-onset DM	⩾ 65	5.9 (median)	United States	Mortality rate was higher in new-onset DM
Bethel *et al*[22]	59335	NGT and new-onset DM	⩾ 65	10 (median)	United States	Microvascular and macrovascular complication rates were higher in new-onset-DM
Panzram *et al*[23]	2381	New-onset DM	All	10 (median)	German	Mortality rate was related to age of onset of DM and was higher in men
Croxson *et al*[24]	861	NGT, IGT, new-onset DM and long-standing DM	65-85	4.5 (median)	United Kingdom	New onset DM was associated with increased mortality
Tan *et al*[25]	10782	NGT and new-onset DM	⩾ 65	4.6 (median)	Scotland	New onset DM was associated with increased mortality in females
Twito *et al*[26]	2994	New-onset DM	⩾ 65	5.5 (mean)	Israel	Mortality rates in new-onset DM were associated with HbA1c levels

NGT: Normal glucose tolerance; IGT: Impaired glucose tolerance; DM: Diabetes mellitus.

duration of the disease as a parameter that should be considered when choosing HbA1c target levels[4]. Vacante *et al*[27] suggested combining the current age of the patient with the duration of DM.

LONG-STANDING DIABETES IN THE ELDERLY

As mentioned above, long-standing DM in the elderly has higher morbidity rates compared to new-onset DM. Therefore, the question is whether good glycemic control in elderly people with long-standing DM will influence the course of the disease.

In young and middle-aged diabetic patients, the role of tight glycemic control is crucial, as was proven in the Diabetes Control and Complications Study (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) in type 1 and type 2 DM, respectively[28-30]. These two studies confirmed the benefit of intensive glycemic control in reducing microvascular and macrovascular complications[31]. Nonetheless, these studies included only new-onset diabetic patients and excluded patients ages 65 years and above at the time of enrollment.

Almost 10 years after the publication of the UKPDS study, and 15 years after the DCCT study, 3 large randomized controlled trials examined the influence of intensive glycemic control on microvascular and macrovascular complications in older subjects with long standing DM. The Action to Control Cardiovascular Risk in Diabetes trial[32] enrolled diabetic patients (mean age 62.2 ± 6.8 years) 10 years after diagnosis, 35% with previous cardiovascular disease. This trial was terminated after 3 years because of excessive deaths in the intensive glycemic control arm. The Action in Diabetes and Vascular Disease trial[33], which had also enrolled people with advanced disease (mean age 66 ± 6 years, average duration of diabetes 8 years, 32% with previous major macrovascular disease), showed no significant effect of tight glycemic control on major macrovascular events or death from any cause, but there was significant reduction in nephropathy incidence and as a result, reduction in the incidence of combined microvascular and macrovascular events. This reduction was proven only for patients younger than 65 years, according to a sub-group analysis published as part of the trial. The Veterans Affairs Diabetes Trial trial[34] enrolled similar diabetic patients (mean age 60.5 ± 9 years, 11.5 years after diagnosis, 41% with major macrovascular disease) and also showed no significant effect of tight glycemic control on major macrovascular events or death from any cause. As one would expect, adverse events related to intensive glycemic control, such as hypoglycemia, were more common in the elderly[35].

Few studies are directed to glycemic control in the old age. For example, the retrospective diabetes and aging study[36] enrolled participants above 60 years of age (38% between 70-79 years and 15% age 80 or older); 57% had diabetes for more than 4 years at enrollment. There was a U-shaped relationship between

Table 3 Studies on the role of tight glycemic control in long-standing diabetes mellitus

Study	No. participants	Years since diagnosis	Mean age at enrollment (yr)	Length of follow up (yr)	Population	Results
ACCORD trial[32]	10251	10 (median)	62.2 ± 6.8	3.4 (median)	United States and Canada	Terminated after 3.5 yr; excessive deaths in the intensive glycemic control arm
ADVANCE trial[33]	11140	8 (mean)	66 ± 6	5 (median)	Asia, Australasia, Europe, and North America (20 countries)	No significant effect of tight glycemic control on major macrovascular events or death; significant reduction in nephropathy incidence
VADT trial[34]	1791	11.5 (mean)	60.5 ± 9	5.6 (median)	United States military veterans; 97% males	No significant effect of tight glycemic control on major macrovascular events or death
Diabetes and aging study[36]	71092	8.3 (mean)	71 ± 7.4	3.1 (mean)	California, United States	U-shape relationship between mortality and HbA1c, with the lowest mortality rate at HbA1c 6%-8%

ACCORD: Action to Control Cardiovascular Risk in Diabetes; ADVANCE: Action in Diabetes and Vascular Disease; VADT: Veterans Affairs Diabetes Trial.

mortality and HbA1c, with the lowest mortality rate at HbA1c 6%-8%, in all age-groups (Table 3).

The benefit of tight glycemic control for prevention of microvascular complications is not immediate. As proven in the UKPDS studies, the difference in outcome between the tight glycemic control group and the control group only appeared 9 years after randomization[14,31]. In other words, for patients with life expectancy of 7 years or less, the benefit in this area is doubtful.

The complexities of diabetes care in old age, with benefits alongside potentially serious adverse events, led researchers to quality-adjusted life year (QALY) trials. Vijan *et al*[37] compared the QALY gained with intensive glycemic control versus moderate glycemic control, in different age-groups. They concluded that older patients, age 75 years and older, experience smaller benefit from glycemic control compared to younger patients, and their expected gain in QALYs for a 1-point change in HbA1c was minimal, even with the favorable assumption that the benefits of glycemic control extend to the elderly.

The results of the aforementioned studies resulted in changes in the clinical guidelines regarding treatment goals for elderly with long-standing DM. However, the guidelines offer general instructions and leave a large margin for clinical judgment.

The 2014 ADA guidelines[4] recommend a standard glycemic goal of HbA1c below 7% for adults, and a less stringent goal, such as < 8% for patients with a more complex status, which is defined according to disease duration, life expectancy, important comorbidities, risk for adverse events and existing vascular complications. The International Association of Gerontology and Geriatrics and the European Diabetes Working Party for Older People published similar recommendations[38].

The consensus report of the ADA and the American Geriatrics Society from 2012[14], offered 3 levels of glycemic control for the old patient: HbA1c < 7.5% for healthy patients, < 8% for patients with intermediate health status (multiple chronic illnesses, 2+ instrumental impairments or mild cognitive impairment), and < 8.5% for patients with poor health status (end-stage chronic illnesses or moderate-to-severe cognitive impairment or 2+ activities of daily living dependencies). The International Diabetes Federation 2013 guidelines[39], suggest a similar categorization. They also emphasize that for end-of-life situations, the goal should be merely to avoid symptomatic hyperglycemia (Table 4).

CONCLUSION

This review summarizes the current evidence about glycemic control in the elderly. Similar to young and middle-aged adults with DM, it seems that the elderly patient with diabetes has higher risks for morbidity and mortality compared to a non-diabetic person of the same age. Even so, new-onset DM is less severe in elderly patients compared to young adults and easier to control. The impact of pre-diabetes state on morbidity and mortality risk in the elderly is doubtful, and the role of screening and treatment in these patients is questionable. Finally, the importance of tight glycemic control on long-standing DM in the elderly in not well-established and the preferred level of glycemic control should be considered in the overall context of the patient's health status. The optimal level of control among elderly patient subgroups requires

Table 4 Guideline recommendations for glycemic control in the elderly

Article	Age	HbA1c target	Population
The 2014 ADA guidelines[4]	Any age	< 7%	Adult patients without serious comorbidities
		< 8%	Patients with a more complex status (see text)
			Age itself not a criteria
The consensus report of the ADA and the American Geriatrics Society 2012[14]	> 65 yr	< 7.5%	Healthy old patients
		< 8%	Patients with intermediate health status (multiple chronic illnesses or 2+ instrumental impairments or mild cognitive impairment)
		< 8.5%	Patients with poor health status (end-stage chronic illnesses or moderate to severe cognitive impairment or 2+ ADL dependencies)
The 2013 IDF guidelines[39]	> 70 yr	7%-7.5%	Functionally independent old patients
		7%-8%	Functionally dependent old patients
		< 8.5%	Frail elderly or dementia
		Any HbA1c; just to avoid hypoglycemia	Patients at end-of-life

ADA: American Diabetes Association; ADL: Activities of daily living; IDF: International Diabetes Federation.

further evaluation.

Beyond all the above, the heterogeneity of the elderly population presents a significant challenge in clinical decision making. Old diabetic patients can be healthy or with much comorbidity and the risks of adverse events from medications increases with age. The decision regarding an individual patient's glycemic goal should be made, ideally with the patient himself, after considering all the comorbidities, together with current cognitive state, risk of adverse events, quality of life aspects and life expectancy.

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MINIREVIEWS

Statin use and risk of diabetes mellitus

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Abstract

The 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors, statins, are widely used in the primary and secondary prevention of cardiovascular diseases to lower serum cholesterol levels. As type 2 diabetes mellitus is accompanied by dyslipidemia, statins have a major role in preventing the long term complications in diabetes and are recommended for diabetics with normal low density lipoprotein levels as well. In 2012, United States Food and Drug Administration released changes to statin safety label to include that statins have been found to increase glycosylated haemoglobin and fasting serum glucose levels. Many studies done on patients with cardiovascular risk factors have shown that statins have diabetogenic potential and the effect varies as per the dosage and type used. The various mechanisms for this effect have been proposed and one of them is downregulation of glucose transporters by the statins. The recommendations by the investigators are that though statins can have diabetogenic risk, they have more long term benefits which can outweigh the risk. In elderly patients and those with metabolic syndrome, as the risk of diabetes increase, the statins should be used cautiously. Other than a subset of population with risk for diabetes; statins still have long term survival benefits in most of the patients.

Key words: New onset diabetes mellitus; Statins; Hyperglycemia; Cardiovascular risk; Dyslipidemia

Core tip: The use of statins in diabetics has long term benefits in terms of decreasing morbidity and mortality. Recent studies have shown that statins increase the incidence of new onset diabetes. The issue became debatable after Food and Drug Administration released changes to statin safety that they increase glycosylated haemoglobin and blood glucose levels. At the same time statins are beneficial in preventing cardiovascular events. Most of the investigators are of the opinion that the risk of diabetes with statins can be outweighed by the long term benefits in preventing complications. In patients with high risk of diabetes, statins should be cautiously used.

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INTRODUCTION

Statins, the 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, inhibit the rate limiting step of conversion of HMG-CoA to mevalonate and thus limit cholesterol synthesis. Lower hepatic cholesterol levels subsequently increase expression of low density lipoprotein (LDL)-receptors in liver cells. This in turn leads to enhanced clearance of LDL-particles from blood. Lowering of plasma LDL-cholesterol by statins reduce production and increase catabolism of apo B 100[1]. A range of products like coenzyme Q10, heme-A, and isoprenylated proteins are generated by mevalonate pathway[2] which have an important role in cell biology and human physiology. The role of statins has been hypothesized to be widespread as in inflammatory markers and nitric oxide (NO)[3], polyunsaturated fatty acids[4], immunomodulation[5], neuroprotection[6], cellular senescence[7], *etc.*

STATINS IN DIABETES

Statins are used for primary and secondary prevention of cardiovascular diseases. Other benefits due to statins are not mediated by their lipid lowering properties[8] but due to its pleiotropic effects. In conditions like heart failure, cardiac arrhythmias, vascular disease and hypertension the non-lipid lowering pleotropic benefits of statins have been observed[9]. These pleiotropic effects mediated by statins can be due to inhibition of isoprenoid synthesis which in turn inhibits intracellular signaling molecules Rho, Rac and Cdc42. The predominant mechanism that has been postulated is inhibition of Rho and its activation to Rho kinase[10].

Type 2 diabetes is characterized by hyperglycemia, insulin resistance and insulin deficiency. The insulin resistance contributes to the abnormal lipid profile associated with type 2 diabetes[11]. Dyslipidemia contributes to increased cardiovascular events in patients with type 2 diabetes[12]. A linear relationship exists between cholesterol levels and cardiovascular diseases in diabetics even if we ignore the baseline LDL[13]. By predominantly lowering LDL-Cholesterol and due to minor effects on other lipoproteins, statins appear to be beneficial[12]. In Heart Protection Study which was done in diabetics, the decrease in cardiovascular events like first major coronary event, stroke were to the tune of 22% as compared to placebo[14]. It was recommended by American Diabetes Association that statin therapy should be initiated in individuals with diabetes and other cardiovascular risk factors with target LDL cholesterol of 100 mg/dL[15]. Investigators are also of the opinion that statin therapy should depend not on the LDL levels but the cardiovascular complications accompanying diabetes[16]. Other studies which showed reduced coronary events with statins in patients with diabetes mellitus are Cholesterol and Recurrent Events and Long-term Intervention with Pravastatin in Ischaemic Disease studies of pravastatin[17,18]. In The Collaborative Atorvastatin Diabetes Study, statins significantly reduced acute coronary events by 36% and stroke by 48%. The beneficial effects of statins were so clear in this study that it was halted two years in advance[19]. West of Scotland Coronary Prevention Study also showed that the risk of diabetes was reduced by 30% in patients on pravastatin 40 mg/d[20].

STATINS AND DIABETES RISK

In February 2012, Food and Drug Administration released changes to statin safety label to include that statins have been found to increase haemoglobin (HbA1C) and fasting serum glucose levels[21]. This release has brought in the debate of using statins in patients with cardiovascular risk factors like diabetes.

In Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin study participants with LDL cholesterol levels of less than 130 mg/dL and elevated high-sensitivity C-reactive protein levels were included. They received rosuvastatin or placebo for a period of two years. It was observed that rosuvastatin significantly reduced the rates of a first major cardiovascular event and death from any cause as compared to placebo. A 54% lower risk of heart attack, 20% lower risk of stroke and 20% lower risk of death from any cause was noted in statin group[22]. An increase in new onset diabetes, *i.e.*, 3% in statin arm and 2.4% in placebo arm was reported. This was accompanied by increase in median value of glycated haemoglobin and was one of the earlier studies to report the increase in new onset diabetes in patients on statins. Women's Health Initiative trial was a post hoc analysis and included 153840 postmenopausal women without diabetes mellitus. Even after adjustment for potential confounders, statin therapy was associated with an increased risk of new-onset diabetes mellitus[23]. There was no difference between women with and without overt cardiovascular disease, which could have influenced the risk-benefit ratio of statins[23]. Authors suggest that statin-induced diabetes mellitus is a medication class effect[23]. Another study also reported that as compared to placebo, statin group showed a higher risk of physician reported incident diabetes and it was also observed that risk was higher in women as compared to men[24].

Meta-analysis of randomized controlled trials by Sattar *et al*[25] involving 91140 non-diabetic patients showed that statin therapy was associated with 9% increased risk of incident diabetes. After a period of four years during which 255 patients were treated, there was one extra case of diabetes mellitus[25]. Authors did not find any apparent difference between lipophilic and hydrophilic statins in association with

diabetes risk[25].

Though some studies put forth this as a class effect, others showed different effects with different statins and at different doses. A number of studies showed dose dependent association between statin administration and incident diabetes. A meta-analysis with 32752 participants was done in which the risk of intensive dose statin therapy was compared with moderate dose statin therapy on incident diabetes. It revealed that intensive dose of statins was associated with high incidence of new - onset diabetes, though it decreased cardiovascular events as well[26]. In those receiving intensive dose of statins, 18.9 ± 5.2 diabetic cases per 1000 patient years were observed *vs* 16.9 ± 5.5 cases per 1000 patient years with moderate doses of statin therapy[26].

In PROVE-IT TIMI 22 trial 3382 patients without pre-existing type 2 diabetes mellitus were included. The levels of HbA1C increased by 0.12% in patients treated with pravastatin 40 mg, while in those receiving atorvastatin 80 mg showed a significant difference and the levels increased by 0.30%[27]. Another study comparing glycaemic control between diabetic patients receiving atorvastatin 10 mg, pravastatin 10 mg or pitavastatin 2 mg/d showed that it was only the atorvastatin-treated patients in which the blood glucose and HbA1C levels increased[28]. Treatment with atorvastatin and simvastatin may be associated with an increased risk of new onset diabetes as compared to pravastatin[29]. Pitavastatin has shown favourable profile in patients with diabetes by improving insulin resistance and minimally impairing glucose metabolism[30]. Increased incidence of diabetes was seen with atorvastatin in the Anglo-Scandinavian Cardiac Outcomes Trial[31] and impaired glucose metabolism in some cases of type 2 diabetes[32]. Increased insulin resistance secondary to statins was demonstrated in a prospective non randomised study in patients with coronary bypass surgery[33]. So, many studies have reported an increase in new onset diabetes and there is a variation in response depending on the statin administered and the dose of statin.

MECHANISM OF STATINS IN HYPERGLYCEMIA

Statin-induced insulin resistance can be due to inhibition of isoprenoid biosynthesis and downregulating C/EBPα production[34]. Decreased synthesis of isoprenoids can produce downregulation of GLUT4 expression on adipocyte cells[35]. It can lead to decrease in insulin-mediated cellular glucose uptake and possibly manifest as intolerance to glucose[36]. Acceleration of type 2 diabetes can be seen secondary to downregulation of GLUT4/SLC2A4 in adipocytes[37]. Over-production of NO by inducing cytokines can cause β-cell apoptosis[38].

On incubating rat pancreatic cells with statin ,there was a decrease in insulin secretion due to inhibition of glucose stimulated increase in free cytoplasmic calcium[39]. Statins also inhibit the insulin secretion due to reduced production of ATP by suppressing the synthesis of ubiquinone (CoQ10)[39]. Clinical doses of atorvastatin in animal model of type 2 diabetes led to inhibition of adipocyte differentiation, decreased SLC2A4 expression in both differentiating and mature adipocytes, and impaired insulin sensitivity and post-challenge glucose tolerance[34]. Animal models have also shown that there is an association between development of insulin resistance and statin-induced myopathy[40].

Other mechanisms hypothesized for the possible effect of statins on new-onset diabetes are that statins by inhibiting phosphorylation interfere with intracellular signal transduction pathways of insulin, reduce action of of small GTPase, decrease peroxisome proliferator activated receptor gamma by inhibiting the differentiation of adipocytes, inhibit β-cell proliferation and insulin secretion by inhibiting leptins[41]. Atorvastatin, a lipophilic statin, may decrease insulin secretion due to increased HMG-CoA inhibition or cytotoxicity[42]. The actions of statins on beta cells of pancreas can be summarised[43] as shown in Figure 1.

RECOMMENDATIONS FOR USE OF STATINS IN DIABETES

Authors have put forth the recommendations from time to time regarding the use of statins in patients with cardiovascular diseases. For patients with cardiovascular risk factors, statins prevent cardiovascular event 8 times more likely than they can cause a case of incident diabetes[44] shifting the risk-benefit ratio in favour of statin therapy[44]. Modest increase in blood glucose levels by statins will not be an issue of concern if they decrease morbidity and mortality due to macrovascular and microvascular complications[45]. In patients with low cardiovascular risk factors, statins should be cautiously used, less aggressive LDL-C-lowering targets should be kept and monitoring of fasting blood glucose levels should be done routinely[46].

In high risk patients with impaired glucose tolerance and established cardiac risk factors, statins and diuretics increased the risk of new onset diabetes. As both the drugs have a propensity to increase blood glucose levels, there is a need of regular monitoring[47]. As compared to other cardiovascular medications like thiazide diuretics and beta blockers, statins can three times less likely cause diabetes[48].

When statins are being used in primary prevention patients at high risk of diabetes, pravastatin should be preferred over other statins[29]. One of the meta-analysis comparing high-dose statin therapy with moderate dose found that the former is associated with improved cardiovascular outcomes, though at the same time there was a 12% increased risk of new-onset diabetes mellitus[49]. Such studies have stimulated the controversy about the treatment of patients not attaining target lipid profile on moderate dose of statins[49].

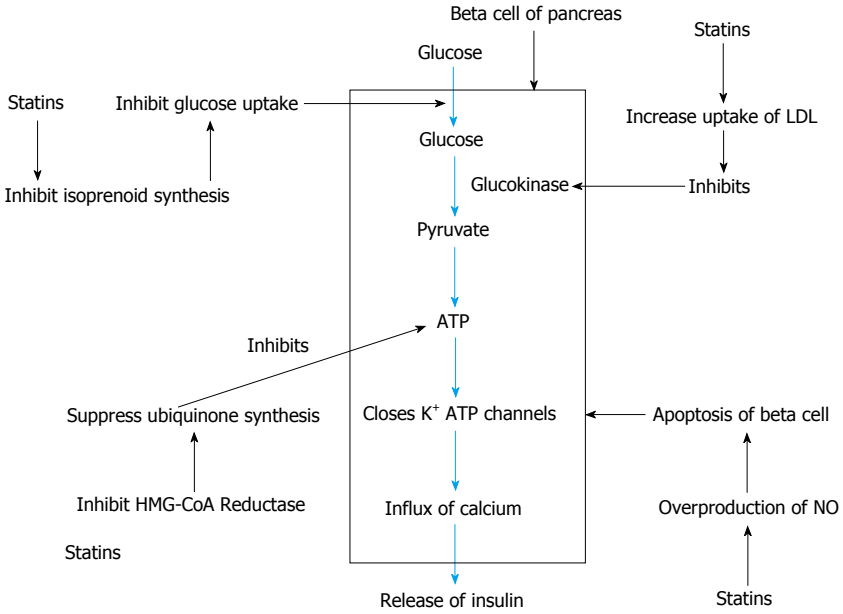

Figure 1 Actions of statins on beta cell of pancreas[43]. HMG-CoA: 3-hydroxy-methylglutaryl coenzyme A; NO: Nitric oxide; LDL: Low density lipoprotein.

Increasing age increases the risk of diabetes and benefits secondary to statins can decrease. There is a need to be vigilant in these patients[50]. Factors like older age, increased weight, and higher blood sugar levels before the use of statins predict that whether a patient will develop diabetes mellitus. The use of statins can unmask diabetes mellitus in patients with other risk factors[51]. So in obese patients and those with metabolic syndrome these findings may be relevant[52]. While analysing one of the initial studies which suggested the link between statins and diabetes, it was found that rate of reduction of cardiovascular events outbalanced the risk of incident diabetes even in patients at highest risk for diabetes though the absolute risk increase was small (placebo 1.2%, rosuvastatin 1.5% developed diabetes[53]). Meta-analysis of individual data of over 170000 persons from 27 randomized trials also put forth the risk benefit ratio in favour of statins[54]. In patients on statins, there was an improved outcome after cardiac surgery[55]. Current guidelines recommend use of statins in patients undergoing coronary artery bypass graft[56]. Statins can reduce cardiovascular complications like atrial fibrillation and MI after cardiac surgery, but at the same time poor glycemic control may lead to deterioration of non-cardiovascular complications like infections and renal complications[33].

Elevated triglycerides and low HDL-C are associated with type 2 diabetes mellitus. The evidence for drugs targeting this type of dyslipidemia is not as strong as those targeting LDL-C[57].

To conclude, physicians should be cautious about development of diabetes in patients on intensive statin therapy[26]. Lifestyle management should be considered in patients with low risk of cardiovascular diseases[58] and the use of statins should be reconsidered[58]. In patients with cardiovascular risk factors, the benefits of statins supersede the risk of diabetes[59]. There is a need of randomized clinical trials to find the role of statins on microvascular complications as the existing evidence only shows a benefit on macrovascular complications[60]. Overall evidence at present shows that the risk of new onset diabetes is less as compared to the long term benefits of statins in patients with cardiovascular risk factors. But there is a small subgroup of population in whom a more careful use of statins is mandatory.

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ORIGINAL ARTICLE

Case Control Study

Association of gene variants with susceptibility to type 2 diabetes among Omanis

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Abstract

AIM: To investigate the association of 10 known common gene variants with susceptibility to type 2 diabetes mellitus (T2D) among Omanis.

METHODS: Using case-control design, a total of 992 diabetic patients and 294 normoglycemic Omani Arabs were genotyped, by an allelic discrimination assay-by-design TaqMan method on fast real time polymerase chain reaction system, for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634) *CAPN10* (rs3792267) and *HHEX* (rs1111875). T2D patients were recruited from the Diabetes Clinic (n = 243) and inpatients (n = 749) at Sultan Qaboos Univesity Hospital (SQUH), Muscat, Oman. Adult control participants (n = 294) were volunteers from the community and from those visiting Family Medicine Clinic at SQU, for regular medical checkup. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study. Almost all volunteers questioned had a relative

with diabetes mellitus. Inspite of the small number of normoglycemic controls in this study, this sample was sufficient for detection of genes and loci for common alleles influencing T2D with an odds ratio of $\geqslant$ 1.3 reaching at least 80% power. Data was collected from June 2010 to February 2012.

RESULTS: Using binary logistic regression analysis, four gene variants showed significant association with T2D risk: *KCNJ11* (rs5219, $P = 5.8 \times 10^{-6}$, OR = 1.74), *TCF7L2* (rs7903146, $P = 0.001$, OR = 1.46), *CDKAL1* (rs10946398, $P = 0.002$, OR = 1.44) and *CDKN2A/B* (rs10811661, $P = 0.020$, OR = 1.40). The fixation index analysis of these four gene variants indicated significant genetic differentiation between diabetics and controls {[*KCNJ11* (rs5219), $P < 0.001$], [*TCF7L2* (rs7903146), $P < 0.001$], [*CDKAL1* (rs10946398), $P < 0.05$], [*CDKN2A/B* (rs10811661), $P < 0.05$]}. The highest genotype variation % between diabetics and controls was found at *KCNJ11* (2.07%) and *TCF7L2* (1.62%). This study was not able to detect an association of T2D risk with gene variants of *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Moreover, no association was found between *FTO* gene variants (rs9939609 and rs8050136) and T2D risk. However, T2D risk was found to be significantly associated with obesity ($P = 0.002$, OR = 2.22); and with the Waist-to-Hip ratio ($n = 532$, $P = 1.9 \times 10^{-7}$, OR = 2.4), [among males ($n = 234$, $P = 1.2 \times 10^{-4}$, OR = 2.0) and females ($n = 298$, $P = 0.001$, OR = 6.3)].

CONCLUSION: Results confirmed the association of *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661) gene variants with susceptibility to T2D among Omani Arabs.

Key words: Type 2 diabetes; Genetics; Oman; Case-control; Association; Gene; Variants

Core tip: To investigate the association of 10 known common gene variants with susceptibility to type 2 diabetes mellitus (T2D) among Omani Arabs using case-control design. A total of 992 diabetic patients and 294 normoglycemic Omani Arabs were genotyped for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634) *CAPN10* (rs3792267) and *HHEX* (rs1111875). Four gene variants showed significant association with T2D risk: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). The highest genotype variation % between diabetics and controls was found at *KCNJ11* and *TCF7L2* gene variants.

Al-Sinani S, Woodhouse N, Al-Mamari A, Al-Shafie O, Al-Shafaee M, Al-Yahyaee S, Hassan M, Jaju D, Al-Hashmi K, Al-Abri M, Al-Rassadi K, Rizvi S, Loic Y, Froguel P, Bayoumi R. Association of gene variants with susceptibility to type 2 diabetes among Omanis. *World J Diabetes* 2015; 6(2): 358-366 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i2/358.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i2.358

INTRODUCTION

Type 2 diabetes mellitus (T2D) is one of the most common non-communicable diseases globally. Insufficient compensatory insulin secretion due to insulin resistance causes T2D. Insulin resistance is, mostly, an early event due to environmental factors, such as obesity. Decline in β-cell function is gradual but generally a late event[1]. In addition to the environmental factors, there is strong evidence that genetic factors play an important role in the pathogenesis of T2D[2].

Candidate gene approach identified few T2D susceptibility gene variants: *Pro12Ala* (rs1801282) in the coding region of peroxisome proliferator-activated receptor γ gene and it is the more common proline allele that is associated with T2D[3]; *E23K* (rs5219) in the coding region of the subunit kir6.2 of the ATP-sensitive potassium channel gene of β-cells (*KCNJ11*)[4] and a series of polymorphisms and haplotypes (UCSNP-43 or rs3792267; UCSNP-19 or rs3842570 and UCSNP-63 or rs5030952) in the coding region of the cysteine protease calpain 10 (*CAPN10*)[5].

Genome-wide association studies (GWAS) of T2D susceptibility genes and loci and their meta-analysis identified a large number of gene variants and confirmed the previously discovered ones[6]. The common intronic variants within the transcription factor 7-like 2 (*TCF7L2*) gene was reported as the strongest genetic risk factor for T2D[7]. Other loci most consistently associated with T2D risk include variants within or near the solute carrier family 30/zinc transporter (*SLC30A8*), hematopoietically expressed homeobox (*HHEX*), cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*), insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*), a genomic region between cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) and fat mass and obesity associated protein (*FTO*)[6,8].

In total, approximately, forty four common T2D susceptibility gene variants and loci have been identified to-date, but all these variants could only explain approximately 10%-15% of the heritability of T2D; which suggests that more variants remain to be discovered[9]. Rare large-effect mutations have recently been re-cognized as causes of many complex diseases[10-12].

According to the international diabetes federation, six out of the world's top ten countries with the highest prevalence (%) of T2D among adults aged 20-79 years, in 2011, are in the Middle East and North Africa region; Kuwait (21.1%), Lebanon (20.2%), Qatar (20.2%), Saudi Arabia (20.0%), Bahrain (19.9%) and

United Arab Emirates (19.2%). Small studies were conducted among Arabs, with a limited number of participants, to investigate genetic susceptibility for T2D[7,13-24].

The prevalence of diabetes in Oman, in 2011, was estimated to be 10.8%; with a further 9.7% of the population at high risk of diabetes with impaired glucose tolerance, (http://www.idf.org/diabetesatlas/content/what-is-diabetes). Oman has a high inbred population and consanguineous marriages are about half of all marriages[25]. Therefore, genetic factors might play an important role in the pathogenesis of T2D among Omanis.

In the present study, 10 known common gene variants, described previously, were examined for their association with susceptibility to T2D among Omani Arabs using case-control study design. Selection of variants was predominantly based on earlier GWAS studies, which extensively investigated T2D and showed a significant association of those variants with the highest odds ratios (ORs) among all the genes/loci discovered[4-8,26].

MATERIALS AND METHODS

Sample size

For determining the sample size, we employed the Power Calculator for Genetic Studies developed by Skol *et al*[27] (2006) in their Website http://www.sph.umich.edu/csg/abecasis/CaTS/index.html. We used a T2D prevalence of 10% in the adult population of the Region as reported previously[28,29]. We also anticipated disease allele frequencies of $\geqslant$ 0.25, and assumed a multiplicative disease model[30]. An optimum one-stage sample was deduced from the Power Calculator: 1000 cases and 1000 controls, will guarantee detection of genes and loci for common alleles influencing T2D with an OR of $\geqslant$ 1.2 reaching at least 80% power. However, we could not collect the required sample size, and only 992 cases and 294 controls were collected. This sample will guarantee detection of genes and loci for common alleles influencing T2D with an OR of $\geqslant$ 1.3 reaching at least 80% power.

Study population

A total of 992 T2D Omani Arab patients and 294 normoglycemic Omani Arab controls were included in this study. T2D patients were recruited from the Diabetes Clinic (n = 243) and inpatients (n = 749) at Sultan Qaboos Univesity Hospital (SQUH), Muscat, Oman. A history of T2D among patients was ascertained from the diagnosis and medical history deposited in the electronic records of the hospital information system. Exclusion criteria for T2D patients included: patients diagnosed with type 1 diabetes; maturity onset diabetes of the young; positive diabetic antibodies (islet cell antibodies and glutamic acid decarboxylase antibodies) or patients diagnosed with any type of cancer. Adult control participants (n = 294) were volunteers from the community and from those visiting Family Medicine Clinic at SQU, for regular medical checkup. The inclusion criteria for controls were: Omani, age $\geqslant$ 35 years, no family history of diabetes (first degree relatives) and with fasting glucose value of < 6.1 mmol/L, according to the World Health Organization 2006 criteria. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study. Almost all volunteers questioned had a relative with diabetes mellitus (DM). Data was collected from June 2010 to February 2012. Participants were informed about the project and written consents were obtained. The study was approved by the Ethics and Research Committee of the College of Medicine, Sultan Qaboos University, Muscat, Oman.

Anthropometric and biochemical parameters

T2D patients and normoglycemic control participants underwent demographic, anthropometric and biochemical investigations, summarized in Table 1. Anthropometric variables measured were: weight, height, waist and hip circumference. Obesity status was defined according to the international classification of an adult's weight (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html), [normal body mass index (BMI): 18.5-24.99 kg/m^2, overweight: 25.00-29.99 kg/m^2 and obese $\geqslant$ 30.00 kg/m^2]. The biochemical investigations included: fasting glucose level and HbA_{1c}. To compare T2D patients and normoglycemic control participants' obesity status, we selected 294 T2D patients; age and sex matched with the normoglycemic control participants (n = 294). Waist-to-Hip ratio (WHR) was also calculated among T2D patients and control participants. Health risk based solely on the WHR, was identified according to the ranges specified at waist-to-hip ratio chart (http://www.bmi-calculator.net/waist-to-hip-ratio-calculator/waist-to-hip-ratio-chart.php) for males (low risk = 0.95 or below, moderate risk = 0.96-1.0, high risk= over 1.0) and females (low risk = 0.80 or below, moderate risk = 0.81-0.85, high risk = over 0.85).

Genotyping

All participants (n = 1286) were genotyped for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Genotyping was done by an allelic discrimination assay-by-design TaqMan method on 7500HT fast real time polymerase chain reaction system (Applied Biosystems, United States). Accuracy was achieved by duplicating approximately 10% of the samples.

Statistical analysis

The SPSS statistical package software (v20.0) was

Table 1 Anthropometric and biochemical characteristics of Omani type 2 diabetes mellitus patients and controls

	T2D patients			Controls		
	Mean ± SD or median (range)[1]			Mean ± SD or median (range)[1]		
	Males	Females	Total	Males	Females	Total
Total number (*n*)	473	519	992	121	173	294
Age (yr)	56 ± 11	56 ± 10	56 ± 11	41 (35-80)[1]	44 (32-79)[1]	43 (32-80)[1]
Weight (kg)	78.8 ± 15.4[NS]	75.1 ± 17.0	76.8 ± 16.4	76.8 ± 14.2	70.3 ± 13	73.7 ± 14.7
Height (cm)	165 ± 9[a]	152 ± 9	157 (106-182)[1NS]	167 ± 8	154 ± 6	159 ± 9
BMI (kg/m^2)	29.1 ± 4.8[b]	32.8 ± 10.3	30 (15-58)[1]	27.6 ± 4.3	29.5 ± 5.2	29.3 ± 5.8
Waist circumference (cm)	100 ± 12	100 ± 13	100 ± 13	95 ± 13	91 ± 11	92 ± 12
Fasting blood glucose (mmol/L)	8.7 (3-24)[1]	9.0 (3-25)[1]	8.8 (3-25)[1]	5.1 ± 0.31	4.9 ± 0.36	5.1 ± 0.41
HbA_{1C} (%)	8.3 ± 1.8	8.3 (4.9-15.5)[1]	8.2 (4.1-18.6)[1]	5.7 ± 0.46	5.7 (4.0-7.9)[1]	5.7 ± 0.44
Obesity status (%)						
Underweight	-	0.5	0.3	2.1	1	1.4
Normal weight	16.8	12.5	14.5	22.2	18.2	19.1
Overweight	45	30.2	36.1	43.8	34.5	38.9
Obese	38.2	56.8	49.1	29.9	45.8	39.6
Missing	-	-	-	2.1	0.5	1

[1]Median (range = minimum-maximum) displayed in the table when the variable does not follow a normal distribution pattern. In all parameters, $P < 0.001$ between diabetics and controls, except: NS; [a]$P < 0.05$ between diabetics and controls; [b]$P < 0.01$ between diabetics and controls. NS: No significant difference between diabetics and controls; T2D: Type 2 diabetes mellitus; BMI: Body mass index.

used for statistical analysis of measured parameters. The measured anthropometric and biochemical parameters were tested for normal distribution using one sample Kolmogorov-Smirnov test. Independent sample *t*-test was used to test the significance of the difference in the mean values for the measured anthropometric and biochemical parameters between T2D patients and control participants with a normal distribution, while the Mann-Whitney *U* test was used for variables with skewed distribution.

The frequencies of the risk allele for each gene variant were calculated for T2D patients and normoglycemic control participants. The proportions of the genotypes of the gene variants were tested for departures from Hardy-Weinberg equilibrium (HWE) for both groups using population genetics software GenAlEx 6.3 (Genetic analysis in Excel, version 6.3)[31]. However, in case-control studies, HWE should be applied only to controls because a deviation from HWE in cases may indicate a genetic association[32,33].

Genotyping data were further analyzed using GenAlEx 6.3[31] and Arlequin 3.1 software[34]. For each polymorphism, GenAlEx was used to calculate fixation index (F), heterozygosity (He) and Fst, which provides a measure of genetic differentiation among subpopulations (T2D patients and control populations). Arlequin was used to calculate genotype's % variation among the subpopulations and its level of significance.

Binary logistic regression analysis on the SPSS statistical package was used to test the association between each gene variant and susceptibility to T2D, adjusted for age, sex and BMI. Bonferroni correction was applied for multiple testing and adjusted *P* values were calculated to be 0.005. Beta coefficients, ORs and 95%CI were also estimated. An OR is a measure of association between an exposure and an outcome. It is measured as an exponential function of the regression beta coefficient value ($e^{beta\ coefficient}$).

The association between obesity status of participants and *FTO* gene variants (rs9939609 and rs8050136) was also tested. In addition, obesity and health risk status, based on the WHR, were tested for their association with T2D risk.

RESULTS

Anthropometric and biochemical characteristics of all participants are summarized in Table 1. About 48% of the T2D patients and 41% of the control participants were males. The mean age of T2D patients (56 years) was higher than that of the normoglycemic control participants (45 years). T2D patients had significantly higher weight, BMI, waist circumference, fasting glucose values and HbA_{1C} % levels compared with control subjects (Table 1). Eighty five percent of the T2D Omani patients were overweight to obese in comparison to 78.5% of the control Omani participants. Half of the T2D patients and 39.6% of the control participants were obese. T2D risk was found to be significantly associated with obesity ($P = 0.002$, OR = 2.22); and with the WHR ($n = 532$, $P = 1.9 \times 10^{-7}$, OR = 2.4), [among males ($n = 234$, $P = 1.2 \times 10^{-4}$, OR = 2.0) and females ($n = 298$, $P = 0.001$, OR = 6.3)].

Among control participants, there were no significant deviation in the proportions of gene variant frequencies from HWE except in *SLC30A8* (rs13266634) ($P = 2.35 \times 10^{-4}$, $\chi^2 = 13.5$) gene variant. However, among T2D patients, there were significant deviations in: *KCNJ11* (rs5219) ($P = 4.14 \times 10^{-9}$, $\chi^2 = 34.6$), *CDKAL1* (rs10946398) ($P = 0.008$, $\chi^2 = 6.9$) and *IGF2BP2* (rs4402960) ($P = 0.038$, $\chi^2 = 4.3$) gene variants.

The risk allele frequencies of the tested variants for diabetic and control participants are summarized in Table 2. Using binary logistic regression analysis,

Table 2 Risk allele frequencies for the tested gene variants among Omani type 2 diabetes mellitus patients and control participants

Gene	Gene variant (SNPs)	Risk/non-risk allele	Risk allele frequency (f)		[1]*P* value	OR	95%CI for OR
			T2D patients (*n* = 992)	Controls (*n* = 294)			
KCNJ11	rs5219	T/C	0.320	0.222	[2]5.8×10^{-6}	1.74	1.37-2.22
TCF7L2	rs7903146	T/C	0.445	0.354	[2]0.001	1.46	1.16-1.83
CDKAL1	rs10946398	C/A	0.364	0.311	[2]0.002	1.44	1.15-1.80
CDKN2A/B	rs10811661	T/C	0.836	0.799	0.020	1.40	1.06-1.84
FTO	rs9939609	A/T	0.480	0.435	0.358	1.11	0.899-1.37
FTO	rs8050136	A/C	0.458	0.425	0.770	1.03	0.829-1.29
IGF2BP2	rs4402960	T/G	0.400	0.357	0.286	1.13	0.904-1.41
SLC30A8	rs13266634	C/T	0.857	0.855	0.329	1.16	0.859-1.57
CAPN10	rs3792267 (-43)	G/A	0.802	0.790	0.445	1.11	0.850-1.45
HHEX	rs1111875	T/C	0.301	0.280	0.636	1.06	0.839-1.33

[1]*P* value: Level of significance; [2]*P* value remained significant after correction for multiple testing (< 0.005). The *P* value, OR and 95%CI were calculated for the association between each gene variant with T2D risk. f: Frequency; T2D: Type 2 diabetes mellitus; SNPs: Single nucleotide polymorphisms.

Table 3 Fixation index, heterozygosity, Fst and % variation among Omani type 2 diabetes mellitus patients and control participants

Gene	Gene variants	T2D patients		Controls		Fst	% variation among T2D and controls	[1]*P* value
		F	He	F	He			
KCNJ11	rs5219	0.190	0.352	0.091	0.314	0.012	2.07	0.000
TCF7L2	rs7903146	0.017	0.486	0.000	0.476	0.009	1.62	0.000
CDKAL1	rs10946398	0.084	0.424	0.056	0.405	0.003	0.48	0.020
CDKN2A/B	rs10811661	0.000	0.275	0.003	0.320	0.002	0.37	0.042
FTO	rs9939609	0.000	0.502	0.000	0.497	0.002	0.31	0.050
FTO	rs8050136	0.006	0.493	0.000	0.503	0.001	0.12	0.147
IGF2BP2	rs4402960	0.066	0.448	0.000	0.476	0.002	0.23	0.089
SLC30A8	rs13266634	0.061	0.230	0.188	0.201	0.000	0.00	1.000
CAPN10	rs3792267	0.057	0.300	0.000	0.335	0.000	0.00	0.517
HHEX	rs1111875	0.055	0.398	0.052	0.382	0.001	0.00	0.341

This provides a measure of genetic differentiation among population; [1]*P* value: Level of significance; F: Fixation index; He: Heterozygosity; Fst: The inbreeding coefficient within subpopulation, relative to total; T2D: Type 2 diabetes mellitus.

four gene variants out of 10 showed statistically significant association ($P < 0.05$) with susceptibility to T2D: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661), Table 2. After correction for multiple testing, *KCNJ11*, *TCF7L2* and *CDKAL1* gene variants still showed a significant association ($P < 0.005$) with T2D.

Fst values, showed statistically significant genetic differentiation between T2D patients and controls (Table 3), in the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). These findings confirmed the results obtained using binary logistic regression analysis.

This study was not able to detect an association of T2D risk with gene variants of *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Moreover, no association was found between *FTO* gene variants (rs9939609 and rs8050136) and T2D risk.

DISCUSSION

In this study, four gene variants showed significant association with T2D risk using binary logistic regression analysis after adjustment for confounding factors of age, sex and BMI: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). The association of *KCNJ11* (rs5219), *TCF7L2* (rs7903146) and *CDKAL1* (rs10946398) gene variants with T2D risk remained significant after correction for multiple testing. Fst, a measure of genetic differentiation among subpopulations (diabetics and controls), confirmed the significant risk difference between diabetics and controls at the four gene loci [*KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661)]. The highest genetic variation between diabetics and controls was found in *KCNJ11* and *TCF7L2* gene variants (Table 3). However, none of the other gene variants previously reported in GWAS were found to be associated with risk to T2D in Omanis.

*KCNJ11 (*rs5219) gene variant was found to be associated with T2D risk among Omani Arabs with an OR of 1.74, which is higher than that reported in previous European studies. Our findings were consistent with what was reported among Saudi Arabs (OR = 1.7), although the risk allele frequency was found to be lower among Saudis compared to Omani Arabs[16]. However, in both Arab studies the association may be overestimated due to the small number of participants included or due to a high-inbred population.

In contrast, no association of this gene variant with susceptibility to T2D was found among Tunisian Arabs[14]. Large scale studies and meta-analysis of the *KCNJ11* gene variants have shown that the lysine variant of the rs5219 gene loci resulting in a 1.15 times higher risk of developing T2D[4,35] and GWAS studies confirmed this association[26,36,37]. The rs5219 variant is located within the N-terminal of the subunit kir6.2 of the ATP-sensitive potassium channel gene of β-cells and can cause spontaneous hyperactivity of pancreatic beta-cells and reduced sensitivity of KATP channels to ATP, resulting in impaired insulin secretion[38].

Although this study is relatively small, the association of *TCF7L2* (rs7903146) gene variant with T2D risk among Omani Arabs is consistent with previous large GWAS studies[14,15,22,36,37,39-44]. The association of *TCF7L2* gene variants with susceptibility to T2D was marginal between the rs12255372 gene variant and T2D risk among Emirati Arabs; but not in Saudi Arabs[13,17]. In contrast, subsequent studies among North African Arabs (Tunisians and Moroccans), Palestinians and Iranians, confirmed the association of *TCF7L2* (rs7903146) gene variant with susceptibility to T2D[7,14,15,22]. Comprehensive genotyping studies across the *TCF7L2* gene showed that the rs7903146 variant to be consistently associated with T2D among European with an OR of 1.37 (1.28 to 1.47)[45,46]. Meta-analysis of 27 different studies found a global OR of 1.46 (95%CI: 1.42-1.51)[7]. The common variants in the *TCF7L2* gene predispose to T2D by reducing beta-cell function and insulin secretion. *TCF7L2* mRNA levels in human pancreatic islets increase with the number of risk alleles and are fivefold higher in T2D patients than in controls pancreatic islets, and over-expression of *TCF7L2* leads to reduced glucose stimulated insulin secretion[47].

CDKAL1 (rs10946398) gene variant's OR was found to be 1.44 among Omani Arabs, which may be overestimated due to the small number of participants. Variants at *CDKAL1* gene loci showed an association with T2D risk among Caucasians[26,36,37], Asians[48,49], African Americans[50] and Arabs[22,24]. A recent meta-analysis of *CDKAL1* (rs10946398) gene variant showed a significant association of this variant with susceptibility to T2D risk (OR = 1.12)[51]. Another study among Russian population showed a significant association of the C allele with higher risk of T2D[52]. The *CDKAL1* gene encodes cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1. The CDK5 is a serine/threonine enzyme that inhibits both Ca^{2+} efflux into the beta-cell and insulin secretion, while inhibition of this enzyme results in enhanced insulin secretion[53].

The association of *CDKN2A/B* (rs10811661) gene variant with susceptibility to T2D risk among Omanis was also confirmed in this study (OR = 1.40), in agreement with a recent large study among North African Arabs[22]. In contrast, this gene variant was not found to be associated with T2D risk in other Arab studies[24,54]. Previous studies among European populations have shown an association with an OR of 1.20[26,36,37]. A recent meta-analysis concluded that the T allele of rs10811661 is a risk factor of T2D both in Asians and Europeans[55]. *CDKN2A* and *CDKN2B* genes encode $p16^{INK4a}$ and $p15^{INK4b}$, which inhibit CDK4 and CDK5, respectively. CDK4 and CDK5 play an important role in β cell function and regeneration[55].

FTO gene variants (rs9939609 and rs8050136) have been shown to associate with BMI and obesity[8,56] and GWAS studies of T2D have also suggested the involvement of *FTO* gene in T2D pathogenesis through obesity[8,26]. It is surprising, therefore, that we could not detect an association of T2D risk with *FTO* gene variants (rs9939609 and rs8050136). This could be attributed to the fact that both diabetics and controls have similar distributions of body weight. The impact of the *FTO* gene variants on T2D risk through obesity, seen in other populations, was not observed here. Hennig *et al*[57] tested the effect of *FTO* variants on measures of BMI in a population of lean Gambians (Africans) and also found no association. However, this study showed a significant association between obesity and T2D risk; and between health risk, based on the WHR, and T2D risk among males and females.

In spite of the small number of participants examined in this case-control study, we were able to confirm the effect of four common gene variants on T2D risk among Omani Arabs. Oman has a homogeneous population due to a high level of inbreeding and the tradition of consanguineous marriages. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study, where, almost everybody has a relative with DM. This might have raised risk allele frequencies of *T2D* gene variants and made it easier to detect.

All previous GWAS identified common gene variants, which could only explain 10%-15% of the heritability of T2D. Large studies with new strategies, other than the classic case-control study design, are required to find the hidden heritability due to rare variants behind developing T2D among Omani and other Arab populations.

Limitation of this study was the lack of oral glucose tolerance test, where we could not run the test among the control group. However, the strength of this study was that the control participants were with no family history of diabetes.

This study confirmed the effect of four common gene variants on T2D risk among Omani Arabs: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). However, we could not detect the association of other known common gene variants with susceptibility to T2D.

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COMMENTS

Background

Type 2 diabetes mellitus (T2D) is one of the most common non-communicable diseases globally. Insufficient compensatory insulin secretion due to insulin resistance causes T2D. In addition to the environmental factors, there is strong evidence that genetic factors play an important role in the pathogenesis of T2D.

Research frontiers

Oman has a high inbred population and consanguineous marriages are about half of all marriages. Therefore, genetic factors might play an important role in the pathogenesis of T2D among Omanis.

Innovations and breakthroughs

In the present study, 10 known common gene variants were examined for their association with susceptibility to T2D among Omani Arabs using case-control study design. Selection of variants was predominantly based on earlier Genome-wide association studies, which extensively investigated T2D and showed a significant association of those variants with the highest odds ratios among all the genes/loci discovered.

Applications

Large studies with new strategies, other than the classic case-control study design, are required to find the hidden heritability due to rare variants behind developing T2D among Omani and other Arab populations.

Terminology

T2D is one of the most common non-communicable diseases globally, and it is a result of insufficient compensatory insulin secretion due to insulin resistance. Candidate gene approach focuses on associations between genetic variation within pre-specified genes of interest and phenotypes or disease states. Genome-wide association studies scan the entire genome for common genetic variation.

Peer-review

This is a well-written and interesting paper evaluating the association between a variety of gene polymorphisms and the risk for type 2 diabetes mellitus.

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